

The Regulatory Role of Macrophages in Antigenic Stimulation

Part Two: Symbiotic Relationship between Lymphocytes and Macrophages

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I. Historical

In 1972, I reviewed for this series the regulatory role of phagocytes in antigenic stimulation, i.e., in immune induction (Unanue, 1972). The information gathered at that time clearly pointed to a highly significant role of accessory phagocytic cells in the multiple cellular interactions taking place during the early development of an immune response. To recapitulate briefly, a number of *in vivo* experiments had indicated a direct relationship between the uptake of antigens by the phagocyte system and the extent of the immune response. Thus, changes in antigen molecules that resulted in enhanced or decreased uptake by the phagocytes resulted in higher or lower immune responses, respectively. After the development of methodologies for obtaining live exudate cells rich in phagocytes and for pulsing these with antigen, the immune response to macrophage-associated antigens was

possible to assay, using combinations of *in vivo* and *in vitro* methods. This approach led to the result that the presentation of antigen bound to live macrophages to the lymphocytes was a highly efficient mode of generating an immune response. Invariably, a strong antibody response was produced to antigen molecules transferred to a host in syngeneic, live macrophages. Concurrently with this approach, the analysis of cellular interactions employing culture methods pointed to a requirement for adherent phagocytic cells in order for lymphocytes to proliferate or to make antibody. In 1972, the basis for the strong immunogenicity of macrophage-associated antigens and for the requirements of macrophages for *in vitro* immune responses were not clear, nor were the cellular and molecular events understood. We felt strongly, based in part on our own studies on antigen handling, that antigens associated with macrophage surface were involved in antigen presentation, yet how, in what form, and with what class of lymphocyte remained unexplained. Whether this antigen simply served as a device to focus T and B cells or played a more essential role was not known. The role of macrophages in directly regulating growth and differentiation of lymphocytes could only be speculated, based on suggestive experiments on the effects of adjuvants on macrophages. Although great steps had been taken in our basic knowledge of the biology of phagocytes, many issues were still to be resolved, most notably those of macrophage differentiation, antigen handling, and secretion.

The 8 years after the earlier review witnessed an incredible number of studies on phagocyte biology and on macrophage-lymphocyte interactions. These studies have placed the macrophage, now more than ever, as a critical regulatory cell with functions never before suspected. Our progress in this field came as the result of improvement in tissue culture methods, in techniques for isolating and identifying lymphocytes, in procedures to probe various cellular interactions, plus major advances in macrophage biology, and, most notably, in basic cellular immunobiology. Progress in immunobiology developed in parallel in the 1970s in various areas, i.e., transplantation genetics, immune response genetics, T-B cell collaboration, cytolytic T cell-tumor effects, and macrophage-lymphocyte interactions. The major findings and discoveries are now beginning to be integrated with each other, but it is obvious that the advances in immunogenetics and in histocompatibility have been of major impact in all of immunology. Indeed, in the course of a brief period of time, three basic cellular interactions, that of macrophages with T cells (Rosenthal and Shevach, 1973), T cells with B cells (Kindred and Shreffler, 1972; Katz *et al.*,

1973), and cytolytic T cells with their targets (Zinkernagel and Doherty, 1974), were found to be regulated by the major transplantation locus of the species. I consider the series of studies by Alan S. Rosenthal and Ethan M. Shevach published in 1973 the most important study on macrophage-lymphocyte interaction in the 1970s and perhaps one of the most seminal ones regarding the genetic control of immune responses. These studies indicated a role of macrophages in events controlled by the *I* region of the major histocompatibility gene complex, a feature never previously suspected, and signaled an *essential* interaction between macrophages and the thymus-derived T lymphocyte. The explosive series of studies in macrophage biology that followed represented, to a great degree, extensions of their basic findings. It may turn out that the common denominator for all the phenomena of cell-to-cell interaction is the process of antigen handling regulated by the transplantation gene locus.

Phagocytes are now viewed as cells capable of exerting a fine control on their environment, particularly on the lymphoid system. This control is exerted not only by carrying out antigen presentation, an essential function, but also by regulating growth and differentiation of lymphoid cells by way of a number of secretory products. Clearly, immune induction depends on a critical interrelationship between the phagocytes and the lymphocytes, the former being the nonspecific cell probably descendent of the primitive amebocytes, whereas the latter are cellular elements that give specificity to the immune response. Both cell types control and regulate each other, and the key in this regulation are the *I*-region products.

This chapter is organized into two major sections. The first reviews the various experimental systems in which macrophages have been involved, such as T-cell proliferation to antigen and lectins, T-B cell interactions, cellular immunity reactions. Some of these I have described more extensively than others. For example, T-cell proliferation and T-B cell interaction are analyzed in depth inasmuch as they were the basic systems for study of macrophage-T cell effects. In contrast, the involvement of macrophages in the development of the cytolytic T-cell response to viruses and tumors is treated briefly, this being a highly complex response involving a multiplicity of interactions and still very much under current study. The second part analyzes the biology of the phagocytes, the synthesis and expression of *I*-region-associated antigen, the issue of macrophage heterogeneity, and the function of antigen presentation and secretion of active products.

II. Analysis of the Regulatory Role of Macrophages

A. INTERACTIONS WITH T CELLS IN ANTIGEN-INDUCED PROLIFERATION

The proliferative response of T cells to antigen has been one of the systems most extensively employed for studying macrophage-lymphocyte interactions in culture. The first series of analyses in man (Hersch and Harris, 1968; Cline and Sweet, 1968) and in the guinea pig (Seeger and Oppenheim, 1970) on lymphocyte proliferation have already been reviewed (Unanue, 1972). Most recent experimental studies have used the guinea pig and mouse as the species of choice. Populations of lymphoid cells rich in T lymphocytes were obtained from lymph nodes, draining the depot of antigen, spleen, or the peritoneal cavity. The cells from the peritoneal cavity were an excellent source of T cells developing a very strong and notable antigen-specific response much higher than that shown by lymph node T cells (Rosenstreich *et al.*, 1971; Rosenstreich and Rosenthal, 1973). This may be because the immune T cell may migrate selectively into sites of inflammation as indicated by the studies of Koster and associates (1971) in antibacterial T-cell immunity carried out at the Trudeau Institute. Accordingly, in our own studies examining the proliferative response of T cells to *Listeria monocytogenes*, we found that an injection of proteose peptone intraperitoneally was *essential* in order to induce the exudate rich in strong T cells (Farr *et al.*, 1979a). Other studies in the mouse used thioglycolate broth (Schwartz *et al.*, 1978). Mineral oil was the choice inflammatory agent in experiments using the guinea pig (Rosenstreich and Rosenthal, 1973). It is worth recalling that the first successful transfer of contact sensitivity, the classical experiments of Landsteiner and Chase (1942), utilized lymphoid cells harvested from the peritoneal cavity.

Regardless of the source, the T cells have been isolated by separating out the phagocytes and B cells using combinations of brief culture on dishes to remove the bulk of adherent cells, followed by passage through glass beads or, preferably, nylon-wool columns as per the technique described by Julius *et al.* (1973). The technique of Julius requires a critical amount of nylon wool per input number of cells; if carried out correctly, it results in yields of about 25–35% of lymph node or splenic lymphocytes, the bulk of which bear T-cell markers. A good separation results in about 95% T cells, without phagocytes, and, at the most, 2–3% B cells.

Using stringent procedures to remove the macrophages and to en-

rich for T cells invariably resulted in T cells that did not respond to antigen in culture even with media that contained growth-promoting components, such as fetal calf serum and 2-mercaptoethanol (Section II,C). In the guinea pig, the studies of the proliferative response were the first to indicate clearly the essential role of phagocytes. Seeger and Oppenheim (1970) first found that passing lymph node cells through glass-bead columns resulted in the total loss of antigen-induced DNA synthesis. These analyses were then extended by Waldron *et al.* (1973), studying the response to purified protein derivative (PPD). The lack of response of macrophage-depleted lymph node cells could be reconstituted fully by adding peritoneal macrophages; in fact, the degree of reconstitution was proportional to the amounts of phagocytes added, i.e., from 1 to 30%, the last dose used. I accentuate this point because, in contrast to the studies using murine cells, guinea pig phagocytes showed little inhibitory effect even at high doses. Waldron *et al.* also used macrophages pulsed briefly with PPD. The response of the lymphocytes to PPD administered macrophage-bound was identical to that induced by PPD in soluble form, in the presence of optimal amount of phagocytes. These studies, therefore, confirmed the efficiency of antigen presentation by way of the macrophage but added the important new point that this step was essential in order for the lymphocyte to respond.

In the murine system, the requirement for phagocytes or other accessory cells was such that as little as 1% contamination with phagocytes still enabled a T-cell proliferative response to develop. Thus, depletion procedures had to be extremely efficient. A single passage of lymphoid cells through nylon wool, for example, or a single cycle of adherence, did not completely eliminate macrophages. Most investigators are now employing removal by one or two cycles of adherence, together with one or two passages through nylon wool. Under such procedures, the T-cell proliferation to antigen, as in the guinea pig, was strictly macrophage dependent (for example, Rosenwasser and Rosenthal, 1978b; Richman *et al.*, 1979; Farr *et al.*, 1979a; Kammer and Unanue, 1980).

Many of the analyses of antigen presentation using the mouse have employed accessory cells pulsed with antigen, which are then added to the lymphocytes, thus bypassing the need for extensive macrophage depletion of the lymphocyte populations. One important consideration in the mouse, however, is the amounts of macrophages added to the cultures. Too many macrophages invariably inhibited proliferation (Section III,D). This was particularly striking if the macrophages were activated. In most instances, the reasons for the suppressive effects of

macrophages were not investigated. Some experiments, however, attribute the inhibition to prostaglandins and other soluble molecules (Section III,C). Major representative results in experimental animals are shown in Table I.

There are few analyses of an antigen-driven proliferation in man, but these have clearly shown the requirements for monocyte-macrophages (Hersh and Harris, 1968; Cline and Sweet, 1968; Rodey *et al.*, 1979; Bergholtz and Thorsby, 1977, 1978, 1979; Breard *et al.*, 1979).

For effective T cell-macrophage collaboration, two essential features are required: (*a*) that the proliferating T cell and the macrophage be histocompatible, sharing part of the *I* region; (*b*) that the phagocyte bear the *I*-region-associated antigens (Ia) of the major histocompatibility complex (MHC) of the species.

The *I* region of the major histocompatibility gene complex was discovered by McDevitt and associates as they attempted to map the locus within the mouse *H-2* that controlled the immune response to the branched polypeptides poly(Tyr,Glu)-poly(DL-Ala)-poly(L-Lys) [(T,G)-A-L] and poly(His,Glu)-poly(DL-Ala)-poly(L-Lys) [(H,G)-A-L] (McDevitt *et al.*, 1972). They identified, using a number of congenic strains of mice, a gene segment between *H-2K* and *S* which was termed *I*. This observation followed their initial study relating the capacity to make an immune response to the *H-2* type of the responding strain (McDevitt and Chinitz, 1969). Subsequently, more *Ir* genes have been linked to this segment, which was subsequently subdivided into various subregions, i.e., *I-A*, -*B*, -*J*, -*E*, -*C* (reviewed by Shreffler and David, 1975; Klein, 1975; Klein and Hauptfeld, 1976; Benacerraf and Germain, 1978; Snell, 1978). After this study, five different laboratories within a brief period of time raised antibodies to the *I*-region products using appropriate *I*-region congenic strains of mice. These antibodies identified a new surface antigenic system, that of the "I-region-associated antigens" (Sachs and Cone, 1973; David *et al.*, 1973; Gotze *et al.*, 1973; Hauptfeld *et al.*, 1973; Hammerling *et al.*, 1974). The Ia antigens were identified on B cells and in a few activated T cells and also in macrophages (Section III,A). Ia antigens have now been identified corresponding to *I-A*, *I-J*, and *I-E/I-C* regions. The *I-A* and *I-E/C* products were characterized as two polypeptide chains of about 33,000 (α chain) and 26,000 (β chain) molecular weight (Cullen *et al.*, 1974, 1976; B. D. Schwartz *et al.*, 1976). (Whether *I-E* and *I-C* are two distinct regions is in dispute; hence the notation now in use: *I-E/C*.) *I-A* and *I-E/C* show extensive serological polymorphism with various allelic forms. The Ia antigens are the main surface proteins responsible for the mixed leukocyte reaction. The HLA-D region in

man codes for the same stimulatory protein and is thus regarded as the equivalent of the *I* region. The major differences between the two inbred lines of guinea pigs, the strains 2 and 13, lies in the *I* region, so that cross-immunization with cells, i.e., strain 2-anti-strain 13 lymphoid cells results in antibodies that are essentially anti-Ia reagents (Geczy *et al.*, 1975; B. D. Schwartz *et al.*, 1976).

1. Histocompatibility Requirements

The requirements for *I*-region histocompatibility between phagocytes and T cells in order for both cells to interact were found both for antigens under *Ir* control as well as for those with no obvious *Ir* gene effects. Antigens under *Ir* gene control are those antigens that induce high immune responses in some strains and low responses in others, the state of responsiveness being linked to the *I* region of the MHC strain. These antigens include synthetic polypeptides of limited antigenic heterogeneity, some weakly immunogenic alloantigens or conventional proteins administered at low doses (reviewed by Katz, 1977; Benacerraf and Germain, 1978). The first observations on the histocompatibility requirements for macrophage-T cell interactions were made by Rosenthal and Shevach (Rosenthal and Shevach, 1973; Shevach and Rosenthal, 1973). They obtained T cells—depleted of phagocytes—from the peritoneal cavity or lymph nodes of immunized inbred guinea pigs, either strain 2 or strain 13, and cultured them with macrophages of either strain pulsed briefly with the antigen under study (Table II). The optimal response to PPD or dinitrophenyl (DNP)-albumin, antigens to which there was no obvious *Ir* gene effect, was elicited only if the T cells from one strain were challenged with the antigen in the macrophages from the same homologous strain. While T cells from an F_1 cross [$(2 \times 13)F_1$] could respond perfectly well to PPD in either strain 2 or strain 13 macrophages, T cells from strain 2 responded only to PPD associated with macrophages from strain 2, or $(2 \times 13)F_1$, but not from strain 13. Rosenthal and Shevach went on to show that this lack of responsiveness to allogeneic macrophages could not be ascribed to differences in the kinetics of the response nor to a suppressor-type effect: excellent proliferation could be elicited from T cells mixed with syngeneic and allogeneic antigen-pulsed macrophages.

Further experiments indicated that antibodies to the MHC gene products could block the macrophage-lymphocyte interaction [such antibodies had been previously found to block antigen-driven T-cell proliferation (Shevach *et al.*, 1972)]. Macrophages from $(2 \times 13)F_1$ pulsed with PPD stimulated proliferation of T cells from strain 2, but

TABLE I
EXPERIMENTS SHOWING ANTIGEN PRESENTATION BY MACROPHAGES FOR T CELL PROLIFERATION^a

Antigens	Species	Investigators	Main results
Tetanus toxoid and PPD	Guinea pig	Seeger and Oppenheim, 1970	Poor response in macrophage-depleted cultures; reconstitution with PEC
PPD	Guinea pig	Waldron <i>et al.</i> , 1973	Complete absence of response without phagocytes; reconstitution with PEC
Horseradish peroxidase	Guinea pig	Rosenstreich and Rosenthal, 1973	1–2% of macrophages bind the antigen and serve as antigen-presenting cells
PPD, DNP-albumin	Guinea pig	Rosenthal and Shevach, 1973	Antigen presentation by macrophages requires histocompatible macrophages; anti-Ia antibodies inhibit
DNP-GL, GT, PPD	Guinea pig	Shevach and Rosenthal, 1973	Macrophages from "responder" strains are required to present antigen
GT, GL	Guinea pig	Shevach, 1976	I-region control of macrophage T cell interactions in outbred guinea pigs
[∞] TNP	Guinea pig	Thomas <i>et al.</i> , 1977; Thomas and Shevach, 1977	Macrophages are required. Primary sensitization can be elicited if allo-reactive T cells are eliminated; anti-Ia blocks the macrophage presentation
Ovalbumin	Guinea pig	Thomas and Shevach, 1976; Paul <i>et al.</i> , 1977	Two independent sets of F ₁ T cells responding to antigen in macrophages from either of the parental strains
DNP-ovalbumin, GAT, etc.	Mouse	Yano <i>et al.</i> , 1977, 1978; Schwartz <i>et al.</i> , 1978	I-region restrictions mapped to I-A in the interaction between spleen antigen-presenting cells and T cells; macrophages from responder strains are required
Insulin	Guinea pig	Barcinski and Rosenthal, 1977; Rosenthal <i>et al.</i> , 1977	Determinant selection by the macrophage
DNP-GL, PPD DNP-albumin	Guinea pig	Yamashita and Shevach, 1977	Ia-positive macrophages are required for antigen presentation
PPD, GAT, OVA	Mouse	Suzuki <i>et al.</i> , 1978	Macrophages present the antigen; non-Ir genes modulate the response

DNP-OVA, hemocyanin, PPD and GLT	Mouse	Rosenwasser and Rosenthal, 1978a,b	The response to multideterminant antigen requires macrophages; allogeneic macrophages can present; a macrophage factor can replace the macrophages
PPD, hemocyanin	Mouse	Cowing <i>et al.</i> , 1978b	Spleen accessory cells present antigen and bear I-A and I-E/C determinants
PPD, TNP	Guinea pig	Stingl <i>et al.</i> , 1978a,b	Langerhans cells of the skin present antigen
Fibrinopeptide	Guinea pig	Thomas <i>et al.</i> , 1979a,b	Determinant selection by the macrophage
Insulin, DNP, ovalbumin	Guinea pig	Yokomuro and Rosenthal, 1979	T cells from guinea pigs can be selected by <i>in vivo</i> immunization with DNP-ovalbumin transferred in one or the other parental macrophage; only "responder" macrophages present insulin
<i>Listeria monocytogenes</i>	Mouse	Farr <i>et al.</i> , 1979a; Beller and Unanue, 1979; Weinberg and Unanue, 1980	Presentation shows I-region restrictions at I-A and requires Ia-positive macrophages; thymic macrophages and alveolar macrophages present <i>Listeria</i>
CLPhe	Mouse	Schwartz <i>et al.</i> , 1979	Two complementing <i>Ir</i> genes must be expressed in the same macrophage
PPD	Mouse	Lee <i>et al.</i> , 1979	Ia-bearing macrophages present antigen; differences found among Ia-positive cells
Myoglobin	Mouse	Richman <i>et al.</i> , 1980	T cells proliferate to antigen presented in Kupffer cells from responder strains
TNP-OVA, PPD Hemocyanin	Guinea pig Mouse	Rogoff and Lipsky, 1980 Kammer and Unanue, 1980	Kupffer cells present antigen The response to hemocyanin requires syngeneic macrophages and is not replaced by a macrophage factor

^a PEC, peritoneal exudate cells; PPD, purified protein derivative; DNP, TNP, di-, trinitrophenyl; GT, GL, GAT, copolymers, respectively of L-glutamic acid and L-tyrosine; L-glutamic acid and L-lysine; and L-glutamic acid, L-alanine, and L-tyrosine.

TABLE II
THE REQUIREMENT FOR HISTOCOMPATIBLE MACROPHAGES IN ANTIGEN-MEDIATED
DNA SYNTHESIS IN IMMUNE GUINEA PIG LYMPH NODE LYMPHOCYTES^a

Macrophage		Lymphocyte DNA synthesis: [³ H]TdR incorporation (cpm × 10 ⁻³)		
		Strain 2	Strain 13	(2 × 13)F ₁
Strain	Antigen pulse			
2	0	0.92 ± 0.28	5.68 ± 1.08	1.60 ± 0.30
2	+	26.38 ± 8.27	8.61 ± 2.08	6.98 ± 0.80
13	0	4.63 ± 1.86	1.66 ± 0.37	1.78 ± 0.47
13	+	3.12 ± 0.67	19.89 ± 4.47	7.81 ± 1.75
(2 × 13)F ₁	0	1.91 ± 0.99	4.27 ± 0.34	1.66 ± 0.53
(2 × 13)F ₁	+	12.42 ± 3.19	11.81 ± 1.98	12.57 ± 2.33

^a Macrophages from strain 2, strain 13, or (2 × 13)F₁ guinea pigs were incubated with mitomycin C and/or purified protein derivative (PPD) for 60 minutes at 37°C, washed, and mixed with column-purified lymph node cells, as indicated. [³H]Thymidine incorporation was expressed as mean cpm × 10⁻³. [From the study of Rosenthal and Shevach (1973).]

this proliferation was totally ablated by adding anti-2 antibodies to the culture. Shevach and Rosenthal concluded that "the activation of immune lymphocytes by antigen-pulsed macrophages is dependent on the interaction of cell surface structures that are the products of the major histocompatibility complex" (1973).

Noteworthy were the studies of antigens under *Ir* gene control. Shevach and Rosenthal selected for their analysis the copolymers of L-glutamic acid and L-lysine (GL) and of L-glutamic acid and L-tyrosine (GT) previously analyzed by Benacerraf and associates (Bluestein *et al.*, 1971). The T lymphocytes from the (2 × 13)F₁ guinea pigs appropriately immunized with GT or GL were cultured with macrophages from either strain pulsed with one or the other antigen. The F₁ T cells responded only to GT pulsed on strain 13 macrophages and to DNP-GL on strain 2 macrophages, i.e., on the macrophages from the strain that responded immunologically when immunized *in vivo* (Fig. 1). Thus, although the F₁ guinea pig contained T cells capable of responding to GT or GL, these T cells did so only when presented with the antigens in macrophages bearing the appropriate *I*-region haplotype of the responder strain. A final and important experiment in this study concerned the effects of antibodies to the MHC products of strain 2 or strain 13—now known to be anti-Ia antibodies. These antibodies blocked specifically the response of the F₁ T cells to the F₁ macrophages pulsed with the antigen under *Ir*-gene control. Thus,

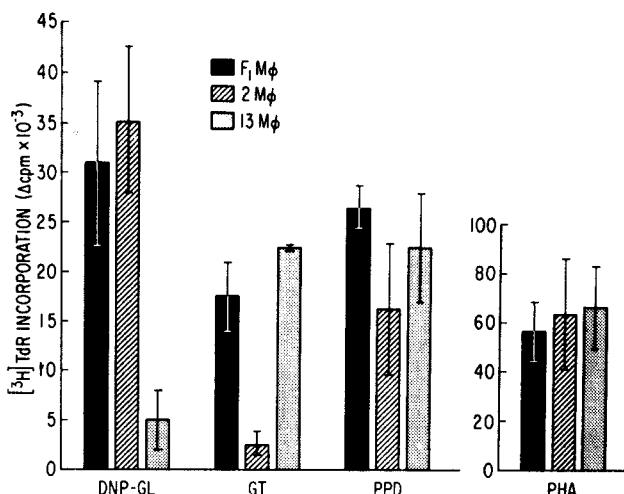


FIG. 1. The graph shows the stimulation of guinea pig (2×13) F_1 T cells by macrophages from each parent or from the F_1 macrophages pulsed with the antigens as indicated. Strain 2 guinea pigs respond to DNP-GL, but not to GT; strain 13 responds to GT but not to DNP-GL. Both respond to purified protein derivative (PPD). The response to phytohemagglutinin (PHA) is also shown. DNP-GL, dinitrophenyl copolymer of L-glutamic acid and L-lysine; GT, copolymer of glutamic acid and L-tyrosine. [From Shevach and Rosenthal (1973).]

anti-2 antibodies blocked the response to DNP-GL (strain 2 is a responder to GL) but not to GT (strain 13 responds to GT). The reverse is also true. The conclusion was that the anti-Ia antibodies inhibited the specific interaction between the T cell and the macrophage with its Ia product.

These series of studies, therefore, indicated that the response of T cells required the phagocyte as an *obligatory* cell and that the interaction taking place between both cells involved a product from the MHC. The antigen-presenting function of macrophages, therefore, acquired a new dimension. It clearly did not represent a simple antigen → T cell-receptor interaction but involved antigen and a specific protein, the Ia product, interacting with the T cells.

Subsequent studies confirmed the important relationship between antigen and the I-region products of macrophages in T cell-macrophage stimulation. A series of experiments have indicated that selective T cells proliferate upon interaction with antigen bound to the appropriate Ia-bearing macrophage. This was best shown using F_1 animals. Thomas and Shevach (1976) developed an assay system in which guinea pig T cells from a (2×13) F_1 guinea pig were cultured

for several days with macrophages of either parental strain pulsed with ovalbumin. At the end of the culture, the T cells were isolated and tested for the capacity to proliferate with the antigen-pulsed macrophages of each strain. The results indicated that the F_1 T cells behaved like two distinct populations, i.e., T cells from the F_1 cultured on macrophages from strain 2 proliferated only to challenge with macrophages of strain 2 but not strain 13, whereas those T cells cultured on strain 13 macrophages proliferated only when challenged with macrophages of strain 13. Paul and associates (1977) explored this issue and presented strong experiments indicating that the T cells from a $(2 \times 13)F_1$ hybrid guinea pig immune to ovalbumin were indeed composed of two independent sets. This was analyzed in two different experimental situations. The first approach was a positive selection procedure first developed by Ben-Sasson *et al.* (1975a,b): ovalbumin-immune $(2 \times 13)F_1$ T cells were placed in culture with ovalbumin-pulsed strain 2 or 13 macrophages; shortly thereafter, the lymphocytes that did not adhere to the macrophages were removed; at the end of 1 week, the T cells were isolated and tested for proliferation to macrophages pulsed with ovalbumin of either strain 2 or 13. In confirmation of the study of Thomas and Shevach, those F_1 T cells cultured initially on macrophages from strain 2 responded only to ovalbumin bound to macrophages of strain 2; the situation was also true for T cells grown initially with macrophages from strain 13. Their second approach consisted of a negative selection procedure: the T cells were cultured briefly with the antigen-pulsed macrophages from either parent and then the proliferating cells labeled by exposure to bromodeoxyuridine (and light); the remaining cells were then tested on fresh, new antigen-pulsed macrophages. The thymidine analog bromodeoxyuridine becomes incorporated into the DNA of the cell and cross-links the DNA strands when activated by light; this results in a block in proliferation. The F_1 T cells first cultured with ovalbumin in strain 2 macrophages only responded to OVA in strain 13 macrophages, i.e., the "clone" of T cells responding to ovalbumin in strain 2 macrophages had been selectively depleted. This experiment suggested that each "clone" was not represented by two cooperating sets of T cells, one recognizing Ia, the other ovalbumin; had this been the case, one or the other would have been eliminated and would not have been able to function in the assay, i.e., in the above example, the putative ovalbumin clone would have been eliminated; therefore, no proliferation would have taken place to ovalbumin bound to strain 13 macrophages.

The two clones of reactive T cells could also be identified *in vivo*

using the macrophage transfer system (Yokomuro and Rosenthal, 1979). F₁ guinea pigs were injected with DNP-ovalbumin on one parental macrophage; days later their T cells were harvested and tested for their proliferation to macrophage-bound ovalbumin; priming *in vivo* with one parental macrophage resulted in T cells that only proliferated with the same macrophages when tested in culture. Similar results to these were found in the mouse in different experimental situations, to be analyzed in the sections to follow.

The most logical interpretation of all these results with F₁ T cells is to postulate that distinct clones of T cells are selected to expand and proliferate upon interaction with antigen and a particular *I*-region product. In the normal immunization, that is, in a syngeneic situation, the clones reactive to the antigen in an allogeneic Ia, even if present, do not expand and, therefore, are not detected. The F₁ experiments also tell us that the *I*-region restrictions cannot be explained by a simple sharing of the MHC surface products of the T cell with that of the macrophage (i.e., the F₁ T cell clone selected on strain 2, for example, would still share MHC determinants with strain 13; the surface expression of MHC products does not show allelic exclusion). Finally, one should recall that, in the studies of cytolytic T cells restricted to their target by H-2K or H-2D products, similar results were found using F₁ T cells.

Analysis of the T-cell proliferative response in the mouse has yielded essentially identical results as in the guinea pig, but it also permitted a more elaborate genetic mapping because of the more extensive information on the murine H-2 and the availability of great number of inbred strains. Schwartz, Paul, and associates examined the proliferative response of T cells to several different antigens and found a perfect correlation between the antibody responses to the antigens *in vivo* and the extent of T-cell proliferation (Schwartz and Paul, 1976; Schwartz *et al.*, 1978). The way in which they studied the genetics of the antigen-presenting accessory cell was to pulse briefly mitomycin C-treated spleen cells with antigens and then to add the live cells to the peritoneal exudate T cells. (Their accessory cell was characterized by its adherence to glass, radioresistance, and lack of B- or T-cell markers.) Using dinitrophenylated ovalbumin or the copolymer of glutamic acid, alanine, and tyrosine (GAT), they confirmed the requirement for histocompatible phagocytes in antigen presentation and mapped this requirement to the *I*-A subregion of the mouse H-2 (Yano *et al.*, 1977, 1978). Table III reproduces results of their experiments using inbred strains of mice with recombinants at H-2. T cells from the B10 or B10.A proliferated upon presentation of the antigens in cells

TABLE III
CONTROL OF ANTIGEN PRESENTATION BY *I*-REGION GENES^{a,b}

Spleen cells		DNP-OVA primed		GAT primed	
Strain	MHC	B10.A	B10	B10.A	B10
B10.A	<i>kkkkkddd</i>	100 (all)	15 (none)	100 (all)	19 (none)
B10	<i>bbbbbbbbb</i>	9 (none)	100 (all)	0 (none)	100 (all)
B10.A (5R)	<i>bbbkdkdd</i>	14 (<i>J, E, C, S, D</i>)	62 (<i>K, A, B</i>)	0 (<i>J, E, C, S, D</i>)	82 (<i>K, A, B</i>)
B10.A (4R)	<i>kkbcccccc</i>	126 (<i>K, A</i>)	16 (<i>B, J, E, C, S, D</i>)	77 (<i>K, A</i>)	8 (<i>B, J, E, C, S, D</i>)
A.TL	<i>skkkkkd</i>	94 (<i>A, B, J, E, D</i>)	14 (none)	47 (<i>A, B, J, E, D</i>)	0 (none)
		A.TL	A.TH	A.TL	B10.A (5R)
A.TL	<i>skkkkkkd</i>	100 (all)	6 (<i>K, D</i>)	100 (all)	7 (<i>J, E, D</i>)
A.TH	<i>sssssssd</i>	0 (<i>K, D</i>)	100 (all)	ND	ND
B10.A (4R)	<i>kbbcccccc</i>	130 (<i>A</i>)	11 (none)	85 (<i>A</i>)	10 (<i>B</i>)
B10.A (5R)	<i>bbbkdkdd</i>	ND	ND	12 (<i>J, E, D</i>)	100 (all)
SJL	<i>ssssssss</i>	23 (<i>K</i>)	106 (<i>K, A, B, J, E, C, S</i>)	ND	ND

^a This table is a summary of the series of experiments of Schwartz, Paul, and associates as reported by them (Schwartz *et al.*, 1978). DNP-OVA- GAT-primed T cells from B10.A or B10 mice were stimulated *in vitro* with the antigens bound to spleen cells from various strains of mice. The proliferative responses were measured by thymidine incorporation, and the data are expressed as percentages of the syngeneic response, i.e., the difference between antigen-pulsed cultures for the allogeneic spleen cells, divided by the difference between antigen-pulsed and nonpulsed cultures for the syngeneic spleen cells, times one hundred ($\Delta \text{cpm allo}/\Delta \text{cpm syn} \times 100$).

^b MHC, major histocompatibility complex. Small letters denote the haplotype source of origin of the alleles at the *K*, *I-A*, *I-B*, *I-J*, *I-E*, *I-C*, *S*, and *D* regions. Capital letters in parentheses following the percentage of the syngeneic response refer to the regions of the MHC that are shared between the responding T cells and the stimulating spleen cells. ND, not determined.

from B10 or B10.A, respectively, not vice versa. Because the T cells from B10.A could not be stimulated by antigen presented in cells from B10.A (5R) but would respond to antigen in cells from B10.A (4R) or A.TL, it was possible to map the *H-2* restrictions to *I-A*. B10.A had identity with B10.A (5R) from *I-J* to *-D*, with B10.A (4R) at the *K* and *I-A*, and with A.TL at *I-A* to *-D*. Other combinations described in Table III confirmed this genetic mapping.

In the murine system, the important issue was raised about the absolute need for macrophages in the response to complex, multideterminant antigens not under obvious *Ir* gene control. We analyzed before that macrophage presentation of complex antigen in the guinea pig showed MHC restriction mapped at the *I* region, implying, therefore, an involvement of this genetic segment in this function. When reevaluating the response of murine lymph node T cells to a number of antigens such as DNP-ovalbumin, hemocyanin, or GLT, Rosenwasser and Rosenthal (1978a,b) found that the responses were completely abolished by depletion of phagocytes and could be reconstituted with peritoneal macrophages, in agreement with past studies. Yet two striking differences were found between the multideterminant antigens DNP-ovalbumin and hemocyanin, and the synthetic polypeptide GLT. The responses to the former two could be reconstituted with allogeneic macrophages or a macrophage culture fluid, whereas the response to GLT strictly required histocompatible macrophages. [The lack of macrophage MHC identity for the response to DNP-hemocyanin was also found in the analysis of the antibody formation *in vitro* (Katz and Unanue, 1973), to be considered in Section II,C.] This is an important point because it raises the issue of whether interactions with macrophages bearing the appropriate Ia products are essential or whether T cells immunized in a syngeneic environment can recognize allogeneic *I*-region products. It is most likely that the response to complex, multideterminant antigens is strictly MHC restricted and that the explanation for the results of Rosenthal and Rosenwasser lies with the number of contaminating phagocytes in their preparation. Their preparation of lymph node lymphocytes still contained a small, yet perhaps significant, number of phagocytes (approximately 0.1–0.5%), sufficient to give a response to PHA. It is now known that complete depletion of macrophages from the T-cell preparations impairs lectin-induced proliferation (Section II,B). We have evaluated this issue ourselves, examining the proliferative response to hemocyanin but employing a more elaborate procedure for depleting macrophages. Under conditions of complete depletion of phagocytes, the proliferative response of lymph node T cells to hemocyanin was found to be dependent strictly on live

phagocytes and to require *H-2* homology between the phagocytes and the T cells; the restrictions mapped at the left-hand side of the *H-2* (*K* to *I-B*); furthermore, the macrophage requirement could not be replaced by a macrophage-conditioned medium (Kammer and Unanue, 1980). The conclusion, therefore, is that, in the mouse as in the guinea pig, the requirement for MHC restrictions at the *I* region for macrophage-T cell interaction is absolute. Of importance is that a very small number of antigen-presenting macrophages, by themselves insufficient to promote antigen-dependent T-cell growth, can function in collaboration with macrophage-derived products. This point requires further elaboration but is inescapable from the analysis of the data described above.

The involvement of *I* region in the macrophage presentation of antigens to T cells has been explored in depth with protein antigens of well-defined structure. These results are considered in Section III,B. Noteworthy have been the studies on insulin (Barcinski and Rosenthal, 1977; Rosenthal *et al.*, 1977; Rosenwasser and Rosenthal, 1979a,b), on human fibrinopeptide B (Thomas *et al.*, 1979a,b), and on myoglobin (Richman *et al.*, 1979).

The few studies carried out in man have indicated the requirements for histocompatible macrophages matched at the HLA-D region (Rodey *et al.*, 1979; Bergholtz and Thorsby, 1978).

2. *Ia*-Bearing Phagocytes

Only a certain percentage of phagocytes bear Ia molecules on their membrane (Section III,A). These Ia-bearing cells are responsible for most macrophage-lymphocyte interactions, including the proliferation of T cells reviewed in this section. Killing the Ia-bearing set of accessory cells with anti-Ia and serum as a complement source results in an impairment of antigen-stimulated T-cell proliferation. The studies have been made on the peritoneal macrophages of the guinea pig (Yamashita and Shevach, 1977) and mouse (Farr *et al.*, 1979a; Kammer and Unanue, 1980) and on the accessory cells of the spleen (Cowing *et al.*, 1978a; Schwartz *et al.*, 1978). A recent report of Lee *et al.* (1979), claimed that Ia-negative macrophages (i.e., the population remaining after killing with anti-Ia) could interact with T cells, but only in conditions where soluble antigen was added to the culture during the entire time. A soluble mediator substituted for the macrophages. My interpretation of this result is that Lee's T-cell population contain residual macrophages—as evidenced by the high response to soluble antigen in the *absence* of macrophages—and that the experiments indicate a cooperation between the T cells and the few residual mac-

rophages with other macrophages or their products. This is a situation akin to that discussed before in the study of Rosenwasser and Rosenthal.

Limited studies have been made on antigen-induced T-cell proliferation in man as concerns the requirement of Ia-bearing macrophages. The results thus far reported indicate that removal of macrophages by killing them with anti-HLA-D antibodies or with heterologous antibodies to the *I*-region product of man abrogated antigen-induced T-cell proliferation (Rodey *et al.*, 1979; Bergholtz and Thorsby, 1978; Breard *et al.*, 1979; Raff *et al.*, 1980; Geha *et al.*, 1979).

In summary, the studies of antigen-induced T-cell proliferation have been extensive and have clearly indicated the key functional role of the macrophage in antigen presentation. In my opinion, these studies have established that (*a*) T cells will not grow in culture upon antigen stimulation in the absence of phagocytes; (*b*) phagocytes are required as an essential antigen-presenting cell; (*c*) the *I* region regulates the interaction between the T cell and the macrophage; (*d*) the phagocytes interacting with the T cell must express Ia antigens on their membranes; (*e*) the *I*-region involvement in macrophage interaction applies to all antigens, even those under no obvious Ir gene control; and (*f*) the interaction between the T cell and the appropriate macrophage selects and maintains the life of the antigen-Ia-reactive T-cell clone.

B. INTERACTIONS WITH T CELLS IN LECTIN-INDUCED PROLIFERATION

The response of T lymphocytes to the lectins phytohemagglutinin (PHA) and concanavalin A (Con A) requires the presence of adherent phagocytic cells. Some of the early reports examining lymphocyte populations depleted of phagocytes failed to find an impairment of the proliferative response to lectins (for example, Hersh and Harris, 1968; Waldron *et al.*, 1973). Rosenstreich and associates (1976), however, made a detailed study of the response of lymph node lymphocytes to PHA and found it to be macrophage dependent. The response was not affected by depleting macrophages by a single passage of lymphoid cells through columns containing nylon wool and glass beads, although the same cells failed to respond to the antigen DNP-ovalbumin to which the guinea pigs were immunized. A second passage of cells through a nylon-wool column resulted in a complete lack of responsiveness to PHA. Addition of purified peritoneal macrophages fully reconstituted the response. Lipsky, Ellner, and Rosenthal confirmed their results (Lipsky *et al.*, 1976; Ellner *et al.*, 1976). In their hands, passage of the lymph node cells twice through nylon wool

resulted in practically a complete depletion of phagocytes (less than one per one thousand). Lipsky *et al.* (1976), made the point that the culture conditions were critical to show the accessory cell dependence. Those situations that favored cell clustering, like high density and round-bottom culture wells, required more stringent macrophage depletion in contrast to having cells at a lower cell density in flat-bottom well dishes. A similar point had been made previously by others analyzing antibody formation *in vitro* (for example, Theis and Thorbecke, 1970). Similar accessory cell dependency of the lectin proliferative response of T cells was later found with murine cells (Habu and Raff, 1977; Ahman *et al.*, 1978b; Rosenwasser and Rosenthal, 1978a; Andersson *et al.*, 1979; Kammer and Unanue, 1980; Larsson *et al.*, 1980a; Gronvik and Andersson, 1980) and human cells (Oppenheim *et al.*, 1968; Levis and Robbins, 1970; Lohrman *et al.*, 1974; Heddocks *et al.*, 1975; Schmidtke and Hatfield, 1976; Taniguchi *et al.*, 1977; Arala Chaves *et al.*, 1978; de Vries *et al.* 1979).

In contrast to the antigen-dependent proliferation, the lectin-proliferative response was reconstituted with syngeneic or allogeneic macrophages (Lipsky *et al.*, 1976; Habu and Raff, 1977; Rosenstreich and Mizel, 1978; Kammer and Unanue, 1980) or even xenogeneic macrophages (Schmidtke and Hatfield, 1976). Interestingly, the reconstitution with phagocytes and other accessory cells was best with the population of macrophages bearing Ia. Thus, Habu and Raff (1977) found that killing the peritoneal macrophage population by exposure to anti-Ia plus fresh serum—as a complement source—markedly impaired their capacity to cooperate with the T cell stimulated by Con A. In our own studies, macrophage populations depleted of the Ia-bearing population did reconstitute the response, although less efficiently than the entire untreated cells (Kammer and Unanue, 1980). Addition of fibroblasts to the culture reconstituted the response of guinea pig T cells but only partially (Lipsky *et al.*, 1976). Habu and Raff (1977) fully reconstituted the response with mouse embryo fibroblasts but not with 3T3 cells. This finding contrasts with the results showing requirements for Ia-positive macrophages—whether the embryo cells bore Ia or contained macrophages was not studied.

The issue of the requirement for an Ia-bearing cell was analyzed in a different way by Ahman *et al.* (1978b). They confirmed the results that the response to Con A was affected by killing the entire unfractionated cells with anti-Ia (and complement) (Niederhuber *et al.*, 1976; Frelinger, 1977; Ahman *et al.*, 1978a; also Larsson *et al.*, 1980a). Such treated cells, however, could proliferate when a preparation of Ia-bearing spleen accessory cells was added to the culture. The accessory

cells were adherent to glass, lacked T-cell determinants, and contained two *I*-region determinants, one mapped to *I-A*, the other mapped from *I-B* to *I-E/C*. The accessory cell lacking Ia reconstituted the response but not as effectively, although, in their studies, a complete titration of each cell was not shown. Interestingly, the response to PHA was not found to be sensitive to treatment with anti-Ia and complement, implying that the Ia-bearing phagocytes were not an essential reconstituting cell. Direct examination of this point with regard to the PHA response has not been made.

A most interesting result with regard to the role of the macrophage in the lectin response was made by the studies of Rosenstreich *et al.* First, they found that 2-mercaptoethanol, an agent believed to replace macrophages in culture (Section II,C) would not replace the need for macrophages, a point confirmed by others (e.g., Ellner *et al.*, 1976; Rosenwasser and Rosenthal, 1978a; de Vries *et al.*, 1979; Kammer and Unanue, 1980). However, mercaptoethanol enhanced the activity of very small numbers of macrophages added to the culture (Rosenstreich and Mizel, 1978): macrophage-depleted T cells responded marginally to PHA if reconstituted with 1% macrophages, yet 1% macrophages in the presence of mercaptoethanol resulted in a very strong DNA synthesis by the T cells (about 35,000 cpm vs. 3000, respectively) (Fig. 2). Noting the lack of effect by mercaptoethanol, Rosenstreich *et al.*, carried out experiments in which the separated T cells or macrophages were pulsed with PHA briefly and then cocultured. Under both circumstances, a strong proliferative response was obtained. The macrophages had to be live and metabolically active. Thus, macrophages could "present" PHA to the lymphocytes; and, alternatively, the T cells, after binding PHA, could interact effectively with the macrophages (Rosenstreich *et al.*, 1976; Rosenstreich and Mizel, 1978).

Further analysis by Rosenstreich and associates indicated that a soluble mediator was involved in the interaction between macrophages and lymphocytes and PHA. Separating macrophages and the PHA-treated lymphocytes by a cell-impermeable membrane resulted in lymphocyte proliferation to about 60% of that found when both cells were mixed. Furthermore, a soluble molecule, named TAF or T cell-activating molecule, released from macrophage cultures replaced the physical need for macrophages. The reconstitution of the lectin-induced response by a macrophage-conditioned medium has now been found by others (for example, Rosenwasser and Rosenthal, 1978a; de Vries *et al.*, 1979; Larsson *et al.*, Kammer and Unanue, 1980; Smith, 1980). The response of T cells to Con A is believed to involve growth factors released by T cells themselves upon interaction with mac-

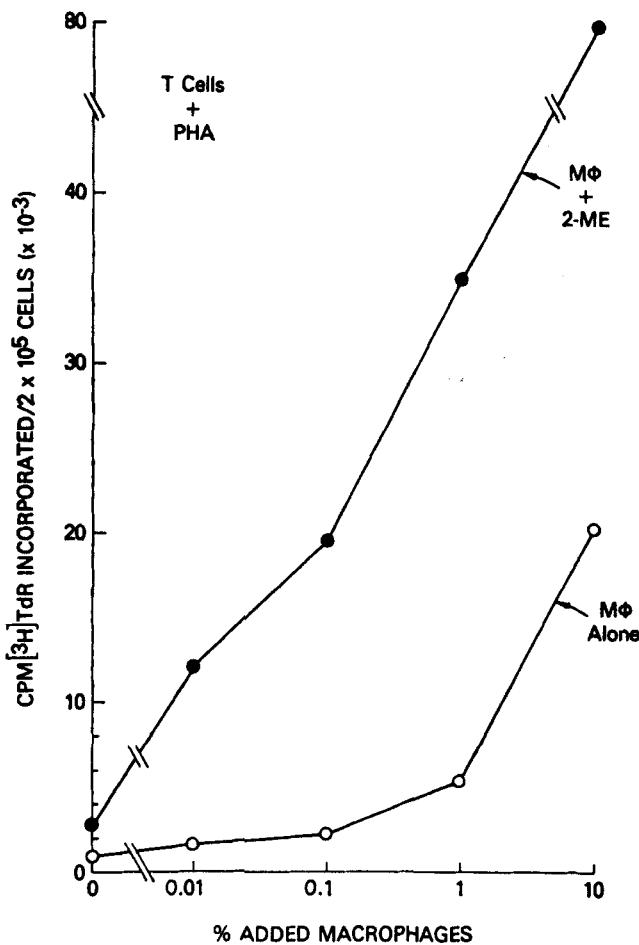


FIG. 2. The figure shows the effects of mercaptoethanol in potentiation of accessory function of macrophages. T cells were incubated with macrophages ($M\phi$) and phytohemagglutinin (PHA) in the presence or the absence of 2-mercaptoethanol. [From Rosenstreich and Mizel (1978).]

rophages. The relationship between the T-cell growth factor and the macrophage products is discussed at more length in Section III,C.

Another polyclonal T-cell response that is strictly macrophage dependent is that induced by generation of aldehyde from cell-surface glycoproteins. Gentle treatment of cells with sodium metaperiodate or sequential treatment with neuraminidase and galactose oxidase induced vigorous T cell proliferation (Novogrodsky and Katchalski, 1972). The T-cell proliferation required macrophages. Actually, mac-

rophages or T cells could be treated with the chemicals and then mixed with the other untreated cells and still generate a proliferative response (Greineder and Rosenthal, 1975b). The response was not MHC restricted inasmuch as it was generated by allogeneic macrophages, but it clearly involved the surface Ia; anti-Ia antibodies directed to the allogeneic macrophages blocked the response (Greineder *et al.*, 1976). Clusters of macrophages and T cells were abundant in the stimulated cultures.

In essence, the polyclonal responses to lectins or to aldehyde generation were found to require macrophages either syngeneic or allogeneic with the T cells. An involvement of the *I* region was not found for the PHA response but was evident for the Con A response. Ia-positive macrophages were better in reconstituting the Con A response of the T cells, yet Ia-negative cells also had an effect. The involvement of the *I* region was well shown in the response to aldehydes by the inhibitory effect of antibodies. The involvement of macrophages in the lectin response involved the release of lymphostimulatory molecules (Section III,C). The nature of the involvement of the *I* region products in the T cell-macrophage interactions induced by lectins or aldehydes is not understood. The results suggest that macrophage-T cell contact induced by nonantigenic stimuli may result in interactions with the Ia antigens that generate the "signals" required for macrophage and T-cell stimulation.

C. INTERACTIONS WITH T AND B CELLS IN ANTIBODY FORMATION

The requirements for phagocytes in the immune response to thymus-dependent antigens have been examined extensively in a number of experimental systems. These systems vary from *in vivo* cell transfer using live macrophages with bound antigen, to cell culture assays, to approaches combining both *in vivo* and cell culture techniques. All the results indicate that macrophage-associated antigen is highly effective in promoting T-B cell collaboration; moreover, in many instances it appears essential for antigen to be bound to phagocytes for T-B cell interactions to develop. The issue of an essential role for phagocytes, however, cannot be generalized to all experimental systems.

The first series of experiments using live macrophages in a transfer system established that macrophage-bound antigen was highly efficient in inducing an antibody response (Unanue, 1972). All the antigens tested were protein antigens now known to be thymus-dependent. Thymectomized mice did not respond to transfer of an antigen like hemocyanin bound to live macrophages, indicating that

macrophage-bound antigen did not bypass the need for T cells and that both T and B cells interacted with the cell-bound immunogen (Unanue, 1970).

1. Hapten-Protein Conjugates

The interactions between T and B cells with macrophage-bound antigens were further analyzed using hapten-protein conjugates. One approach, taken by Kunin *et al.* (1972), was to immunize mice with live thioglycolate-induced macrophages previously pulsed with rabbit albumin and then to assay the spleen cells in cell cultures for the response to DNP-rabbit albumin. The presence of T cells immune to the carrier resulted in an enhanced anti-DNP response. Their results indicated an excellent generation of carrier-primed T cells by immunization with the macrophage-bound antigen. The experimental system was then manipulated using X-irradiated mice that received mixtures of thymus, bone marrow cells, and antigen-bearing macrophages. Excellent carrier-primed cells were found in spleens of X-irradiated mice injected simultaneously with thymocytes and macrophages, followed 8 days later with bone marrow cells; the reverse, however, giving bone marrow cells with macrophages first, followed 8 days later with the thymocytes, did not result in induction of carrier-specific T cells. Kunin *et al.* concluded that carrier priming developed only following T cell-macrophage interaction. A more detailed quantitative approach was followed by Klaus (1974) using the classical thymus-bone marrow Claman-type transfer system. Helper T cells were generated by transferring cortisone-resistant thymocytes into X-irradiated mice, together with soluble hemocyanin or hemocyanin-bearing live macrophages; 5 days later, the helper T-cell activity in the spleen was assayed by cotransferring them together with bone marrow cells and then measuring the anti-DNP response. The macrophage-associated hemocyanin was several thousandfold more immunogenic than soluble hemocyanin in inducing carrier-primed cells *in vivo*, in accordance with earlier studies of the antibody response to the whole molecule (Unanue and Askonas, 1968a). Askonas and Roelants (1974) extended this type of observation, noting that the number of different anti-DNP B cell clones generated *in vivo* was much larger upon immunization with a DNP-protein bound to live macrophages.

David Katz and I (1973) analyzed the response to DNP proteins using the Mishell-Dutton systems, employing spleen cells from immunized mice. We found that DNP-hemocyanin bound to macrophages was about 10,000-fold more immunogenic than a similar amount of soluble or dish-bound protein. Whether DNP-hemocyanin

was bound to macrophages directly or as part of an immune complex made no difference. One interesting result was found when adding soluble antigen together with macrophage-bound antigen to the T-B cell mixtures. In this situation, the response to macrophage-bound antigen was significantly reduced. A similar result had been previously reported in *in vivo* experiments (Spitznagel and Allison, 1971). The results imply that there are two forms of antigen that compete with each other, the macrophage-bound and the soluble antigen. Although not explored, it is possible that the soluble antigen acted by triggering suppressor T cells (Ishizaka and Adachi, 1976; Pierres and Germain, 1978).

Using hapten-conjugated proteins associated with macrophages, it was questioned whether both the haptic and carrier protein had to be linked together in the same molecule or could be placed separately either in the same macrophages or in different ones. To do this, Askanas and Roelants (1974) transferred, *in vivo*, DNP-bovine γ -globulin-primed spleen cells with mixtures of macrophages, one bearing bovine γ -globulin and the second DNP-hemocyanin, and then assayed for the anti-DNP response. Although the control group challenged with macrophages bearing DNP-bovine γ -globulin gave excellent responses, the mixtures of macrophages was very weak. We analyzed this point ourselves by placing the hapten bound to the unrelated carrier protein (in our case, DNP- γ -globulin) and the immunizing protein (hemocyanin), both on the same macrophage (Unanue and Katz, 1973). Using radioactive proteins, we were able to estimate that the amounts of both proteins bound to the macrophage were similar to the amount of DNP-hemocyanin bound to macrophages. The results indicated that lymphocytes would respond weakly to the unlinked hapten and carrier determinants on the same macrophage, but strongly if the determinants are bound in the same molecule (Fig. 3).

2. Depletion of Accessory Cells

A different approach from testing the immunogenicity of macrophage-bound antigen is to examine cultures for antibody formation in the absence or the presence of accessory cell, i.e., to ask how essential the accessory cells are for the B-T cell interactions to take place. An early approach was to deplete accessory cells, usually from spleen, on the basis of their adhesion to culture dishes. Mosier's first report (1967, 1969) showing that depletion of adherent cells blunted the antibody response to SRBC, was rapidly confirmed (Roseman, 1969; Pierce and Benacerraf, 1969; Pierce, 1969; Haskill *et al.*, 1970; Hartmann *et al.*, 1970; Shortman *et al.*, 1970; Feldmann and Palmer,

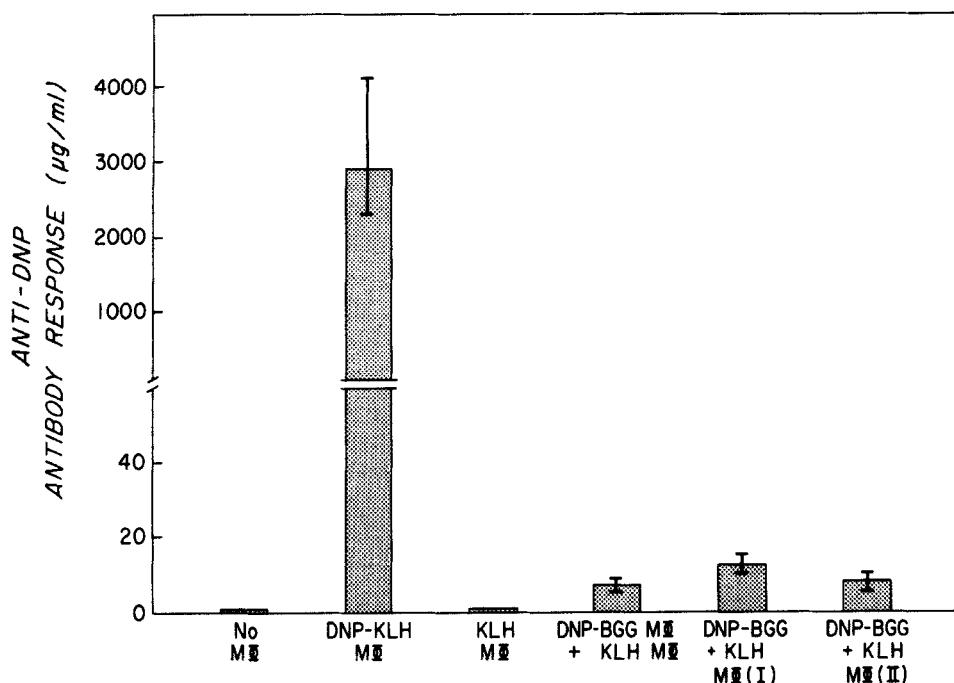


FIG. 3. This experiment shows the immunogenicity of hapten-proteins bound to macrophages. BALB/c mice received 25×10^6 DNP-hemocyanin-primed spleen cells with or without 12×10^6 macrophages. Mice were bled for anti-DNP antibody a week later. DNP-KLH MΦ: macrophages containing $3.1 \mu\text{g}$ of DNP-hemocyanin; KLH MΦ: macrophages containing $12.1 \mu\text{g}$ of hemocyanin; DNP-BGG MΦ + KLH MΦ: received simultaneously one set of macrophages containing $3.7 \mu\text{g}$ of DNP-bovine γ -globulin and another set containing $13.2 \mu\text{g}$ of hemocyanin; DNP-BGG + KLH MΦ(I): both antigens were bound to the macrophages ($2.7 \mu\text{g}$ and $9.7 \mu\text{g}$, respectively); DNP-BGG + KLH MΦ(II): as I but with $0.2 \mu\text{g}$ of bovine γ -globulin and $16.4 \mu\text{g}$ of hemocyanin. The results indicate that the optimal immune response to macrophage-bound antigen was induced by the haptic determinant linked to the carrier molecules. [From Unanue and Katz (1973).]

1971; Shortman and Palmer, 1971; Cosenza and Leserman, 1971; Feldmann, 1972; Sjoberg *et al.*, 1972; Chen and Hirsh, 1972a,b; Moller *et al.*, 1976; Lee *et al.*, 1976; Niederhuber, 1978, 1980; Niederhuber and Allen, 1979; Wong and Hercowitz, 1979).

The response of SRBC has been the most frequently analyzed, probably because of its ease of production and analysis in culture. More recently, however, primary antibody responses in culture have been developed to protein antigens such as TNP conjugated to either synthetic polypeptides, like (TG)-AL or (HG)-AL, or to proteins like

hemocyanin. These primary responses were found to be strictly dependent on accessory cells (Hodes and Singer, 1977; Singer *et al.*, 1978; Hodes *et al.*, 1978). Likewise, the development of helper T cells in culture, to be considered below, was strictly macrophage dependent (Erb and Feldmann, 1975a,b; McDougal and Gordon, 1977). Responses to synthetic polypeptides like GAT are also macrophage dependent (Pierce *et al.*, 1974).

The extent to which the T-B cell interactions leading to antibody formation in culture are accessory cell dependent becomes specially relevant in two circumstances: one is the secondary antibody response and the second with the use of 2-mercaptoethanol (2-ME).

Pierce's study (1969) showed that, while the primary response of cultured spleen cells to SRBC was easily abrogated by depletion of accessory cells, this was not the case for a secondary response. As early as 3 days after priming, no more than 50% depletion was possible. The same spleen from a mouse immune to SRBC would still respond after depletion of adherent cells but would not respond to burro red cells—a non-cross-reactive antigen. Pierce acknowledged that his method of depletion—by adherence—still left behind up to 1% phagocytes. Reports on the anti-DNP-response using spleen cells from mice immune to DNP-hemocyanin (Katz and Unanue, 1973) or DNP- $\phi\chi$ (Bluestein and Pierce, 1973a, 1973b) also indicated significant responses following depletion of accessory cells by the adhesion method of Mosier. In contrast, other studies using more stringent methods of depletion of accessory cells indicated that the secondary responses were markedly reduced—85 to 95%—by removal of accessory cells. These removed accessory cells by dish adherence method (Lamvik, 1969; Theis and Thorbecke, 1969; Radcliffe and Axelrod, 1971), by the use of glass bead columns (Feldmann and Palmer, 1971), anti-macrophage antibodies (Feldmann and Palmer, 1971), iron carbonyl (Sjoberg *et al.*, 1971), or the Sephadex G-10 method (Ly and Mishell, 1974). The latter method has proved to be a simple, fast, and reproducible one for eliminating accessory cells.

3. Studies with 2-Mercaptoethanol

This reducing agent is a promoter of lymphocyte function and has been discussed as a possible substitute for macrophages in culture. Although the effects of thiols have been examined in a number of situations, some of which we have alluded to before, their actions have been particularly emphasized and discussed in the context of antibody formation *in vitro*. Early reports described that addition of 2-ME to cultures enhanced the proliferative response of lymphocytes to ligands

(Fanger *et al.*, 1970) and to allogeneic cells (Heber-Katz and Click, 1972; Bevan *et al.*, 1974); in general, 2-ME potentiated the function of all immunological cells *in vitro*, varying from antibody responses (Click *et al.*, 1972), to antigen-driven T-cell responses (Section I,A), to cytolytic T-cell responses (Cerottini *et al.*, 1974; Harris *et al.*, 1976; Igarashi *et al.*, 1977), to the growth of B lymphocyte colonies (Metcalf, 1976). Click and associates (1972) were the first to examine the effects of 2-ME on the antibody response to SRBC using the method of Mishell and Dutton. They found that addition of 2-ME, at an optimal dose of $2 \times 10^{-5} M$, resulted in a marked increase in the plaque-forming cell response of *unfractionated* spleen cells. This increase was clearly not associated with a promotion of cell viability. Because the reducing agent did not need to be added daily to the culture, Click *et al.* postulated that most likely it was affecting very early events; the half-life of the reducing agent was expected to be short. Kinetic study disclosed that the development of plaque-forming cells, in cultures with 2-ME, progressed at an exponential rate earlier and for longer periods than in cultures lacking the reducing agent.

Chen and Hirsch were the first to examine the action of 2-ME on the plaque-forming cell response of spleen cells depleted of adherent cells (1972a,b). They confirmed that depletion of adherent cells would result in marked reduction of the antibody response and that addition of peritoneal macrophages would reconstitute it. Their experiments indicated that addition of 2-ME resulted in a marked improvement of the response of the accessory cell-depleted spleens and that the 2-ME had to be present for at least 72 hours in cultures. They also found a marked improvement of cell viability by addition of the thiol; while nonadherent cells showed a poor recovery, about 3% of initial input recovered after 4 days, the addition to them of macrophages or 2-ME resulted in about 30% recovery. A logical conclusion was that at least one effect of 2-ME must be through its improvement of cell survival. Although there is no major disagreement with this point (Pierce *et al.*, 1974), the issue is that 2-ME clearly had other major effects that resulted in an increase in cell growth. The results of Click *et al.* are not contradictory to those of Chen and Hirsch inasmuch as the former used unfractionated spleen cells; in Click's experiments, it was possible to observe a marked enhancement of a response by 2-ME without a marked effect on cell viability.

The critical point in Chen and Hirsch's work is the question of whether the response of T and B cells to SRBC in the presence of 2-ME is truly macrophage independent. This issue becomes critical with regard to an essential role of a macrophage-antigen-presenting

step. Chen and Hirsch faced this question attempting to enumerate macrophages in the spleens in various experimental manipulations. Their depletion method used adherence to plastic. Cognizant of the difficulties in enumerating macrophages, they proceeded to test for their presence by counting adherent cells, using either phagocytosis as an index or by the appearance of differentiated macrophages in cultures. In their estimates, the cultures of 5×10^6 nonadherent spleen cells contained from 500 to 2000 phagocytes, and a similar number after addition of 2-ME. Another 24 hours of culture in dishes, or 48 hours—the second 24 hours with 2-ME—resulted in further depletion to the extent of 220 macrophages per culture, respectively. In all instances, the antibody response to SRBC cells with 2-ME in the culture was the same regardless of the number of residual macrophages. Hence, Chen and Hirsch concluded that they had no evidence that the reducing agent was acting by increasing macrophage development in culture and that most likely its effect was by improving culture conditions. Other groups have confirmed these observations again using the SRBC response as the test antigen. Particularly noteworthy are the reports by Opitz and associates, who, by depleting macrophages using iron carbonyl or silica, completely abrogated the SRBC response. The impaired response was fully reconstituted by 2-ME (Lemke and Opitz, 1976; Opitz *et al.*, 1977a,b).

In contrast to these results, other investigators have failed to reconstitute macrophage-depleted lymphoid cells by 2-ME. Ly and Mishell (1974), using their method of depletion by G-10, obtained inconsistent results. Hodes and Singer's (1977) studies on the primary response to TNP-proteins, in which accessory cells were depleted, also by G-10 columns, showed strict dependence on accessory cells without any effect whatsoever of 2-ME. Similar results were obtained by Erb and Feldman (1975a,b,c). The proliferative responses of T cells to antigen or lectins discussed previously were strictly macrophage dependent, and these, again, could not be replaced by 2-ME (Section II,A). The study of Rosenstreich and Mizel (1978), mentioned in Section II,B, is worth repeating in this context. Their results indicated that 2-ME synergized with macrophages to increase the response.

Two final points with regard to thiols are worth discussing before making some final conclusions on their use and significance. How are the thiols acting on the cell? Do they have a direct effect, or is it through a secondary product?

Broome and Jeng (1974) made a very thorough analysis of the effect of various thiols on the growth of normal spleen cells and various lymphoid tumor lines. Normal spleen cells and 13 of 22 tumor lines

had increased growth in cultures when various thiols were added. In contrast, other cell lines—HeLa or fibroblasts, for example—were not influenced. There is general agreement now, from their studies, the early reports referred to before, and from recent publications, that thiols are very important growth potentiators for lymphocytes. These include the normal basal proliferation of lymphocytes (Lemke and Opitz, 1976; Goodman and Weigle, 1977), the growth of T cells upon lectin stimulation (for example, Lemke and Opitz, 1976; Rosenstreich and Mizel, 1978), or of B cells in response to anti-immunoglobulin antibodies (Sidman and Unanue, 1978, 1979; Sieckmann *et al.*, 1978), or to endotoxin (Lemke and Opitz, 1976). Other lymphoid tumor lines have also been shown to require 2-ME for growth (Hewlett *et al.*, 1977, 1979).

Broome and Jeng in their study went on to test various thiols trying to establish a functional-structural relationship. Various thiols and their disulfides promoted growth, in decreasing order of activity—for example, thioglycerol, the most potent of all, 2-ME mercaptopropionic acid, and cysteamine. Several substitutions of the basic thioethane compound resulted in changes, but the relationship of these with the growth potential was not found. For example, addition of a methyl group to the primary carbon did not affect the growth activity, but that of a polar group destroyed it. For more detailed analysis of these points, their paper should be consulted.

Are the thiols acting directly on the cell? The reducing activity of the thiols was brief—a few hours (Broome and Jeng, 1973); therefore, the expectation was that, if the compounds acted directly on the lymphocyte, they should do it during the first few hours of culture. Chen and Hirsch (1972b), however, found that medium containing 10% fetal calf serum treated with 2-ME retained its activity for promoting antibody formation for at least 3 days and suggested possible secondary effects. That the thiols might have direct effects was suggested by experiments in which 2-ME was added to cells in the absence of proteins; nevertheless the optimal growth potential required the addition of serum to the media (Lemke and Opitz, 1976).

Regardless of whether thiols directly act on a cell component, one way in which they promote growth is by the generation of an active molecule from plasma (Opitz *et al.*, 1977a,b; Hewlett *et al.*, 1979; Sidman and Unanue, 1978, 1979). The initial studies of Opitz *et al.* indicated that the fetal calf serum used for culturing lymphocytes, when treated with 2-ME, generated an active molecule about the size of ovalbumin. This molecule substituted for 2-ME in the culture and supported antibody formation to SRBC. The 2-ME-treated serum pro-

tein had to be added within 24 hours of culture and kept throughout the entire period. Our studies indicated that serum treated with 2-ME and then alkylated with iodoacetamide generated a 65,000-MW molecule that migrated electrophoretically like albumin and promoted growth of B cells and T cells (Sidman and Unanue, 1978; also in Braun and Unanue, 1980). The active moiety appeared, therefore, to be closely related to albumin. The later studies of Opitz *et al.* supported this conclusion (Hewlett *et al.*, 1979; Opitz *et al.*, 1978, 1980): Using affinity chromatography with Affi-Gel Blue columns, the 2-ME-generated moiety behaved exactly like albumin; furthermore, purified bovine albumin treated with 2-ME also generated a growth-promoting activity. The biochemical basis for this effect has not been established. Last, it should be noted that incubation of macrophages with fetal calf serum did not generate the active moiety.

In essence, the thiols have been clearly shown to promote growth of all classes of lymphocytes. The issue of whether these compounds substitute for macrophages is not entirely resolved, in part, because of the difficulties in eliminating all phagocytes from heterogeneous cell populations. Taking the overall evidence from analysis of T cell-antigen interactions or the T-B cell responses, particularly to protein antigens, one tends to favor the explanation that the antigen-presenting function of macrophages cannot be replaced by thiols. The thiols increase lymphocyte activity in cultures and, therefore, may replace a growth-maintenance function of macrophages yet may not be able to bypass the essential function of antigen presentation provided by the macrophage. It is possible, however, that in the presence of an agent like 2-ME the antigen-presenting function may be done by the B cell during its handling of antigen, particularly if these B cells are in high frequency, as in primed animals. This applies particularly to the SRBC response. Some direct evidence for B-cell presentation of antigen was presented by Kammer and Unanue (1980) but awaits further studies (see below).

4. Induction of Helper Cells

Continuing the analysis of T-B cell-macrophage interactions in culture, I will now review experiments attempting to analyze and dissect the interaction. Erb, Feldman, and their associates developed a two-stage culture system for the generation of carrier-primed helper T cells (Erb and Feldman, 1975a,b,c,d; Erb *et al.*, 1976, 1979a,b). First, T cells were cultured for 4 days with a protein antigen, resulting in the production of helper cells; the function of the helper T cell was assayed in a second culture by mixing the T cells with unprimed B cells

and the appropriate hapten-protein conjugate, later measuring the anti-hapten response. The anti-hapten response—usually to DNP—was measured by a plaque-forming assay.

Carrier-specific helper T cells were generated, inducing a small to modest number of plaque-forming cells with rather large experimental variations. Nevertheless, the results in the various publications appeared to be highly consistent. The generation of helper T cells to hemocyanin or to (TG)-AL required the presence of accessory phagocytic cells in the culture. The 2-ME did not replace the need for macrophages. Macrophages pulsed with the antigen were also highly efficient in generating helper T cells. McDougal and Gordon (1977) developed a similar system. In their experiments, cortisone-resistant thymocytes developed into helper T cells upon culture with hemocyanin or fowl γ -globulin. An excellent dose-response relationship was obtained, depending on the number of macrophages added to the culture. The primed T cells also generated an important nonspecific helper effect (assayed by challenging with DNP on an unrelated carrier protein) that was most pronounced with high numbers of T cells.

Erb and Feldman made a number of observations regarding the role of the macrophage in the generation of helper T cells. Marked differences were found between the response to hemocyanin added to the T cell-macrophage cultures either in soluble form or bound to Sepharose 2B particles. Thus, macrophages syngeneic with the T cells were required for the response to soluble hemocyanin—or the synthetic polypeptide (TG)-A-L (Erb and Feldman, 1975a,b). By mixing syngeneic and allogeneic macrophages, it was found that the lack of response by allogeneic macrophages could not be attributed to a suppressor effect. Furthermore, using strains of congenic mice with intra-H-2 recombinations, it was possible to map the locus controlling the interactions to the *I*-A region of *H*-2 (Erb and Feldman, 1975c). The response to hemocyanin-Sepharose also required macrophages yet could be partially replaced by 2-ME and, in contrast to the response to soluble hemocyanin, allogeneic macrophages were effective. The implications were that *I*-region restrictions did not take place with particulate antigens.

A second point explored by Erb and Feldman concerned the requirements for macrophage-T cell contact. In their first experiment (1975a), helper T cells were generated in a double-chamber culture where the macrophages were separated by a 0.2- μ m pore filter. This suggested that macrophages operated by releasing a soluble molecule. Direct examination for this material was done by culturing mac-

rophages with the antigens for 4 days and then using the conditioned media to culture the T cells. Culture fluids from syngeneic, but not from allogeneic, macrophages generated antigen-specific T helper cells to soluble proteins when added at ranges from 1 to 10% (volume/volume). This "genetically restricted factor" could be removed from solution by an immunoabsorbent column containing anti-I-A antibodies, but not by one containing anti-Ig antibodies. The conclusion reached by Erb and Feldman was that the macrophage released a molecule with Ia determinants and antigen-binding specificity. A different molecule was formed in cultures of macrophages that were cultured with hemocyanin, or without, that could generate helper T cells to Sepharose-hemocyanin, without any *H-2* restrictions.

Finally, a brief comment on the differences found by Erb and Feldman between soluble and particulate-bound hemocyanin. This lack of *I*-region restriction with particulate antigens cannot be generalized because it was not found for SRBC (Kappler and Marrack, 1976; Sprent, 1978a,b,c; Niederhuber *et al.*, 1979) or for bacteria (Farr *et al.*, 1979a). One possible explanation is that the initial T-cell culture contained sufficient macrophages to serve as antigen-presenting cells, but only in cooperation with the growth factor. This bead-bound hemocyanin is very likely to interact much more efficiently with any few residual macrophages than the soluble antigen.

Important experiments to discuss are those of Ishizaka and Adachi (1976), who examined the generation of helper T cells *in vitro*. They found a major difference if the T cells were incubated with macrophage-bound antigen or with soluble antigen in the absence of macrophages. Normal spleen cells were cultured on ovalbumin bound to syngeneic peritoneal macrophages for 5 days, after which their function as helper cells was assayed by *in vivo* adoptive transfer; the T cells were inoculated with DNP-hemocyanin-primed B cells into X-irradiated mice, which were boosted with DNP-ovalbumin and then assayed for the IgG and IgE anti-DNP response. T cells cultured with macrophages developed helper T cell function. In contrast, when the lymphocytes were incubated with 10 µg of ovalbumin per milliliter in the absence of macrophages, no helper cells developed, but instead T cell suppressors that blocked the response to ovalbumin of normal mice. The results were found with both the IgG and IgE response. This is an important experiment that strongly supports that the mode of antigen presentation is a critical step in immune induction. It brings out good evidence that antigen not presented in the macrophage stimulates preferentially the suppressive pathway. The generation of

suppression *in vivo* by high doses of antigen may well be explained by a bypass of the macrophage requirement. Another experiment supporting Ishizaka's result is that of Pierres and Germain (1978), to be discussed later.

5. MHC Restrictions

The *I*-region-dependent regulation of macrophage-B and T cell interactions was examined in a number of systems, all of which indicated that Ia-bearing phagocytes matching at the *I* region with the T cells were required for the interaction. For example, Hodes and Singer and their associates studied extensively the primary *in vitro* responses of mixtures of T and B cells to TNP-(TG)-AL or TNP-hemocyanin (Hodes and Singer, 1977; Singer *et al.*, 1977, 1978, 1979; Hodes *et al.*, 1978, 1979). The response was strongly dependent on phagocytic accessory cells identified with the use of a cytotoxic assay as bearing both I-A and I-E/I-C determinants (Hodes *et al.*, 1978; Dickler *et al.*, 1980). The response to both proteins required that T cells and macrophages share the *I*-A region of *H*-2 (Singer *et al.*, 1977, 1978).

Kappler and Marrack's laboratory, with a somewhat different approach, reached the same conclusions (Kappler and Marrack, 1976, 1978). In their system, *F*₁ mice from the combination (C57BL/6 × DBA/2) were inoculated *in vivo* with hemocyanin-pulsed macrophages from either of the parental strains; the T cells were subsequently isolated and cultured with B cells and macrophages from one or the other parents, together with haptenated hemocyanin; the anti-hapten response was measured by a plaque assay. The *F*₁ T cells cooperated only with the T cell-macrophage of the parental strain used for priming (Kappler and Marrack, 1976). In a subsequent study, the T cells reactive with the antigen were enriched in culture, by first placing them on a monolayer of macrophages bearing the antigen (Swierkosz *et al.*, 1978). Binding of T cells to macrophages took place if the macrophages were pulsed with the specific antigen. Furthermore, using the system of *F*₁ T cells primed *in vivo*, described earlier, it was possible to identify two populations of *F*₁ T cells, each binding to one or the other parental macrophages. The *F*₁ T cells isolated exhibited helper activity when tested with B cells and macrophages that had the same *K* through *I*-A region as the macrophages used for the isolation (Swierkosz *et al.*, 1979). The antigens used in the system were hemocyanin, (TG)-AL, and SRBC.

A third system of *I*-region-dependent macrophage-lymphocyte interaction is the primary SRBC response. Niederhuber's laboratory analyzed its macrophage requirement and found that macrophages

bearing Ia were essential (Niederhuber, 1978, 1980). The Ia-bearing macrophages contained antigenic determinants coded in *I-A*, *I-E/C*, and *I-J*. Niederhuber's most provocative observation concerned the effect of antibodies to the *I-J* subregion (Niederhuber *et al.*, 1979; Niederhuber and Allen, 1980). They found that a brief 30-minute treatment at 37°C of spleen macrophages with antibodies to *I-J* resulted in a complete loss of function when T-B cells were added; similar treatment with antibodies to *I-A* or *I-E/C* did not affect the macrophages.

The *in vitro* antibody response to the synthetic terpolymer of L-glutamic acid-L-alanine-L-tyrosine (GAT), an antigen under *Ir*-gene control, showed peculiar characteristics insofar as MHC restrictions that merit attention. The response to GAT was dependent on adherent accessory cells (Pierce *et al.*, 1974) and could be reconstituted with GAT pulsed to syngeneic or allogeneic macrophages (Pierce *et al.*, 1976). Furthermore, the removal of macrophages from the spleen cell suspension (of a "responder" mouse strain) resulted in the ready development of specific suppressor T cells (Pierres and Germain, 1978; Germain *et al.*, 1980) (Fig. 4). The suppressor cells were also found in unfractionated spleen cells by adding a large amount of GAT. (The suppressor T cells were assayed by their inhibition of the development of a primary anti-GAT response to syngeneic spleen cells.) This last result again depicts the balance between the development of helper and suppressor T cells, depending on the way in which antigen is introduced: suppression may be favored by antigens not presented in macrophages or presented—as in the case of an excess of soluble antigen—perhaps in the context of a "wrong" antigen-presenting cell.

Further analysis of the GAT system disclosed that, although no MHC restrictions were found insofar as macrophage presentation, these did take place in situations where the T and B cells came from mice primed *in vivo* with GAT-bearing macrophages. Thus, mice were primed *in vivo* with GAT bound to syngeneic or allogeneic macrophages, and their spleen cells were later examined in culture: response took place only with the GAT bound to the macrophages used for priming, whether syngeneic or allogeneic (Pierce *et al.*, 1976). In a different situation, the spleen cells from F₁ mice between a responder and a nonresponder strain, primed *in vivo* with "responder" or "non-responder" strain GAT-pulsed macrophages would make antibody to the GAT pulsed with the macrophage used for priming (Pierce *et al.*, 1977). In contrast, in the T cell proliferative response to GAT, F₁ T cells would proliferate only to GAT bound to responder strain macrophages (Yano *et al.*, 1978). The same results were found by Miller

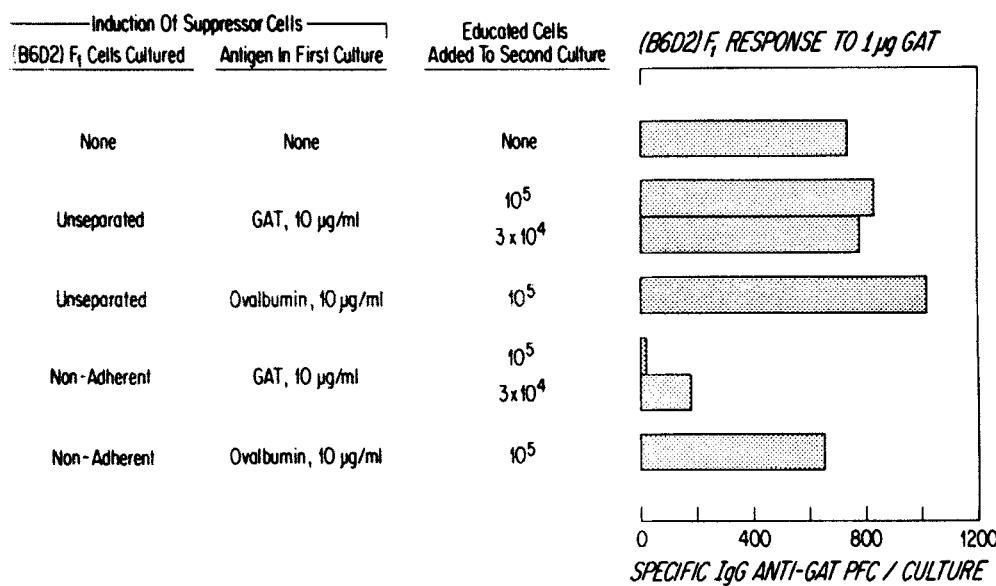


FIG. 4. This experiment shows the induction of suppressor T cells by incubation of macrophage-depleted spleen cells with copolymer L-glutamic acid-L-alanine-L-tyrosine (GAT). Unfractionated or nonadherent spleen cells were cultured for 2 days with the antigens indicated in the graph. The "educated" cells were then added to fresh cultures of syngeneic spleen cells stimulated with GAT. Cells cultured in the absence of adherent cells with 10 µg of GAT generated suppressor activity. [From Pierres and Germain (1978).]

studying delayed sensitivity (Section II,D). Also, Howie and Feldman (1978) reported collaboration to GAT only between macrophages of responder strains interacting with B cells and T helper factors. However, recent studies by Araneo and Kapp (1980), using proliferation as the assay, again found that F₁ T cells proliferated to both responder or nonresponder macrophages, which leaves the issue unresolved. The GAT system may be a unique situation, but it should be considered at a time when the molecular mechanisms underlying the recognition of antigen and Ia are better understood. Perhaps the structure of GAT is such that an association of it with a macrophage I-region product, if indeed taking place, results in similar configurations, regardless of I-region haplotype. The genetic defect of the GAT would be placed then at the level of the lymphocyte.

It should be noted that, in some studies, I-region-restricted interactions between the macrophages and the lymphocytes were not found (Katz and Unanue, 1973; Unanue, 1978). In these experiments, al-

logeneic macrophages presented haptenated hemocyanins effectively to *immune* spleen cells that were not fractionated or were not stringently depleted of macrophages and, therefore contained syngeneic macrophages. The possibility was raised of transfer of antigen from the allogeneic to the syngeneic macrophages (or B cells) (Unanue, 1978). Indeed, this system has been used to support a pathway of presentation of antigen from macrophages to another antigen-presenting cell not involving an MHC-restricted interaction (Section III,B). Pierce and Malek (1980) obtained evidence supporting this possibility in the *in vitro* response of mice to fowl γ -globulin. Syngeneic and allogeneic antigen-pulsed macrophages stimulated about the same response of unfractionated immune spleens. However, only syngeneic macrophages presented antigen when the spleen cells were totally depleted of macrophages by the Sephadex G-10 method of Ly and Mishell (1974). Evidence of release of immunogenic antigen was also obtained in this study.

An *in vivo* demonstration of the MHC-restricted interaction between helper T cells and antigen-presenting cells has been elegantly demonstrated by Sprent. His basic experiments consisted of injecting (CBA \times BL/6)F₁ purified T cells into an X-irradiated mouse of one or the other parental strain together with SRBC. The F₁ T cells were recovered from the thoracic duct lymph and cotransferred with the antigen and parental strain B cells into X-irradiated mice, which were later examined for their plaque-forming cell number (Sprent, 1978a,b). The immune T cells in the initial host were depleted from the thoracic duct cells during the first 2 days, indicating their transient removal from the circulation, but appeared in large number by 6 days. Thus, by examining day 2 or day 6 thoracic duct cells, "negative" or "positive" selection of immune T cells could be shown. The results indicated that F₁ T cells would cooperate only with the B cells of the same parental strain used for the *in vivo* priming and supported the concept that these F₁ T helper cells comprised two distinct groups. Sprent went on to show that the selection was found only when transferring purified T cells (to the first host). Transferring spleen cells showed no preference, suggesting that antigen-presenting cells in the spleen competed with those of the host. This was shown directly by cotransferring peritoneal exudate cells of one or the other parent, together with purified T cells (Table IV) (Sprent, 1978a). In the experiment shown in Table IV, F₁ T cells transferred into BL/6 preferentially helped B cells of BL/6; this preference was not found if the peritoneal exudate cells were added to the T-cell inoculum. Sprent has recently shown that the selection process could be blocked by injection of anti-Ia antibodies (1980b). He

TABLE IV
ANTIGEN PRESENTATION *in Vivo*^a

T-cell group	T cells	PE cells added during positive selection	B cells	Anti-SRBC PFC per spleen at 7 days in irradiated F ₁	
				IgM	IgG
A	F ₁ , T → B6	—	CBA	1,770 (1.12)	1,580 (1.52)
			B6	17,280 (1.14)	37,050 (1.13)
B	F ₁ , T → B6	3 × 10 ⁷ CBA PE	CBA	26,950 (1.16)	67,740 (1.13)
			B6	21,160 (1.19)	47,880 (1.16)
C	F ₁ , T → B6	3 × 10 ⁷ B6 PE	CBA	2,150 (1.34)	8,140 (1.37)
			B6	57,330 (1.24)	114,910 (1.02)
D	F ₁ , T → CBA	—	CBA	19,740 (1.12)	59,400 (1.06)
			B6	1,300 (1.30)	2,160 (1.14)

^a This table is taken from Sprent's study (1978a). (CBA × BL/6)F₁ nylon-wool-purified T cells were injected into X-irradiated BL/6 mice together with sheep red cells (SRBC). In groups B and C, CBA or B6 peritoneal exudate (PE) cells were injected together with the T cells. Thoracic duct lymphocytes were harvested 7 days later and transferred with B cells (of CBA or B6) into F₁ X-irradiated animals plus sheep red cells. The results show the plaque-forming cells (PFC) after subtraction of the background values.

has concluded that the activation of helper T cells was *H2* restricted *in vivo*, that the cells presenting antigens were enriched in spleen and in peritoneal exudates, and that they were radioresistant, belonging most likely to the phagocyte lineage (1980a).

Finally I will analyze very briefly, but speculate freely on, the controversial issue of whether the *I*-region control in the interaction of T and B lymphocytes with macrophages is at the level of the macrophage-T cell interaction exclusively or applies also to the interaction of B and T cells as initially postulated by Katz's laboratory (Katz *et al.*, 1973, 1978). There is no argument that macrophage-T cell interactions result in the development of helper T cells. The point is whether the T-cell clones reactive to an antigen in the context of an *I*-region determinant of an appropriate macrophage need also to interact with a B cell bearing the same *I*-region product. A number of experiments indicated that T cells from F₁ animals selected on macrophages from one parent will collaborate only with the B cells of the same parent (Yamashita and Shevach, 1978; Sprent, 1978a,b; Swierkosz *et al.*, 1978; Andersson *et al.*, 1980). However, other experiments suggest that the primary restrictive *I*-region interaction is only between macrophages and T cells (for example, Singer *et al.*, 1979; McDougal and Cort, 1978). An explanation for these discrepant results

awaits further studies. It may well be that at least in culture there are various pathways to activate B cells. For example, release of nonspecific helper factors from the T cell-macrophage interaction that trigger B cells without an *I*-region restrictive interaction (Section III,C).

There is no *a priori* reason why selected T cells cannot interact with the B cell in an *I*-region-restricted way. B cells take up and handle antigen in a manner analogous to macrophages (Engers and Unanue, 1973), the exception being that only the B cells with specific receptors can interact with antigens, whereas all the macrophages in a population are capable of antigen-binding. An antigen presentation role for the B cell may explain various observations: (*a*) that B cells recognize mostly conformational determinants of a globular protein (Sela, 1969); (*b*) yet T cells recognize unfolded or denatured determinants; but (*c*) hapten and carrier determinants of an antigen have to be linked together for optimal interactions. These three observations could be explained if a B cell recognizes a protein antigen in its tertiary structure, "processes" it, and then presents the "processed" or "carrier" determinants to the T cell in the context of its Ia molecule.

Direct examination of antigen presentation from B cells to T cells has been difficult to test and, in general, has not been critically examined. Technical difficulties in separating macrophages from B cells limit the experiments. Our laboratory has recently presented suggestive evidence that B cells may present hemocyanin to T cells (Kammer and Unanue, 1980). The proliferation of T cells was highly favored by the addition of macrophage-conditioned medium to the culture. More recent results using Ig as antigen support an antigen presenting function of B cells (Chestnut and Grey, 1971). It is possible that the B cell may present antigen, but only for the purpose of receiving back the T cell helper signal that it acquires by direct cell contact. However, the B cell will be limited in its capacity to reciprocate by providing a stimulus required for the activation and growth of the T cell. Thus, the stimulation of the T cell becomes the responsibility of the phagocytes, which are abundant and capable of freely binding antigen. (The inability of B cells to support T cell growth is also shown by their poor role as stimulators in the mixed leukocyte reaction.) This explanation does not exclude that a B cell, under appropriate circumstances, can also be stimulated by macrophage factors and/or non-specific T helper factors in situations that do not involve an *I*-region-restricted step (Section III,C). However, the fact that, for optimal *in vivo* responses, the hapten and carrier determinants must be linked in the same molecules (reviewed in Katz, 1977) argues for the major pathway being that of B

cell stimulation by close contact with T cells. Hence, the scenario that I envision for the three cells is that phagocytes are the primary antigen-handling cells that result in the selection, growth, and stimulation of the *I*-region-restricted T cells. The B cells are stimulated when they recognize intact antigen (either free or bound also to phagocytes) and presents it to the T cell in a process that could be modulated by soluble helper factors from either the macrophage or the T cell.

The studies described in this section permit the conclusion that: (*a*) macrophage-bound antigen is a highly effective mode of antigen presentation for T-B cell interactions; (*b*) the macrophages are required for the primary antibody response to thymic-dependent antigens, either for protein or particulate antigens; (*c*) the requirement for macrophages in secondary response or for primary responses to SRBC in the presence of a growth promoter, e.g., 2-ME, are less stringent; it is even possible that macrophages may not be essential in these situations; (*d*) in most studies, the interactions between T and B cells with macrophage-bound antigen involve the *I* region of the macrophage; that is to say, antigen presentation for T-B collaboration involves Ia-bearing macrophages and shows requirements for *I*-A homology, as in the proliferative systems described before; and (*f*) interactions of T lymphocytes with antigen in the absence of macrophages favor the development of suppressor cells. Finally, in all studies so far, the helper T cells involved in *I*-region-restricted interaction with macrophages bear the Ly1 marker and not the Ly2 antigen characteristic of the suppressor and cytolytic T cells (reviewed by Katz, 1977; and Cantor and Boyse, 1977).

D. INTERACTIONS WITH T CELLS IN CELLULAR IMMUNITY REACTIONS

I am referring in this section to cellular immunity reactions that result in accumulation of phagocytes in foci of immune reactions and in macrophage activation for increased microbicidal and tumoricidal activity. Cellular immunity reactions constitute the basis of resistance to intracellular pathogenic microorganisms, such as the facultative intracellular bacteria, protozoa, viruses, and fungi. An important component of macrophage activation is also found in the response to tumors. Delayed-type hypersensitivity and contact sensitivity reactions are expressions of cellular immunity reactions to protein antigens or contact sensitizers deposited in the skin. These reactions involve immune T cells that, by way of various soluble mediators, call forth and activate macrophages. The activated macrophages are bone marrow-derived

phagocytes that have changed their biology, developing a heightened cytoidal activity. Such activated macrophages will be able to stop growth and/or kill bacteria, protozoa, and tumors (for papers on these issues, see MacKaness, 1962; MacKaness and Blanden, 1967; Dannenberg, 1968; North, 1969, 1978; McGregor *et al.*, 1971; Lane and Unanue, 1972; David and David, 1972; Lefford, 1975; Karnovsky and Lazdins, 1978; Cohn, 1978).

A major and apparently essential component in the stimulation of T cells to bring about macrophage accumulation and activation is an interaction of the T cell with Ia-bearing phagocytes. Several experimental systems have been used to examine this issue, some involving *in vivo* analysis, others involving culture approaches. For didactic reasons, these several approaches are now considered separately.

1. Delayed Sensitivity

The first transfer of delayed-type hypersensitivity reactions involving contact sensitizers or tuberculin were made by Landsteiner and Chase (1942) and Chase (1945) using outbred guinea pigs. They transferred peritoneal cells from outbred immune guinea pigs into nonimmune recipients, which were skin tested hours later. Successful transfer required a large number of lymphoid cells. Subsequent to these pioneering studies, lymphocyte transfers employed cells from inbred animals to avoid primarily tissue rejection. The first indication that the effective transfer of delayed reactions by lymphocytes required an important contribution by the host involving the *Ir* genes came from the studies of Green, Paul, and Benacerraf using outbred guinea pigs (Green *et al.*, 1967). They were pursuing the analysis of the genetics of the immune response to the polylysine compounds (Kantor *et al.*, 1963) and were attempting to transfer the capacity to develop delayed sensitivity from a "responder" to a "nonresponder" guinea pig. Lymphoid cells from responder guinea pigs were able to transfer the reaction to responder, but not to nonresponder, guinea pigs, suggesting to Green *et al.* "that some essential genetic controlling processing step on the antigen, specific for L-lysine sequences, must take place before sensitized cells capable of binding the antigenic determinant can be stimulated by such antigens." Subsequent studies linked the response to poly(L-lysine) compounds to the MHC of the guinea pigs (Ellman *et al.*, 1970; Martin *et al.*, 1970). Responder and nonresponder animals differed primarily at the *I* region (Benacerraf *et al.*, 1971).

In 1975, Miller and his associates reevaluated the transfer of delayed sensitivity using inbred strains of mice (Miller *et al.*, 1975). Mice were immunized with fowl γ -globulin in Freund's adjuvant; their lympho-

cytes were harvested and transferred to various inbred strains syngeneic or allogeneic with the donor of the cells. The recipient mice were later challenged in the ear with a small amount of the antigen; the accumulation of cells radiolabeled by a pulse of [¹²⁵I]iodobromodeoxyuridine (Vadas *et al.*, 1975) was taken as a measure of delayed sensitivity. The basic results of their experiments were that the sensitized T cells could transfer the reaction to syngeneic, but not to allogeneic, recipients; furthermore, using recipient mice from inbred strains sharing part of the H-2, it was found that successful transfer required identity at I-A subregion between the donor and recipient strains of mice. No suppressor mechanism could be ascertained for the restriction of the transfer (Vadas *et al.*, 1977).

A logical candidate for the restriction of the transfer of delayed sensitivity was the macrophage as per the results found by Rosenthal and Shevach's study of 1973 (Section II,A). Delayed sensitivity reactions were known to be very successfully induced—or the reactions elicited in the immune animal—by transferring antigen bound to live macrophages (Unanue and Feldman, 1971; Feldman and Unanue, 1971; Seeger and Oppenheim, 1972). Miller *et al.* proceeded to immunize F₁ mice (between CBA, an H-2^b strain, and BALB/c, an H-2^d) by injecting them with live macrophages from either of the parental strains pulsed with antigen (fowl γ -globulin or hemocyanin). Such mice, as expected, developed strong delayed reactions (Miller *et al.*, 1976, 1979); their lymphocytes were able to transfer the delayed reactivity only to the recipient mice of the same strain as the antigen-carrying macrophage. In other experiments, it was found that a delayed reaction could be elicited in immune mice if injected with live macrophages bearing antigen, but only if the macrophages were syngeneic or semiallogeneic. Overall, these experiments, therefore, pointed to the antigen-presenting event as the one responsible for restricting the transfer of the reaction.

Other experiments used an antigen under Ir-gene control, i.e., an antigen that results in responsive or unresponsive states among inbred strains, like the copolymer of glutamic acid-alanine-tyrosine (GAT). It was found that GAT-immune cells from an F₁ between a responder and a nonresponder would transfer the delayed sensitivity state only to mice from a responder strain (Miller *et al.*, 1977, 1979). Furthermore, F₁ recipients could be sensitized by macrophages pulsed with GAT from a responder strain but not with macrophages from the nonresponder (Miller *et al.*, 1979). Finally, cultured bone marrow cells pulsed with antigen were able to induce a delayed sensitivity state when transferred into normal recipients. Killing the Ia-bearing mac-

rophages by treatment with anti-Ia (plus serum as complement) did not sensitize (Motram and Miller, 1980).

2. Contact Sensitivity

The Langerhans cells of the skin are further reviewed in Section III, A. In this section, we consider this phagocytic cell as a strong candidate for presentation of antigens introduced by way of the epidermis. Langerhans cells are found throughout the epidermis, mainly in the upper layers of the stratum malpighii. They appear in hematoxylin and eosin stains as cells with a clear cytoplasm containing a peculiar organelle—the Birbeck granule—consisting of a rod-shaped structure contained in a membrane-limited vesicle. Langerhans cells can be distinguished by their content of ATPase and their uptake of gold salts (reviewed by Breathnach, 1975; and by Silberberg-Sinakin *et al.*, 1978).

Interest was aroused in the finding that, in man, guinea pigs, and mice, Langerhans cells contained Fc and C3 receptors (Stingl *et al.*, 1977) and bore Ia antigens (Rowden *et al.*, 1977, 1978; Klarskog *et al.*, 1977; Stingl *et al.*, 1978a,b,c; Forsum *et al.*, 1979b; Tamaki *et al.*, 1979). Langerhans cells also took up a variety of soluble and particulate antigens and thus had characteristics with the cells of the mononuclear phagocyte system (Shelley and Juhlin, 1977; Silberberg-Sinakin *et al.*, 1978). In contrast to classical mononuclear phagocytes, the Langerhans cells contained few lysosomes and showed little pinocytic activity. The phagocyte, in turn, lacked the typical Birbeck granule (Birbeck *et al.*, 1961).

Stingl and associates made a major observation by isolating the keratinocytes rich in Langerhans cells and establishing in culture that they could present antigen in systems involving T cell proliferation (Stingl *et al.*, 1978a,b, 1980). They isolated epidermal cells and selected by density gradient those bearing Fc receptors and capable of binding opsonized red cells. This Langerhans cell-rich fraction was added with antigen (ovalbumin) to cultures of immune T cells from syngeneic or allogeneic strains of guinea pigs. A strong proliferative response was obtained by ovalbumin presented in Langerhans cells, but only in syngeneic combinations. Killing the Langerhans cell with anti-Ia resulted in a complete loss of the response. Langerhans cells were also found to be good stimulators in the mixed leukocyte reaction; also, T cells from guinea pigs immune by skin sensitization with picryl chloride were found to proliferate in culture upon interactions with trinitrophenyl conjugated to syngeneic macrophages (Thomas *et al.*, 1977a) or to isolated Langerhans cells (Stingl *et al.*, 1978a, 1980).

Thus, overall, Langerhans cells were behaving as the conventional Ia-bearing macrophage, insofar as their capacity to take up and present antigen.

The finding of a phagocyte-like cell in the epidermis bearing Ia and capable of antigen binding and presentation raises the issue of whether this is the cell responsible for immunization to antigens that contact and bind to the skin (Silberberg *et al.*, 1976). There is evidence that the Langerhans cell increases in the skin after application of a contact sensitizer and migrates into the dermal lymphatics and into the draining lymph nodes (Silberberg *et al.*, 1975; Silberberg-Sinakin and Thorbecke, 1980). These events may be essential steps in transporting and providing the immunogen to the lymphoid elements. Experiments of Macher and Chase (1969) provided evidence that the skin was directly responsible for the presentation of antigen. They painted the guinea pig ears with a contact sensitizer and evaluated the effects of excising the ear on the later development of contact sensitivity. Ear excisions 24 hours after painting, when 80% of the sensitizer had escaped via the circulation, completely abolished the development of delayed sensitivity. This indicated that the antigen that was rapidly removed was not immunogenic. However, removal of the skin site at later times showed progressively less effect until the third and fourth days, at which times there was no effect, i.e., sensitization had taken place. They concluded that the skin depot was responsible for sensitization; to quote, "these results support the concept of interaction between allergen and host tissue at the site as being essential for transformation of the simple chemical into the sensitizing antigen" (Macher and Chase, 1969). Very convincing evidence that this is by way of the Langerhans cell was provided by Toews, Bergstresser, and Streilen (Toews *et al.*, 1980). They sensitized mice with DNFB on areas of the skin varying in their content of Langerhans cells and later measured the delayed reactions by ear swelling after application of the sensitizer. Painting of the tail skin with a content of 100 Langerhans cells per square millimeter resulted in very weak reactivity in contrast to painting on the skin of the abdomen, a site with a high content of Langerhans cells ($700/\text{mm}^2$). Toews *et al.* went on to show that Langerhans cells disappeared after ultraviolet irradiation of the skin and that painting the irradiated skin resulted in no sensitization. As a control, they showed that mice sensitized on areas not directly exposed to ultraviolet light were able to develop a delayed sensitivity state. Very interestingly, not only was the painting on tail skin or ultraviolet-irradiated skin ineffective, but it apparently resulted in a tolerant state; i.e., mice painted with DNFB on tail or on ultraviolet-irradiated

skin were later painted for a second time on normal abdominal skin and subsequently challenged; the first exposure resulted in specific abrogation of sensitization. The data, therefore, clearly supported a cardinal effect of the skin most likely via the Langerhans cells in inducing a local state of immunity.

3. *Lymphokine Secretion*

Cellular immunity reactions are believed to be mediated by a number of mediators released by T cells in the course of antigen stimulation. The secretion of macrophage inhibitory factor by guinea pig or murine T cells challenged by antigen in culture required the presence of macrophages (Wahl *et al.*, 1975a,b; Nelson and Leu, 1975; Ohishi and Onoue, 1975; Landolfo *et al.*, 1977) and was inhibited by anti-Ia antibodies (Ben-Sasson *et al.*, 1974). The same macrophage requirement applied to the secretion of macrophage chemotactic factor induced by antigen (Wahl *et al.*, 1975a), osteoclast-activating factor induced by PHA (Horton *et al.*, 1974), and mitogenic factor induced by human T cells (Larsson, 1978; Breard *et al.*, 1979). The secretion of macrophage-activating factor by murine T cells required Ia-positive macrophages (Farr *et al.*, 1979b). Once secreted, the various lymphocyte mediators acted on macrophages regardless of their strain of origin or Ia content (Fidler, 1975; Riisgaard *et al.*, 1978; Farr *et al.*, 1979b). As in most interactions in culture between macrophages and lymphoid cells, their amount and state of macrophage activation was critical. Lymphokine secretion was suppressed, for example, using activated macrophages from tumor-bearing mice (Varesio *et al.*, 1979). The nature of the suppressive function of the activated macrophages may be multiple (Section III,C).

4. *Antibacterial Immunity and Macrophage Activation*

The only studies of induction and development of cellular immunity to facultative bacteria have used *Listeria monocytogenes*, an organism that induces a severe infection in mice. Resistance to *Listeria* depends on the recruitment and activation of phagocytes resulting from T cell stimulation (MacKaness and Blanden, 1967). I will not review here the process of macrophage recruitment and activation but will focus exclusively on the events leading to T cell stimulation. The first indication of the involvement of the MHC in anti-*Listeria* immunity came from the studies of Zinkernagel and associates (Zinkernagel, 1974; Zinkernagel *et al.*, 1977). The experimental setup consisted of measuring *Listeria* growth in tissues of recipients of *Listeria*-immune T cells; i.e., T cells were harvested from immune mice and injected intravenously

into normal mice, followed a few hours later with live *Listeria* organisms; viable bacteria were estimated by colony counts a day later. It was found that the *H-2* complex restricted the successful transfer of antibacterial immunity; in order for bacterial counts to be reduced by 100- to 1000-fold, it was necessary that the recipient and donors of the T cells match at the *I-A* region. Limited experiments also suggested the presence of two *H-2* reactive T cells in *F₁* recipients. The interpretation of these studies was the same as that given to Miller's, i.e., that the development of T cell reactivity required an initial interaction with host accessory cells regulated by the *I* region.

In our laboratory, we investigated the interactions between *Listeria*-immune T cells and macrophages that resulted in either macrophage or T cell stimulation (Farr *et al.*, 1977, 1979a,b; Ziegler and Unanue, 1979; Beller *et al.*, 1980). *Listeria*-immune T cells were placed in culture, together with peritoneal macrophages and heat-killed *Listeria* organisms. After 24 hours, the culture supernatants were harvested and assayed for a number of mediators, while the macrophages were tested for tumoricidal function. After 24 hours of interaction with T cells, the macrophages were highly cytotoxic and killed ⁵¹Cr-labeled P815 tumor cells. In order for the macrophages to develop cytotoxic function, it was necessary that there be *I*-region homology between T cells and macrophages and that the macrophages bear Ia antigens. By mixing immune *Listeria* T cells from one strain with macrophages from various congenic lines, it was possible to map the *H-2* restriction to *I-A* subregions with a minor involvement in *I-E/C*. At the same time that macrophages developed cytolytic activity, secretion of mediators took place. Among them we found a macrophage-activating factor and a 15,000 MW mitogen identical to the lymphocyte-activating factor (LAF) made by the macrophage (Section III,C). Again, the secretion of all mediators involved a first interaction regulated by the *I* region. Identical results were obtained assaying for T-cell proliferation. Using a functional assay that measures the direct binding of T cells to the macrophages, it was possible to ascertain that T cells would interact with macrophage-bound *Listeria* only if the macrophages showed surface Ia and genetically shared the *I-A* region (Ziegler and Unanue, 1979 (Section III,B)).

In summary, the studies in cellular immunity have indicated that (*a*) macrophage-bound antigen can transfer—or elicit—a delayed-sensitivity state and that these reactions involve the *I* region of the macrophage; (*b*) an antigen-handling step restricts the cellular transfer of delayed reactions and cellular immunity to infection, and this restriction maps to the *I* region; (*c*) skin phagocytes are most likely the

cells responsible for elicitation of contact sensitivity by presenting and carrying the sensitizer to the T cells, again involving an *I*-region function; (*d*) in culture, the production of mediators and the activation of the macrophage requires recognition of antigen bound to a phagocyte. Such recognition has the same characteristics shown for the proliferative response. Finally, the T cell involved in the cellular immunity reactions described above bears the Ly1 antigen and thus belongs to the helper subset.

E. INTERACTIONS WITH B CELLS AND POLYCLONAL STIMULI

The critical issue here is whether polyclonal stimuli, most of which are regarded as thymic-independent antigens, will act on B cells without the need of macrophages or do so only with the intervention of the phagocytes. The macrophage dependency of B cell proliferation and differentiation induced by polyclonal stimuli in cell cultures has not been an easy problem to tackle and, furthermore, has not been pursued as thoroughly as the T-cell responses to antigen. Discrepant results are frequent, even within a single laboratory, most likely because of the difficulties in counting and eliminating phagocytes. The problem of separating phagocytes from B cells has not been adequately resolved, and most methods have some problems: for example, the iron-carbonyl method, while removing macrophages, also eliminates some B cells; overall, whether polyclonal stimuli act directly on the B cell or by way of the macrophage still requires critical evaluation.

Some polyclonal stimuli, most notably *Escherichia coli* lipopolysaccharide (LPS), induce B cell proliferation and differentiation after macrophage depletion and thus appear to be macrophage independent (reviewed in Coutinho and Moller, 1975; Persson *et al.*, 1978). Some of the responses involving LPS, however, do involve the macrophage. The LPS stimulates the release of powerful lymphostimulatory molecules from macrophages, and these molecules definitely have an effect in B- and T-cell responses in culture (see Section III,C). In a system extensively analyzed by Hoffman and colleagues (1979), it was found that spleen cells responded with an antibody response to TNP coupled to autologous red cells, but only in the presence of LPS, confirming earlier reports (Schmidtke and Dixon, 1972b). Such a response, however, required macrophages. The macrophage requirement could be replaced by medium conditioned by macrophages treated with LPS (Hoffman *et al.*, 1979). Therefore, although a powerful molecule like LPS apparently can act on B lymphocytes directly, its effects on macrophages result in responses involving a multiplicity of cells.

DNP-levan (Desaymard and Feldman, 1975) and lanatoside C

(Smith and Hammarstrom, 1977; Hammarstrom *et al.*, 1978) are antigens that have been shown not to require macrophages. Other stimulants, such as the polyanion dextran sulfate require accessory cells (Diamantstein *et al.*, 1973; Persson *et al.*, 1977; Hammarstrom *et al.*, 1978; Smith and Hammarstrom, 1978). There are discrepant results with regard to polymerized flagellin used so extensively by the Australian workers. While many studies showed no impairment of the antibody response in culture (Shortman *et al.*, 1970; Feldman and Palmer, 1971; Shortman and Palmer, 1971), a recent report claims a marked reduction by elimination of phagocytes using the iron-carbonyl method (Lee *et al.*, 1976).

TNP-derivatized Ficoll, a polysucrose compound, induces differentiation of spleen cells to antibody formation in cultures. Although the first report claimed that this response was macrophage independent (Mosier *et al.*, 1974), subsequent studies agreed that macrophages were required (Lee *et al.*, 1976; Chused *et al.*, 1976; Nordin, 1978; Kirkland *et al.*, 1980; Boswell *et al.*, 1980). The antibody response to TNP-polyacrylamide beads was also found to be macrophage dependent in both the mouse (Duclos *et al.*, 1979) and in man (Delfraissy *et al.*, 1977).

The response of human peripheral blood lymphocytes to pokeweed mitogen has been extensively studied. Pokeweed mitogen induced strong B-cell differentiation that required the presence of T cells (Keightley *et al.*, 1976). The response also required a critical number of monocytes (Rosenberg and Lipsky, 1979; Knapp and Baumgarten, 1978; Gmelig-Meyling and Waldmann, 1980); macrophages also inhibited the response. The nature of the inhibition is unexplained but did not appear to be mediated by prostaglandins (Gmelig-Meyling and Waldmann, 1980). The response of human B cells to a water-soluble antigen from *Nocardia asteroides* was not affected by monocyte depletion (Bona *et al.*, 1979; Gmelig-Meyling and Waldmann, 1980).

How the macrophage operates in the response to the macrophage-dependent polyclonal stimuli is not well understood, although there are some experiments indicating that they do this by releasing a growth-promoting component. The antibody response to dextran sulfate developed in macrophage-depleted cultures in the presence of macrophage-conditioned medium (Persson *et al.*, 1977, 1978; Smith and Hammerstrom, 1978). A similar degree of reconstitution could be obtained by using 2-mercaptoethanol (2-ME). The macrophage culture fluids consisted of 24-hour culture of unstimulated macrophages. There is yet no biochemical characterization of the active principle. The experiments of Persson suggest that the B cells required two interactions, one with dextran sulfate, the second with the growth-

promoting molecule from the macrophage-conditioned medium; whether this is the case has not been proved yet nor critically examined. Studying the TNP-Ficoll response, Nordin (1978) found a partial reconstitution of macrophage-depleted spleen cells by either 2-ME or macrophage-conditioned medium.

The interaction of the B cells with the polyclonal stimuli associated with the phagocytes has been the subject of recent studies. Boswell *et al.* (1980), found that the antibody response to TNP-Ficoll was macrophage dependent; spleen cells depleted of adherent or latex-ingesting cells (using the fluorescence-activated cell sorter and fluoresceinated latex beads) did not develop an anti-TNP antibody response. The spleen-adherent cells were pulsed with TNP-Ficoll, then washed to eliminate the unbound compound, and added to Sephadex G-10-fractionated spleen cells; the spleen B cells went on to develop an excellent anti-DNP response. The spleen accessory cells had to be live; but unfortunately, no definite information was given on their uptake of the TNP-Ficoll. An interesting observation on Boswell's study was the response of pulsing the nonadherent spleen cells with the antigen; such cells could act as antigen-presenting cells when added to cultures of spleen cells but not when added to cultures of spleen cells depleted of accessory cells. The conclusion was that the B cells in the nonadherent population were binding antigen and eventually passing it to the accessory cells, which were then responsible for the presentation.

Kirkland *et al.* (1980), have now confirmed most of the points made in Boswell's study, including the last one on the possible B cell transfer of antigenic material; they also reported that treatment with anti-IgM antibodies stopped the putative antigen presentation supporting the contention that B cells were responsible, by way of membrane Ig, as the TNP-receptor. It is obvious that the cellular basis of TNP-Ficoll response is of interest and requires further analysis.

Weigle's laboratory, examining the polyclonal response of B cells to Fc fragment of Ig, have recently found a novel pathway of macrophage-lymphocyte cooperation. They found that Fc fragments of human Ig strongly stimulated proliferation of murine B cells (Berman and Weigle, 1977), as well as their differentiation to antibody formation (Morgan *et al.*, 1980). The Fc fragments could be derived from all classes of Ig except IgE. The proliferative response to the Fc fragments was dependent on adherent phagocytic cells (Morgan and Weigle, 1979a), whereas the differentiation required both phagocytes and T cells (Morgan and Weigle, 1980). The evidence so far obtained indicated that a portion of the Fc fragment was cleaved following its interaction with macrophages; this Fc subfragment was the one re-

sponsible for inducing B-cell proliferation (Morgan and Weigle, 1979b). The experiments consisted of culturing briefly Fc fragment with spleen-adherent cells, after which the culture fluid was tested and fractionated on Sephadex chromatography. The active moiety eluted with an apparent molecular weight of 15,000. It could be removed by an immunoabsorbent containing antibodies to the Fc fragment and subsequently eluted and isolated. The isolated subfragment could stimulate B cell proliferation in the absence of phagocytes. Insofar as the differentiation of B cells to antibody-forming cells, the results indicated that, following interaction of the B cells with the Fc subfragment, there was a stimulation to differentiation involving the T cells (Morgan and Weigle, 1980). These experiments of Weigle and associates, therefore, suggest a cellular amplification reaction by immune complexes involving the macrophage and T cells that are somehow brought into the reaction.

The conclusions from the studies reported herewith are that (*a*) macrophages are definitely involved in some response to many of the conventional polyclonal stimuli, but the molecular and cellular events still await further analysis; (*b*) involvement of the macrophage may be by way of secreted active molecules; (*c*) Fc fragments of Ig act as a polyclonal stimulant only after a processing of the fragment by the macrophages.

F. OTHER INTERACTIONS OF THE MACROPHAGE: MIXED LEUKOCYTE REACTION AND ANTI-TUMOR IMMUNITY

The *mixed leukocyte reaction* involves the interaction of T cells (responding cells) with I-region determinants of allogeneic cells (the stimulator cells). Phagocytes are considered to be the major stimulatory cells in the mixed leukocyte reactions in man (Rode and Gordon, 1974), guinea pigs (Greineder and Rosenthal, 1975a), mouse (Talmage and Hemmingsen, 1975; Davidson, 1977; Shirrmacher *et al.*, 1975; Minami *et al.*, 1980), and rat (Oehler and Herberman, 1977).

Greineder and Rosenthal did a detailed study of the cellular nature of the stimulatory cell and found excellent stimulation with highly purified populations of peritoneal and alveolar macrophages as well as spleen cells. This stimulation was blocked by anti-Ia antibodies (Greineder *et al.*, 1976). The depletion of phagocytes from the spleen cell suspension resulted in a loss of stimulation, making them conclude that B cells, although bearing Ia antigens, were poor in stimulating and/or triggering the T cells. There are several reports in the literature claiming stimulation by B cells, but these did not eliminate the phago-

cytes (a review on most papers on this subject can be found in Davidson, 1977).

Two recent studies using murine spleen cells as stimulators confirmed the observations of Greineder and Rosenthal and added new information. Ahmann *et al.* (1979), found that purified T cells did not respond to allogeneic B cells, but only to a phagocyte-rich adherent population of cells expressing Ia determinants encoded in *I-A* and *I-E/C*. The main subregion of *I* stimulating in their combinations was *I-A* but also to lesser extent *I-B* to *I-C*. Minami *et al.* (1980) confirmed the lack of stimulation by B cells and pointed to the macrophage as the major stimulatory cell. The degree of proliferation correlated perfectly with the number of phagocytic cells.

Steinman and Witmer (1978) have reported strong stimulatory activity by "dendritic" cells isolated from the spleen (Section III,A) and very weak activity from peritoneal macrophages. The lack of stimulation by peritoneal macrophages in their experiments is puzzling. In our hands, we found excellent stimulatory activity. Of interest is that the stimulatory activity of the peritoneal macrophage decayed after 24 hours of culture, correlating with the loss of Ia biosynthesis (Section III,A); this loss of Ia was reversed if the macrophages were given a phagocytic stimulus that resulted in stimulation of T cells (Beller and Unanue, 1980b).

The immune response to tumors, like that to protein antigens and microorganisms, is highly complex, involving interactions among various lymphocytes, particularly T cell subsets. Several effector cells participate in the anti-tumor response: natural killer cells, cytolytic T cells, and activated macrophages. Phagocytes may participate in the anti-tumor response by (*a*) presenting tumor antigens, thereby inducing cellular immunity; (*b*) regulating the level of natural cytotoxicity; and (*c*) participating as effector cells. Activated macrophages are found during development of an anti-tumor response *in vivo*. Such macrophage activation resembles that found during antibacterial immunity and is caused by lymphokines released by immune T cells. The effector function of the activated macrophage will not be reviewed.

Overall, the role of macrophages in presenting tumor antigens for the development of effector T cells has not been critically examined. Whether T cells are generated upon direct contact with the tumor or indirectly by tumor antigens shed into the medium and captured by the phagocytes is not known. Several attempts were made, with some success, to generate tumor-immune lymphocytes by incubating them with tumor (Schechter *et al.*, 1976; Kedar *et al.*, 1977), but the cellular

interactions involved in the reactions were not studied; suppressor cells were also generated in some cases (Small and Trainin, 1976). Treves and associates generated effector T lymphocytes by incubating the lymphocytes for 2-4 days with thioglycolate-induced peritoneal macrophages pulsed with tumor extracts (Treves *et al.*, 1976; Treves, 1978). Syngeneic macrophages were more effective than allogeneic macrophages; fibroblasts were not capable. The effector cells were active *in vitro*, by reducing the number of tumor cells synthesizing DNA, most likely reflecting the presence of cytolytic T cells, and also *in vivo*, by conferring protection. The Treves studies indicated that macrophages could present tumor antigens and sensitize lymphocytes, but they did not address the issue of whether this was the way in which normal tumor antigens were presented to T cells. The expectation is that, at least for the generation of the helper T cells that secrete lymphokines, a presentation of tumor antigens by the macrophage may be essential (Section II,D).

I will now review briefly the macrophage in the induction of cytolytic T cells to tumors, as well as to virally infected cells and modified autologous cells. The induction of cytolytic T cells involves a number of regulatory interactions with various subsets of T cells and with the participation of various growth and differentiation factors (reviewed by Wagner *et al.*, 1980); a large series of studies agreed on the requirement for accessory cells (for example, Wagner *et al.*, 1972; Koren and Hodes, 1977; Woodward and Daynes, 1979; Woodward *et al.*, 1979; Yamashita and Hamaoka, 1979; Taniyama and Holden, 1979; Friedman *et al.*, 1979; Pettinelli *et al.*, 1980). The exact role of the macrophages, whether presenting antigens or supplying stimulatory factors, is not known. Some reports indicated that the generation of primary cytolytic T cells to allogeneic cells or tumors took place with both syngeneic or allogeneic spleen adherent cells, but that, in either case, the Ia-bearing macrophages were essential (Pettinelli *et al.*, 1980; Yamashita and Hamaoka, 1979). In experiments reported by Woodward and associates, the T cells obtained from lymph nodes draining the tumor implant developed into effector cytolytic T cells in the presence of Ia-positive accessory cells but without the need for addition of tumor antigens, suggesting that the function of the macrophage was to promote growth and/or differentiation independent of antigen presentation (Woodward and Daynes, 1979; Woodward *et al.*, 1979). One report examining the secondary cytolytic T-cell response to syngeneic tumors required syngeneic macrophages (Taniyama and Holden, 1979). Finally, macrophages activated by cellular immune reactions, such as by infection with bacillus Calmette-Guérin (BCG)

were found to inhibit development of cytolytic T cells by mechanisms not yet known (Klimpel and Henney, 1978; Klimpel *et al.*, 1979).

The phagocytes are important regulators of natural killer (NK) cells. NK cells are believed to be involved in the innate resistance to tumors. Although their exact lineage is in dispute, the NK cells have a series of distinctive properties including surface markers. The relationship between NK cells and macrophages can be gathered from three major observations: (*a*) that the increase of NK cell activity *in vivo* brought about by interferon inducers is impaired by the injection of agents that are toxic for macrophages; (*b*) that, in culture, the increase in NK activity produced by incubation of spleen cells with interferon inducers is ablated if macrophages are depleted; (*c*) that macrophage culture fluids contain a molecule that induces NK activity *in vivo* and *in vitro*. It is likely that this molecule is interferon, a molecule known to be secreted by macrophages and found in the culture fluids that induce NK activity. Some details on these points are now given.

NK activity *in vivo* increased by severalfold by administration of interferon or interferon inducers (Schultz *et al.*, 1977; Gidlund *et al.*, 1978; Djeu *et al.*, 1979a). That the action of the interferon inducers might be by way of the macrophage was suspected by the effects of silica or carrageeinin, agents known to be toxic for phagocytes. These agents administered 4 hours before poly(I : C) prevented the increase in NK activity in the spleen, usually observed after 18 hours (Djeu *et al.*, 1979b). The effects of carrageeinin on spleen NK activity was also reported by Cudcowicz and Hochman (1979).

Since poly(I : C) also increases NK activity on cultured spleen cells, it became possible to test more definitively the involvement of the macrophage (Djeu *et al.*, 1979b). Thus, removal of macrophages from the spleen cells, or treatment of spleen cells with silica or carrageeinin, impaired the generation of NK activity induced by poly(I : C), as well as the production of interferon. Furthermore, a conditioned medium from macrophages treated with poly(I : C)—containing interferon—induced NK activity in spleen cells. Other experiments established that the generation of NK activity by interferon did not require macrophages (Djeu *et al.*, 1979b).

Essentially similar results were obtained by Tracey (1979). Infection with BCG resulted in a pronounced activation of the macrophages and also in an increase in NK cells (Wolfe *et al.*, 1976; Tracey *et al.*, 1977). Injection of silica prior to infection stopped the increase in NK activity. Furthermore, intraperitoneal injection of macrophages from BCG-infected mice—but not from normal mice—or a conditioned medium from such activated macrophages resulted in the generation of NK

activity. The active principle in the macrophage-conditioned medium was not known but was presumed to be interferon. In a recent study, Tracey and Adkinson (1980) found that the BCG-activated macrophages secreted prostaglandins (Section III,C) that could inhibit NK activity. Indomethacin, which stops prostaglandin synthesis, increased the NK-inducing activity of the activated macrophages, both *in vivo* and *in vitro*.

G. INTERACTIONS NOT MEDIATED BY ANTIGENS

Mononuclear phagocytes may be involved in the antigen-independent differentiation of lymphocytes and also of granulocytes. Their role in such processes, exclusively studied in culture, may be related to the production and secretion of growth-promoting and -differentiating molecules, as well as to the presence of surface-bound Ia molecules.

In Section III,C, we mention that macrophages secrete colony-stimulating factor that results in the development of granulocyte/macrophage colonies in culture (reviewed by Kurland and Moore, 1977). Colonies of B cells will develop in soft agar (Metcalf *et al.*, 1975) from B lymphocytes harvested from the spleen. The growth of these B cell colonies in soft agar is stimulated by a feeder layer of macrophages or by molecules secreted by macrophages (Kurland *et al.*, 1976; Kincade *et al.*, 1978), as well as inhibited by macrophage-derived prostaglandins (Kurland *et al.*, 1976). Growth of B cells around macrophages has also been reported in liquid cultures (Garland and Owen, 1978).

A most important function of phagocytes may be in the intrathymic maturation of T cells. The maturation of the T cell in the environment of the thymus gland has been the subject of intense study for many years. How such maturation takes place is not known, but speculations have centered on the role of thymic "hormones" and thymic "epithelial" cells. The thymic gland is made up of thymocytes at various maturational stages and a stroma highly rich in reticular—or epithelial—cells (Raviola, 1975). Among the stromal cells are star-shaped or oval cells, with thin cytoplasmic-dendritic prolongations, particularly in the cortex. These stellate dendritic cells have Ia antigens as detected by immunofluorescence of frozen sections of thymic tissue (Rouse *et al.*, 1979; Janossy *et al.*, 1980). Some of the stromal cells studied by electron microscopy have macrophage characteristics, and a few contain the Birbeck granule, the cytoplasmic marker of the special phagocyte of the skin, the Langerhans cells (Sections II,A and

III,A) (Hoefsmit *et al.*, 1980). Typical macrophages were abundant in the medulla and in the corticomedullary areas.

The cells found by tissue immunofluorescence bearing Ia are indeed related to the phagocytes. Thus, David Beller isolated, from suspensions of murine thymus cells, two distinct types of phagocytic cells: one, represented by 85% of the cells, had typical macrophage morphology, bore Fc receptors, and took up latex particles; the remaining 15% had dendritic appearance, also showed Fc receptors, but were poorly phagocytic (Beller and Unanue, 1980a). Both populations had a high content of cells with surface-bound Ia antigens. The dendritic cells are probably the counterpart of the Langerhans-type phagocytes. Excellent antigen presentation to immune T cells was found by a mixture of these two cells (Beller and Unanue, 1980a). Stromal cells have been isolated from the thymus after delicate handling so as not to grossly disrupt their architecture. Small clusters of very large reticular-type cells with extensive cytoplasmic extensions surrounded by thymocytes could be identified. Those large "nurse cells" contained Ia antigens (Wekerle and Ketelsen, 1980; Wekerle *et al.*, 1980). The nature of the cells in the cluster and its relation to phagocytes is not clear.

An effect of thymic phagocytes in thymocyte differentiation has now been documented in culture. Generally speaking, attempts to reproduce the entire sequence of thymic maturation in culture have not been satisfactory. Some differentiation limited to the expression of thymic surface proteins by immature prothymocytes was found using soluble products obtained from the thymus gland (Komura and Boyse, 1973). However, results using thymic epithelial cultures have, on the whole, not given positive results. Cultures of thymic epithelia have been poorly characterized as to their culture types and frequently are highly contaminated by fibroblasts and macrophages (Loor, 1979; Beller and Unanue, 1978). An early study of Mosier and Pierce (1972) found that thymocytes lost more Thy.1 antigen—an expression of maturation—when cultured on "epithelial" cells or on adherent cells from the spleen. Part of the results were explained by a selective growth of mature thymocytes rather than by a true maturational process. In our studies (Beller and Unanue, 1977, 1978), thymocytes were separated into mature and immature sets by density using sedimentation in gradients of bovine albumin (Konda *et al.*, 1973). Immature thymocytes were characterized by their low content of H-2, the presence of TL antigens, and, functionally, by not responding in the mixed leukocyte reaction or to PHA. Immature thymocytes cultured on a layer of thymic macrophages adhered very avidly to the macrophages

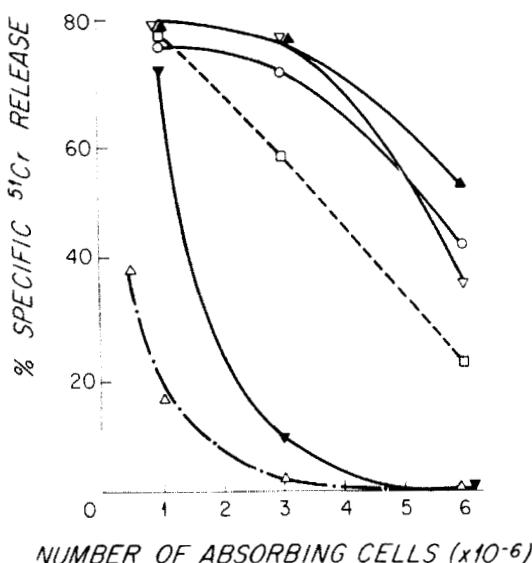


FIG. 5. This figure shows the H-2 maturational changes found in thymocytes cultured on thymic macrophages. Fractions rich in immature thymocytes were cultured without added cells (\circ), on fibroblasts (Δ) or on thymic macrophages (∇) for 48 hours, after which the amounts of H-2 antigen in the cell were measured by their capacity to absorb a standard anti-H-2 serum. The serum was titrated by its capacity to lyse ^{51}Cr -labeled cells. The immature thymocytes showed helper content of H-2D after their culture on macrophages. The content of H-2D on mature thymocytes (Δ) and on mixtures of mature and immature ones (\square) is shown for comparison. [From a study by Beller and Unanue (1978).]

(Section III,B) and dramatically changed within a period of 48–72 hours. Maturation was evidenced by changes in the amounts of surface H-2, which increased severalfold to the level shown in mature thymocytes, as measured by quantitative absorption, by a loss of sensitivity to killing by anti-TL antibodies, and by a capacity to respond in the mixed leukocyte reaction (Fig. 5). The increase in H-2 antigens was found in thymocytes treated with mitomycin C and therefore not synthesizing DNA, hence ruling out the possibility that the previous results were accounted by a selective growth of a few mature cells contaminating the fractionated population. The maturational changes seen by macrophages could be reproduced by a conditioned medium containing a 40,000 MW material without interferon activity (Beller and Unanue, 1977) and also quite distinct from the 15,000 MW thymocyte mitogen secreted by macrophages (Section III,C). Curiously, thymic maturation was not accomplished by cultures of purified thymic “epithelial” cells devoid of macrophages (Beller and Unanue,

1978). Changes in reactivity of guinea pig thymocytes to Con A were reported after incubation with macrophages (Van den Tweel and Walker, 1977), but the nature of the change, either selection of cells or maturation, was not studied.

In summary, the thymus gland contains a large number of cells with Ia antigens, some of which clearly belong to the phagocyte series. The reasons for the high concentration of Ia-bearing cells, their nature, and their role are not known and require evaluation. Ia-bearing stromal cells are found in the thymus from early fetal life (Jenkinson *et al.*, 1980); Ia-bearing macrophages were reported from birth (Lu *et al.*, 1980). If Ia antigens are involved in antigen-independent maturational events, then it is likely that these cells may well be the substrate for such events (Section III,B). Clearly, part of the differentiative process of immature thymocytes can be reproduced in culture using phagocytes or a differentiation factor derived from them. The extent to which the T cells are functional after maturation on thymic macrophages has not been fully examined. This is a point of particular importance, particularly as it concerns the MHC involvement.

H. MACROPHAGES IN SUPPRESSIVE PHENOMENA

There are three basic situations to analyze where macrophages are involved in suppressive-type effects: (*a*) in the generation of suppressor T cells; (*b*) in a direct suppressive effect on lymphocytes in culture; and (*c*) as a mediator or carrier of T-cell suppressor factors.

While presentation of antigen by way of the macrophages is the essential way to elicit helper T cells, the interaction of antigen with T cells in the absence of macrophages resulted in generation of suppressor T cells (Ishizaka and Adachi, 1976; Feldman and Kontiainen, 1976; Pierres and Germain, 1978). This is an important point, analyzed in Section II,C, which, at face value, implies that the induction of T suppressors may take place by direct interaction with antigen without any other cellular mediation. Sherr *et al.* (1980) reported that suppressor T cells to fowl γ -globulin were induced *in vivo* by transfer of Ia-positive adherent spleen cells containing the antigen, a palmitoyl derivative of fowl γ -globulin. Mice that received as little as 0.1 μ g did not respond upon immunization with TNP-fowl γ -globulin (plus adjuvant); and moreover, their spleen cells injected into normal mice suppressed the specific response. The responsible cell was adherent and bore I-A determinants, not I-J determinants. Interestingly, immunization with the antigen coupled to any cell type, including red cells, but at a 100-fold higher dose, induced an unresponsive state without any detectable suppressors. This is an interesting study worth pursuing. How the Ia-positive macrophages with the antigen enter into the suppressor pathway requires more analysis. Sherr speculated

that perhaps Ia-positive macrophages may induce helpers, which, in turn, regulate the generation of suppressor cells such as those postulated in the studies of Eardley *et al.* (1978).

Macrophages in cultures, as was indicated in the preceding sections, presented antigen but inhibited lymphocyte functions, particularly at high numbers or if activated by cellular immune reactions. The inhibitory effect of macrophages was explained to a great extent by their release of prostaglandins, oxygen intermediates, and perhaps other factors (discussed in Section III,C). Interestingly, the release of the inhibitors was increased by situations that stimulated the macrophage, such as phagocytosis of the antigen and interaction with T cells, or its products, or both. A whole loop of interactions could be followed starting with a T cell induced by antigen presented in macrophages to secrete lymphokines; these, in turn, activated macrophages, which then released prostaglandins; the prostaglandins inhibited proliferative as well as T-cell secretion of mediators. It would appear that the system regulates itself by these multiple interactions.

Finally, macrophages may regulate the action of suppressor factors. One of the best-analyzed systems was that worked by Pierce and associates: suppressor T cells (Ly2 bearing) secreted a protein that, upon oxidation by a small metabolite from the macrophage, converted into a highly active inhibitor (Section III,C). In a different system, it was shown that suppressor factors released by T cells would bind to macrophages, resulting in the inhibition of the transfer of contact sensitivity (Asherson and Zembala, 1974; Zembala and Asherson, 1974; Ptak *et al.*, 1977, 1978a,b; Kojima *et al.*, 1979). As worked out by Ptak *et al.* (1978a), cells immune to trinitrobenzene sulfonic acid transferred the contact-sensitivity state if injected into normal mice. A brief 1-hour incubation of these cells with a suppressor factor (induced by incubation of tolerant T cells with the antigen) abolished their capacity to transfer the reaction. The point is that the suppressive factor required the macrophages (Kojima *et al.*, 1979) that would bind and remove the activity (Ptak *et al.*, 1978a). The macrophages with the bound factor inhibited the immune T cells. Ptak *et al.* (1978b) have brought out preliminary evidence that the macrophage might cause the suppression by releasing a nonspecific inhibitor. As envisioned by Asherson, Ptak, and Gershon, the macrophage would act, therefore, as a conduit of suppressor signals.

III. Basis for Macrophage Regulation

The role of macrophages in immune induction can be explained by two distinct, yet interrelated, functions: handling and presentation of antigen to lymphocytes, and secretion of lymphostimulatory mole-

cules. Key molecules in the antigen-handling process are the *I*-region products. Section III,A considers some general properties of phagocytes. It also reviews other accessory antigen-presenting cells. Section III,B reviews antigen handling, and Section III,C reviews the secretory function of macrophages.

A. PROPERTIES OF MACROPHAGES AND OTHER ACCESSORY CELLS

The classical mononuclear phagocytes are responsible for the interactions described in the preceding section. In most studies, these cells were isolated from the peritoneal cavity. Cultured bone marrow, liver, lung, and spleen macrophages have been used. In this section, we first review some general characteristics of the phagocytes and then consider the expression and synthesis of the Ia proteins.

1. The Mononuclear Phagocyte and the Ia Antigens

Mononuclear phagocytes develop from self-renewing stem cells found in bone marrow and, to lesser extent, in lymphoid organs. The cell line is represented by cells in various stages of differentiation, from the early monocyte, to the mature, well-differentiated macrophage found in the various tissues. Maturation in the line is characterized by a progressive development of lysosomes, increase in Fc and C3 receptors, and in various functions such as phagocytosis, pinocytosis, and adhesiveness to culture dishes. Mononuclear phagocytes are affected by the local environment and by systemic stimuli such as bacterial products and lymphocyte mediators. These local and systemic factors contribute to the functional heterogeneity of phagocytes harvested from various tissues, under different conditions.

An issue still unresolved is the migration of phagocytes into tissues and the factors that control it in steady-state and in pathological conditions. There is little doubt that the bone marrow contains a large number of cells that give rise to mature phagocytes (Volkman and Gowans, 1965a,b; Cline and Moore, 1972; Cline and Summer, 1972; van Furth and Cohn, 1968; van Furth and Diesselhoff-den Dulk, 1970). Thus, destruction of dividing cells by systemic irradiation resulted in a loss of inflammatory exudates, but these exudates developed if the irradiated animal was infused with bone marrow cells (Volkman and Gowans, 1968a,b; Volkman, 1970). [Also, macrophages were found to develop in cultures of bone marrow cells grown with appropriate growth factors (reviewed by Stewart, 1980).] While kinetic analyses supported the sequence of bone marrow precursor → monocytes → inflamed tissue (van Furth *et al.*, 1973; Volkman and Collins, 1974), not yet clear is the extent to which tissues renew their phagocyte population in the normal steady state; that is to say, whether the mac-

rophages in tissues—all or part of them—derive from bone marrow via blood monocytes or from local cells. Most *in vivo* studies using radiolabeled thymidine indicated a small (2–5%) but still significant proportion of dividing cells in exudates (Volkman and Gowans, 1968a; van Furth and Cohn, 1968; Volkman and Collins, 1968). Furthermore, one study failed to trace the flow of monocytes into peritoneal exudates in *normal* conditions (Volkman, 1976), implying that in steady-state conditions macrophages were renewed by local precursors. The issue of self-renewal in tissue and the control of monocyte-macrophage populations is important not only because it may relate to the response to inflammation, but it may be crucial as it pertains to the immunogenic function of macrophages.

As is evident from the previous sections, phagocytes are essential cells, mediating the interactions of lymphocytes with antigen. Within the phagocyte population, however, there is a definite heterogeneity in function. One major reason for this heterogeneity is the difference in the expression of Ia within the macrophage population. As was analyzed in the preceding section, it is the presence of the Ia proteins that gives the phagocyte the capacity to interact with T lymphocytes. Early studies identified Ia antigens in some, but not all, macrophages using immunofluorescence (Unanue *et al.*, 1974) or cytotoxicity (Hammerling *et al.*, 1975). There is now general consensus following more extensive analysis that the mononuclear phagocytes in all tissues can be grouped into two sets, one bearing and the other lacking Ia molecules (for example, R. H. Schwartz *et al.*, 1976; Yamashita and Shevach, 1977; Dorf and Unanue, 1978; Cowing *et al.*, 1978a,b; Lee *et al.*, 1979; Beller *et al.*, 1980). The methods for detecting Ia in macrophages included immunofluorescence or cytotoxicity using anti-Ia antibodies. Immunofluorescence studies required careful consideration of the nonspecific binding by the antibody to the macrophage Fc receptor. Nevertheless, the nonspecific binding was totally minimized using strong alloantibodies (Cowing *et al.*, 1978a) and, particularly, monoclonal anti-Ia antibodies (Beller *et al.*, 1980), both of which gave excellent and very specific localization to the surface Ia. Figure 6 is an example from our studies using the monoclonal anti-I-A^k raised in the Herzenberg's laboratory (Oi *et al.*, 1978). Ia-bearing macrophages were also identified using cytotoxicity, in particular with strong cytolytic serum as the source of the complement (Hammerling *et al.*, 1975; Cowing *et al.*, 1978; Dorf and Unanue, 1978). Macrophages bearing Ia antigens were also recognized using fluorescent antibodies in the fluorescence-activated cell sorter (R. H. Schwartz *et al.*, 1976; Dickler *et al.*, 1980).

The presence of Ia in only a fraction of macrophages has raised several questions, not all of which are currently answered. What is the

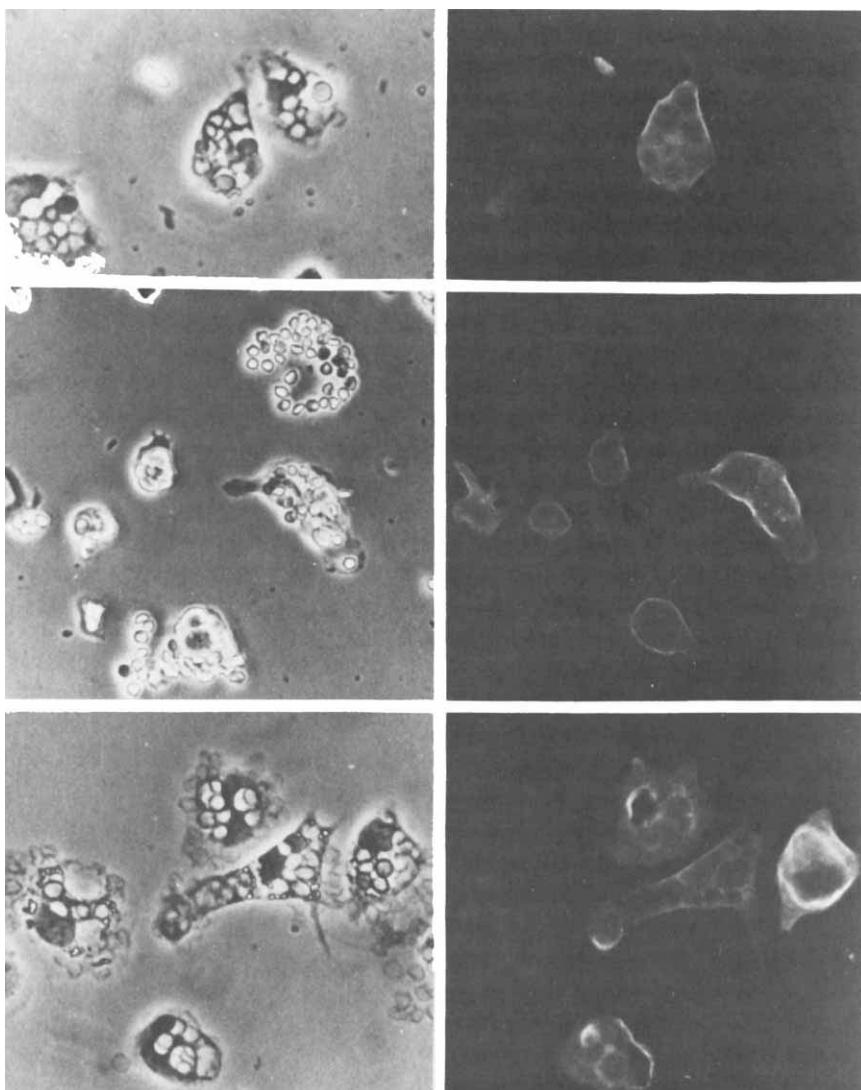


FIG. 6. Immunofluorescence of macrophages using anti-I-A antibodies. In the upper and lower panels, peritoneal macrophages were incubated at 37°C with red cells opsonized with IgG antibodies, then fixed and stained with anti-I-A. Both Ia-positive and Ia-negative macrophages phagocytized the opsonized red cells. In the middle panel, the macrophages were incubated at room temperature. Binding of red cells took place, without phagocytosis, to both Ia-positive and Ia-negative macrophages. [From Beller *et al.* (1980).]

relationship between Ia-positive and Ia-negative macrophages? Are the two cells related? Do the two cells have distinct functions? Are there regulatory mechanisms controlling the percentage of Ia-positive and Ia-negative in various tissues?

Cytological examinations disclosed that both macrophages, i.e., for ease of terminology, Ia-positive and Ia-negative, had about the same general properties. Both sets of macrophages were morphologically identical, adhered to plastic surfaces, and took up particles (Yamashita and Shevach, 1977; Cowing *et al.*, 1978a; Beller *et al.*, 1980). Both had Fc and C3 receptors, although most Ia-positive showed less when quantitated using appropriately opsonized red cells (Beller *et al.*, 1980).

a. *Tissue Distribution.* The percentage of Ia-positive and Ia-negative macrophages varied among the different tissues and exudates of mice; but within a given tissue or exudate, the distribution was highly consistent (Table V). It is important, however, in studying distribution of Ia-positive macrophages that consideration be given to the fact that environmental stimulation changed the percentage (see below). This may account for the occasional reference to high numbers of Ia-positive macrophages, for example, in the peritoneum (Unanue *et al.*, 1974; Hammerling *et al.*, 1975).

A brief comment on the distribution of macrophages in the various tissues follows. The *peritoneal cavity* was frequently examined because of the ease for sampling and obtaining a pure population of phagocytes. Cultured peritoneal macrophages were practically all classical phagocytes, synthesized Ia (see below), and, as noted in Section II, were highly active in antigen-presenting function. The peritoneal macrophages were harvested from normal animals or from animals injected with mineral oil, peptone, or thioglycolate broth; the former was used mainly for studies in the guinea pig, and the last two were used particularly in the mouse (Table V). The percentage of Ia-bearing macrophages in the inflammatory exudates was about the same as in the resident population (Yamashita and Shevach, 1977; Beller *et al.*, 1980). Overall results using T cell proliferation as the readout to measure macrophage-T cell interactions showed comparable antigen-presenting function by the various exudates. In general, however, the more activated the macrophage, the more critical was the number in culture because of their inhibitory effects. Thus, in the mouse, resident macrophages (i.e., from noninduced peritoneal exudates) or macrophages from peptone, thioglycolate, or oil exudates had good antigen-presenting function, but the thioglycolate-induced macrophages were more inhibitory. Other culture assays that explored at the same time the antigen-presenting role and a second macrophage function, such as secretion or activation for tumoricidal activity, did

TABLE V
TISSUE DISTRIBUTION AND FUNCTION OF Ia-POSITIVE MACROPHAGES^a

Source	Stimuli	Ia-positive (%)	Antigen-induced T cell proliferation	Secretion of LAF ^b	Activation for cytotoxicity
Peritoneum	None (resident)	10-20	++	+++	1±
	Peptone	10	++	++	++
	Thioglycolate	5-10	+	-	++
	Oil	5-10	++	NS	
	<i>Listeria</i> infection	50-75	++	++	++++
Spleen	None	50	++	++	NS
	<i>Listeria</i> infection	>75	NS	NS	NS
Skin	None	90	++	NS	NS
	Contact reactions	> In absolute numbers	NS	NS	NS
Liver	None	25-30	++	NS	NS
Thymus	None	75	++	++	NS
Lung	None	10	++	++	++
		80 (guinea pig)	++	NS	NS
	L-Conditioned medium	30	++	++	++
Bone marrow	None	75 (man)	++	NS	NS

^a References are given in text. All studies were done in the mouse except as indicated.

^b LAF, lymphocyte activity factor; NS, not significant.

show considerable variation depending on the type of exudate macrophage. The secretion of lymphostimulatory molecules resulting from macrophage-T cell interactions (Section III,C) took place best with resident macrophages, to a lesser extent with peptone-induced macrophages, and very poorly with thioglycolate-induced macrophages; in contrast, the inflammatory macrophages in the same culture developed tumor cytotoxicity, while the resident macrophages were practically inactive (Farr *et al.*, 1979a,b; Unanue *et al.*, 1980).

Whether or not there is heterogeneity within the Ia⁺ population insofar as antigen-presenting function is an important point that has not been critically addressed. A study by Lee *et al.* (1979), suggested that this might be the case. They separated macrophages on the basis of size, using velocity sedimentation in albumin gradients, and found some fractions that were better than others, despite the presence of Ia-bearing cells in all. Detailed cytological studies were not reported, nor was the issue of nonspecific suppression analyzed. Thus, the reasons for this heterogeneity remained unexplained.

The spleen Ia-bearing macrophages were first studied by Cowing and associates (1978a,b). They isolated a population of adherent phagocytic cells capable of taking up latex beads and with a high representation of Ia using immunofluorescence or cytotoxicity. The figures in Cowing's studies amounted to about 50% of the phagocytes. Figures about the same range were found in other studies (Beller *et al.*, 1980; Lu *et al.*, 1980). The spleen macrophages synthesized Ia in culture and expressed determinants both in I-A and in I-E/C region (Cowing *et al.*, 1978a). Dickler *et al.* (1980), isolated the population of Ia-bearing phagocytes using the fluorescence-activated cell sorter and found excellent antigen-presenting function. Other accessory cells found in spleen besides the mononuclear phagocyte include the interdigitating cells, the Langerhans cells, and the dendritic-type isolated by Steinman and Cohn (1973). These will be discussed in Section III,A,2.

The skin Ia-bearing Langerhans cell was discussed in Section II,D and will also be considered in the next section.

The liver Kuppfer cells were isolated and tested for percentage of Ia and antigen-presenting function (Richman *et al.*, 1979, 1980; Forsum *et al.*, 1979a; Lipsky and Rogoff, 1980; Rogoff and Lipsky, 1979, 1980). Berzofsky's laboratory isolated the Kuppfer cells from the mouse by treatment of liver slices with collagenase (Richman *et al.*, 1979). The cells were typical phagocytes, 50% of which bore Ia. The Ia-positive cells contained molecules coded in the I-A and I-E/C subregions. Isolated Kuppfer cells were essential for the proliferative response of purified T cells to sperm whale myoglobin (Richman *et al.*, 1969). Lipsky's laboratory isolated the Kuppfer cells from guinea pig liver

using collagenase and trypsin (Rogoff and Lipsky, 1980; Lipsky and Rogoff, 1979, 1980). They found that about 27% bore Ia. These liver macrophages were essential for the proliferative response to lectins and antigens and showed typical MHC restrictions, but were less active when compared to peritoneal macrophages. Both groups commented on the biological significance of an antigen-presenting function by the Kupffer cell. Clearly, the Kupffer cells have the potential to interact with lymphocytes, but evidence *in vivo* suggests that antigens that flow through the liver lose their immune potential. It is not clear how both phenomena can be related.

There are two current studies on Ia in *alveolar macrophages*. Our laboratory found about 10% Ia-positive cells in the macrophages from nonstimulated murine lungs (Weinberg, 1980; Weinberg and Unanue, 1981), while Uhr's laboratory, working in the guinea pig, found about 80% (Toews and Lipscomb, 1980; Lipscomb *et al.*, 1981). Alveolar Ia-positive macrophages presented antigens and were as active as peritoneal macrophages. Curiously, in our own studies, we found that alveolar macrophages lacked the capacity to bind *Listeria monocytogenes* and, therefore, presented poorly these bacteria to immune T cells in functional assays. Murine alveolar macrophages also lacked C3 receptor (Alblas and van Furth, 1979). Both C3 receptor and the structures that bind to *Listeria* are trypsin sensitive, and it may be that the normal proteases of the lung fluid are affecting the macrophage surface and modifying its function. Opsonization of *Listeria* allowed for its binding to the trypsin-resistant Fc receptor of the macrophage with the development of antigen-presenting function (Weinberg, 1980; Weinberg and Unanue, 1981).

Thymic macrophages were found to contain a very high percentage of Ia-positive cells (Beller and Unanue, 1978, 1980; Lu *et al.*, 1980) (Section II,G).

Monocytes were used as antigen-presenting cells mostly in human studies. As referred to in Section II, their antigen-presenting function resided in the Ia-bearing population. The percentage of monocytes bearing Ia varied from 50 to 75 (Albrechtsen, 1977). A recent study of Raff and associates (1980) employed a monoclonal antibody that discriminated two populations of glass-adherent monocytes, each containing Ia determinants; of great interest is that clearly functional dichotomy was found after testing the two populations. Elimination by cytolysis, with complement, of macrophages bearing the 120,000-dalton determinant (termed Mac-120 antigen) markedly diminished the antigen-induced proliferation but not the capacity to induce an alloreactive mixed leukocyte reaction. The Mac-120-positive cells were highly active in stimulating basal proliferation of T cells. Cytological and biological examination of both subsets have not yet

been reported, except with regard to their similar uptake of latex particles. Mac-120 was found in 40% of the monocytes.

Macrophages have been cultured from bone marrow in the presence of medium containing growth-promoting molecules. The cells isolated from the marrow were pure macrophages bearing Fc and C3 receptors and having phagocytic properties. Bone marrow-grown macrophages were tested in various antigen-presenting systems and found to be highly effective (Stern *et al.*, 1979; Lee and Wong, 1980; Erb *et al.*, 1980b; Mottram and Miller, 1980; Calamai and Unanue, 1980). These included induction of helper T cells (Stern *et al.*, 1979); presentation of tuberculin, hemocyanin, and flagellin for T-cell proliferation (Lee and Wong, 1980); presentation of *Listeria monocytogenes* assaying T-cell proliferation, macrophage secretion of lymphostimulatory molecules, and macrophage activation for cytoidal activity (Calamai and Unanue, 1980); and macrophage induction of delayed sensitivity *in vivo* (Mottram and Miller, 1980). The antigen-presenting function has been definitely ascribed to the Ia-bearing macrophage population—about 30%—isolated by cell-sorting experiments (Erb *et al.*, 1980b,c).

b. Regulation of Ia in Macrophages and of Macrophage Ia Population. Three recent important observations indicate that the biosynthesis of Ia by macrophages takes place during a brief period of time, that the activity or stimulation of the macrophage may regulate the expression of membrane Ia, and that the relative percentage of Ia-positive and Ia-negative macrophages in tissues is under regulation. These are results that are mostly coming from studies with David I. Beller and other members of our laboratory (Beller *et al.*, 1980; M. G. Scher *et al.*, 1980; Beller and Unanue, 1980, 1981).

The time that Ia-bearing macrophages expressed Ia was brief and limited, under basal conditions, to a few hours. Fluorescent antibody studies of peritoneal macrophages in culture indicated progressive loss of Ia so that, by 24–72 hours, most of the Ia-bearing cells were no longer detectable (Beller and Unanue, 1980a). Biosynthetic studies indicated strong synthesis of Ia by the macrophages during the first day but then a progressive loss with time. This loss of Ia surface expression and biosynthesis could not be accounted for by loss of macrophages from the culture dish. This progressive loss of the capacity to synthesize Ia explains the early results of B. D. Schwartz *et al.* (1976), who reported a negligible amount of Ia made by cultured macrophages. In their experiments, biosynthesis was studied only after 3 days of culture. Also noteworthy are the studies of Cowing *et al.* (1978b), who found a loss of Ia-positive spleen macrophages after 1 week of culture but could not differentiate between selective loss of cells and lack of expression.

The loss of Ia biosynthesis in Beller's experiments was selective.

Total protein synthesis was not impaired at the time that Ia was not being synthesized. Furthermore, a different membrane protein, the H-2K protein, was synthesized continuously in culture (Fig. 7).

In vivo studies disclosed a loss of Ia by the macrophages. These experiments were done by examining X-irradiated mice (Beller and Unanue, 1981). Such mice showed a marked loss of Ia-bearing macrophages time after irradiation with kinetics very similar to that found in culture. To ascertain truly that the loss of Ia represented selective loss by the macrophage—not the disappearance of a subset of macrophages—cell transfers were done. C57BL/6 mice were irradiated, then transplanted intraperitoneally with macrophages from (C57BL/6 × A)F₁ mice; at times thereafter, the peritoneal cavity was examined, and the transplanted macrophages were identified immunocytochemically by examining for surface-bound H-2K^k and I-A^k molecules (corresponding to the haplotype of the A strain). Indeed, the transplanted macrophages could be easily identified by the presence of H-2K, yet the I-A^k molecule had completely disappeared with time after examination. Because the percentage of Ia-positive macrophages of the donor macrophages was very high, the results strongly indicated a selective loss of this protein from the recovered macrophages. The implication of this finding as regards the effects of X-irradiation in immunity is discussed below.

When examining the loss of Ia in culture, the question was asked whether the biosynthesis of Ia was affected by the stimulation of the macrophage (Beller and Unanue, 1980b). Although nonstimulated macrophages lost Ia during the first 48 hours of culture, the uptake of latex beads, dead *Listeria monocytogenes* organisms, opsonized red cells, or soluble antigen-antibody complexes resulted in continuous biosynthesis. After phagocytosis, there was progressive expression of Ia to the surface of the macrophage as well as incorporation of labeled amino acids into the newly synthesized Ia protein. Interestingly, the biosynthesis of Ia stimulated by particle uptake was taking place only in the set of macrophages that originally expressed surface Ia. Elimination of such Ia-positive macrophages by antibody (with complement) resulted in no expression of Ia after particle phagocytosis by the remaining Ia-negative macrophages.

A different series of observations indicated an *in vivo* control of the Ia-positive and Ia-negative macrophage populations. Beller *et al.* studied the peritoneal exudate for possible changes in Ia-positive macrophages under different inflammatory conditions (Beller *et al.*, 1980). Mice were inoculated with a number of inflammatory molecules—mineral oil, peptone, thioglycolate broth, endotoxin—and were also infected with *Listeria monocytogenes* organisms. While the first group of materials produced an increase in macrophages, this increase con-

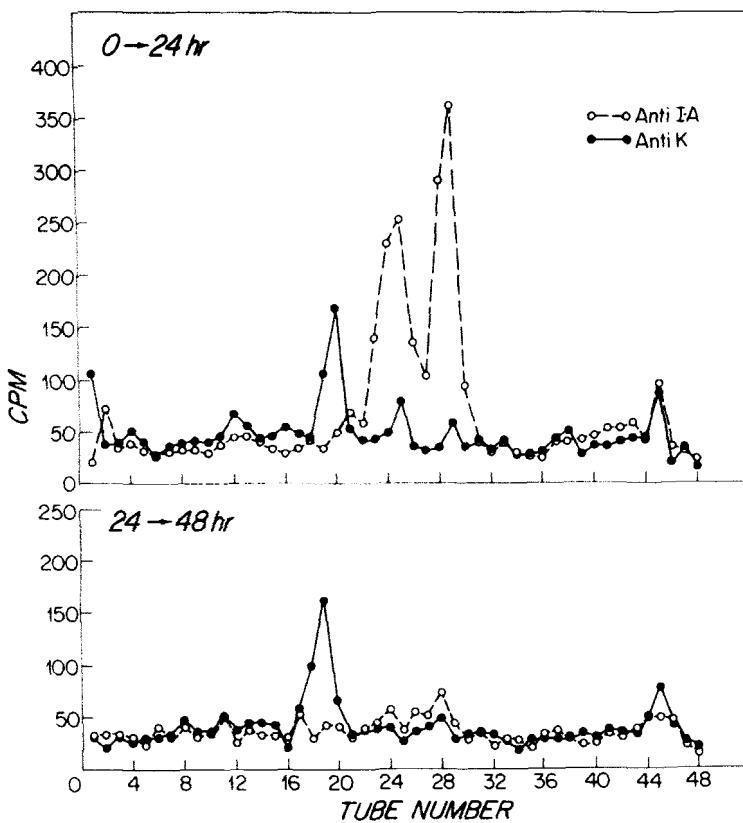


FIG. 7. Macrophages were cultured from zero to 24 hours or from 24 to 48 hours after planting with [³H]leucine, after which the cell-bound I-A or -K products were isolated and precipitated with specific monoclonal antibodies and examined by polyacrylamide gel electrophoresis. During the first 24 hours, the macrophage synthesizes both K and I-A proteins; after 24 hours, the synthesis of I-A is markedly reduced. (From Beller and Unanue, 1981.)

sisted predominantly of Ia-negative macrophages. The absolute number of Ia-negative macrophages was actually increased by about 10-fold 3 days after the intraperitoneal injection. In contrast, infection with live *Listeria* resulted in a complete shift in the macrophage population to 50–90% Ia-positive macrophages. Similar results were obtained after infection with *Mycobacterium tuberculosis* (Beller *et al.*, 1980) or with *Trypanosoma cruzi* (Behbehani *et al.*, 1981). The marked increase in Ia-positive macrophages following infection resulted from T cell stimulation. An early indication that this was the case came from analysis of immune mice. Mice immune to hemocyanin or *Listeria* were challenged intraperitoneally with either hemocyanin or dead *Listeria* organisms, and the percentage of Ia-

positive macrophages was studied days later. Either of the two antigens did not induce a noticeable change in the distribution of Ia-positive macrophages in unimmunized mice. However, a *secondary* boost resulted in a marked increase in Ia-positive macrophages, but only following specific challenge, i.e., hemocyanin-primed mice did not respond to dead *Listeria* but only to hemocyanin. Further analysis disclosed that T cells transferred the capacity to develop an exudate rich in Ia-positive macrophages. T cells were harvested from immune mice and transplanted intraperitoneally together with the specific antigen: 3 days later, there was a marked increase in Ia-positive macrophages in the peritoneal exudate, but not in the spleen.

T cells regulated the appearance of Ia-positive macrophages by way of a soluble mediator (Scher *et al.*, 1980). Peritoneal exudate cells from *Listeria*-immune mice composed of macrophages and T cells were cultured with dead *Listeria* organisms; the conditioned medium from such cultures, when injected intraperitoneally, induced the appearance of Ia-positive macrophages. The production of the mediator—termed macrophage Ia-recruiting factor or by the acronym MIRF—was dependent on the interaction in culture of Ia-positive macrophages and T cells, together with the specific antigen. While the production of MIRF, like all mediators, required the involvement of the *I* region, its action was unrestricted by the *H-2* (Fig. 8). Preliminary studies indicate that MIRF is a nondialyzable protein, stable to 56°C and to repeated freeze-thawing (Scher *et al.*, 1980).

How are the T cells—or MIRF—regulating the appearance of Ia-positive macrophages? We have now found, in studies not yet published, that the action of T cells in recruiting Ia-positive macrophages is radiosensitive. The experiments in X-irradiated mice are informative inasmuch as they show one possible mode of action of the various phlogogenic stimuli in regulating macrophage populations. Normal and irradiated mice were injected with T cells and antigen, or mineral oil, and the peritoneal exudates were examined a few days later. The X-irradiated mice, in contrast to the normal mice, did not develop either exudates rich in Ia-positive macrophages in response to T cells, or exudates rich in Ia-negative macrophages, in response to the mineral oil. Such exudates were found if the X-irradiated mouse was reconstituted with bone marrow stem cells. The experiments indicated that the two responses, to immune or nonimmune stimuli, operated on the bone marrow precursors, but not at the level of the mature tissue macrophages (Scher *et al.*, 1981).

More recent studies have tested the response in culture of Ia-negative macrophages to lymphocyte mediators (Steinman *et al.*, 1980; Steeg *et al.*, 1980). After several days of incubation with the lymphokines, a high number of the Ia-negative macrophages went on to

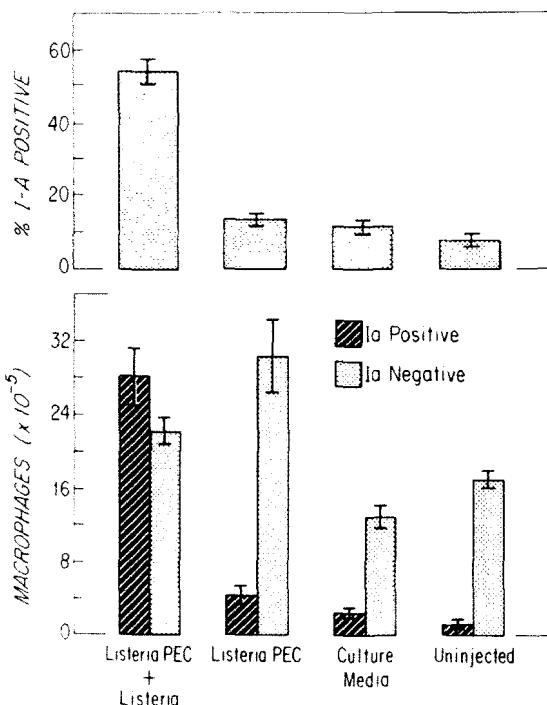


FIG. 8. Recruitment of Ia-positive macrophages by a soluble mediator. Mice were injected intraperitoneally with (a) a culture fluid from mixtures of macrophages and *Listeria*-immune T cells with heat-killed *Listeria monocytogenes* organisms (*Listeria* PEC + *Listeria*; (b) a culture fluid from the same cells but not challenged with *Listeria* (*Listeria* PEC); and (c) regular medium. The percentage of Ia-positive macrophages and the absolute numbers of Ia-positive and Ia-negative in the peritoneum were investigated 12 hours later. The active factor that recruits Ia-positive macrophages is secreted *in vitro* only by challenge of immune T cells with macrophage-bound antigen. PEC, peritoneal exudate cell. [From Scher *et al.* (1980).]

synthesize and express Ia, indicating that most macrophages have the potential to make these modulatory proteins. In agreement with the *in vivo* studies of Scher *et al.* (1981), the expression of Ia by the cultured phagocytes depended highly on their state of maturation. Most monocytes and exudate macrophages readily expressed Ia but only a small minority of the resident macrophages (Beller and Unanue, unpublished observations).

c. *Ontogeny.* The control of the Ia-positive and Ia-negative macrophage subgroups is evident in studies on their ontogeny in the mouse. Up to the second week to the fourth week of life, the macrophage population is made up mostly of Ia-negative cells (Lu *et al.*, 1979; Nadler *et al.*, 1980). Lu's studies examined the peritoneal and spleen macrophages for their uptake and presentation of *Listeria*

monocytogenes. The macrophages took up the bacteria but were unable to stimulate proliferation of immune T cells from an adult. The macrophages from the neonate were phagocytic, could be activated by lymphokines, and secreted lymphostimulatory molecules when stimulated with endotoxin. Their lack of antigen-presenting function was best attributable, as would be expected, to their low content of Ia-positive macrophages. No suppressive effects were noted when the macrophages from the neonate were mixed with adult spleen cells. In Nadler's study, the immunoincompetence of the spleen accessory cells was studied in the primary antibody response to haptenated hemocyanin, and, as in Lu's experiments, it correlated with the absence of Ia-positive cells. These results may explain previous studies indicating a deficient function of adherent cells from neonates in the primary antibody response to SRBC (Landahl, 1976; Nakano *et al.*, 1978); to be noted is that an initial study by Fidler *et al.* (1972) had failed to see a defect in the adherent cells. It is of interest that Argyris in 1968 reported that injection of macrophages from adult mice into newborns resulted in the development of an antibody response to SRBC. This observation was confirmed (Hardy *et al.*, 1973; Blaese and Lawrence, 1977).

Another interesting point concerning the ontogeny of Ia-positive cells is their appearance in the thymus. In the thymus, Ia-positive macrophages with effective antigen-presenting function were found since birth, in contrast to their absence in the spleen (Lu *et al.*, 1980). In a different study, Jenkinson *et al.* (1980) reported finding thymic stromal cells bearing Ia antigens since day 14 of gestation. We have speculated that the Ia-positive accessory cells found in the neonate since very early life function not to present antigen, but rather to modulate the antigen-independent differentiation of T cells (Lu *et al.*, 1980) (Section II,G).

Recent studies may explain the defect in Ia expression by the macrophages of the neonate (Snyder *et al.*, 1981). Neonates injected intraperitoneally with MIRF did not respond with exudates rich in Ia-positive macrophages. Furthermore, a cell was identified in the neonatal spleen that, when injected into adults, interfered with the development of exudates rich in Ia-positive macrophages induced by T cells. This "suppressor" cell did not bear surface markers of T or B cells, was actively proliferating, and poorly adherent. That the macrophage from the neonate has the potential to express Ia was evident in the cell culture systems where Ia could be induced in them by a T cell-conditioned medium. Thus, it appears that the lack of Ia expression in the neonate is caused by an active suppression induced by dividing hematopoietic cell and not be an intrinsic defect of the phagocyte.

The lack of Ia-positive macrophages in the secondary lymphoid tissues and in exudates appears, at face value, to place the neonate at a disadvantage during a critical time of life. It may be, however, that this lack of Ia-positive macrophages is what regulates the lack of response to self antigens during this important developmental stage, and it ensures the establishment of self-tolerance.

d. Summary of Ia Macrophage Subsets. The more recent data indicate that macrophages synthesize Ia molecules for a brief period of time, after which they lose the capacity to express this important regulatory molecule. The loss of Ia by tissue macrophages implies that a certain amount of the Ia-negative macrophages were cells that initially contained Ia. However, it is not resolved whether *all* Ia-negative macrophages derive from Ia-positive cells. Indeed, the Ia-negative macrophages from fresh exudates induced by nonimmune stimuli were young bone marrow-derived cells that did not contain Ia from the time that they left the marrow; whether these cells contained Ia during their intramarrow life is not known. It may well be that the phagocyte is a single cell lineage in which Ia is expressed at only a certain stage, which can be regulated by external stimuli. It may also be that the line splits at some point, one branch never expressing Ia, while the other is capable of making the protein at least during a period of time. The recent experiments cited before, in which a majority of monocytes and early macrophages express Ia following culture with lymphocyte mediators, support the former statement.

The fact that Ia is synthesized briefly establishes that the maintenance of Ia-positive macrophages in tissues requires a continuous supply of new cells from their precursors. Indeed, the X-irradiated mouse showed, together with the loss of stem cells, the loss of Ia from its radioresistant tissue macrophages (Beller and Unanue, 1980b). Although macrophages harvested from irradiated mice presented antigen (Schmidtke and Dixon, 1972a), one would predict that macrophages harvested from mice several days after irradiation would function poorly as antigen-presenting cells in an MHC-restricted system. Indeed, macrophages from X-irradiated mice presented antigen poorly in an early experiment done by Galilly and Feldman (1967), perhaps explained by the loss of Ia.

In essence, the macrophage Ia phenotype is regulated by (*a*) the activity of the macrophage; (*b*) immune and nonimmune stimuli; (*c*) unknown tissue conditions that result in varying ratios; and finally (*d*) ontogenetic events. This regulation of Ia expression underlines the importance of controlling the synthesis of such an important regulatory protein in a multifaceted cell like the macrophage.

2. Accessory Cells besides the Macrophages

A number of cells have been identified in lymphoid organs with some characteristics in common with classical phagocytes, but also with some differences. We now review their characteristics, role as accessory cells, and possible relationship with the classical phagocyte.

The Langerhans cells of the skin were discussed in Section II,D. These are cells with typical morphological appearance: a dendritic appearance in the skin, a clear cytoplasm, a few lysosomes, and a typical organelle, the Birbeck granule. Langerhans cells bear Fc and C3 receptors and present antigen in a typical MHC-restricted fashion. Cells resembling Langerhans cells have also been described in the secondary lymphoid organs, in the thymus, and in the afferent lymph. Thus, histological analysis of lymphoid organs identified in the thymus-dependent areas stellate cells, called interdigitating reticular cells, in intimate contact with the surrounding lymphocytes (Veerman, 1974; Van Ewick *et al.*, 1974; Hoefsmit *et al.*, 1980; Veerman and Van Ewick, 1965). These cells had morphological features of the Langerhans cells, and many contained Birbeck granules. Interdigitating cells increased in number after antigenic stimulation (Silberberg-Sinakin *et al.*, 1976; Kamperdijk *et al.*, 1978). The studies of Silberg and Thorbecke, mentioned in Section II,D, indicated a rapid increase in cells in dermal lymphatics and in nodes draining the sites of contact reactions. Such cells were classified, on the basis of Birbeck granules, as Langerhans but were indistinguishable with the interdigitating cells. Other studies indicated that the interdigitating cells of the lymph nodes derived by way of the afferent lymphatics inasmuch as the cells disappeared after the ligation of the afferent vessels (Kamperfjik and Hoefsmit, 1978). To this effect, it is noteworthy that Langerhans cells were found to originate from bone marrow precursors in experiments using transplanted bone marrow cells (Katz *et al.*, 1979; Frelinger *et al.*, 1979). A histological study identified stellate Ia-bearing cells in the thymus-dependent areas of lymph nodes; these cells were compatible in morphology with the interdigitating cells (Hoffman-Feizer *et al.*, 1976) (Section II,G). In the thymus, cells with morphological features of interdigitating cells were identified, some of which contained Birbeck granules (Hoefsmit *et al.*, 1980). Cells with dendritic morphology bearing Ia were identified in frozen section and actually isolated (Section II,G).

Cells with characteristics akin to classical phagocytes and Langerhans cells were found in lymph from afferent lymphatics of rabbits (Kelly *et al.*, 1978), pigs (Drexhage *et al.*, 1980), and man

(Sokolowski *et al.*, 1978; Spry *et al.*, 1980). These were medium-size mononuclear cells, some having C3 and Fc receptor, and a few having Birbeck granules. The cells emitted long cytoplasmic projections; thus, the term "veiled cell" given to them. Veiled cells were particularly abundant in the lymph after skin irritation. In man, the large mononuclear cells with veiled cell characteristics contained Ia molecules (Spry *et al.*, 1980).

The Langerhans-interdigitating-veiled cell should be compared to a fourth cell with dendritic appearance, the dendritic cells isolated by Steinman and Cohn (Steinman and Cohn, 1973, 1974; Steinman *et al.*, 1974, 1975). These investigators isolated from the spleen a glass-adherent cell with dendritic extensions and scanty cytoplasm containing few organelles. The dendritic cells did not exhibit Fc and C3 receptors but had a high content of Ia antigens (Steinman *et al.*, 1979). They were isolated after initial adherence to culture, after which they spontaneously detached. In culture, the dendritic cells were found to be strong promoters of the mixed lymphocyte reaction (Steinman and Witmer, 1978) and also stimulated growth of autologous lymphocytes, the so-called syngeneic mixed lymphocyte reaction (Nussenzweig and Steinman, 1980). The dendritic cell isolated by Steinman is also bone marrow derived and tends to cluster with lymphocytes in a manner similar to that shown for the veiled cells.

A comparison of the various cells discussed above is shown in Table VI, which, in great part, I derived from a recent and very excellent review by Thorbecke (Thorbecke *et al.*, 1980). To me, the evidence is strong that the interdigitating cell, the Langerhans cell, and the veiled cell are the same: their characteristic morphology, the presence of Fc and C3 receptor, and the Ia content argue for this. It is my opinion, too, that the dendritic cell of Steinman is most likely part of this lineage: its morphological resemblance and presence of Ia also is in favor. However, the lack of Fc and C3 receptors argues against it. Also, no Birbeck granules have been reported in the cells isolated by Steinman. Although these arguments are powerful, they are not conclusive. Fc and C3 receptors are dynamic membrane structures that may fluctuate in their expression as a cell develops, matures, or is activated. The lack of Birbeck granules must be taken with caution: the amounts of this organelle among skin dendritic cells vary considerably, and some even lack them (see Thorbecke *et al.*, 1980, page 33). Some species, particularly the mouse, appear to have few Birbeck granules.

It may well be, therefore, that all the four cells discussed above belong to the same lineage. The definite answer awaits until such a time as detailed culture analysis and surface phenotype studies be-

TABLE VI
COMPARISON OF VARIOUS TYPES OF CELLS^a

Property	Macrophages	Langerhans cells	IDC ^b	Veiled cells	Thymus dendritic cell	Spleen dendritic cell (Steinman)	Germinal center dendritic cell
Surface markers							
Fc receptor	++	+	NK	±	++	—	?
C3 receptor	++	+	NK	±	—	—	++
Ia	++	++	+ (?)	+	++	++	NK
Glass adherence	++	++	NK	±	+	+	NK
Latex phagocytosis	++	±	NK	±	—	—	NK
Lysosomes	++	±	+	NK	NK	±	±
Birbeck granule	—	±	Some	Some	Some	—	—
Pinocytic vesicles	+++	++	++	++	NK	+	±
Antigen presentation	++	++	NK	NK	NK	NK	NK
Stimulator: MLC	++	++	NK	NK	NK	++	NK
Origin	Tissue and bone marrow precursors	Bone marrow	NK	NK	NK	Bone marrow	NK

^a References are included in the text.

^b IDC, interdigitating cells; NK, not known.

come available. The relationship of the Langerhans cell—and others—with the classical phagocyte also needs evaluation. The issue is whether both sets of cells represent two completely distinct lineages, two lines that split from a common precursor, states in the differentiation of the phagocyte, or adaptation of the classical phagocyte to a particular tissue microenvironment. Although the phagocytes have shown a great degree of plasticity in responding to stimuli, no direct evidence has been brought up that macrophages in culture develop characteristics of Langerhans cells. Therefore, the first two possibilities are the most likely.

Critical comparison of the function of the two cells should inform us of what may be functions in common or particular to each type. So far, Langerhans cells and the typical mononuclear phagocyte presented antigen to the same extent and were both active as stimulators in the mixed leukocyte reaction. Both cells most likely cooperate in bringing about a dynamic response to antigen. The macrophage, because of its antigen-handling properties, should be the major cell involved in the response to bacteria, virus, protozoa, and tumors, as well as proteins, whereas the Langerhans group of phagocytes may be more involved in the response to soluble sensitizers that attach to the skin. The Langerhans group of cells may represent a line of phagocytic cells that selectively homes to certain specialized tissues and is endowed with less inflammatory properties—hence its presence in skin and in the thymus. The macrophages, on the other hand, have the added function of more actively responding to various inflammatory agents.

Finally, a comment on the antigen-trapping mechanism of the germinal center seems pertinent. Not many studies have been made in the past few years, so its mechanisms and role remain to be elucidated. Antigen trapping to germinal center requires antibody and, particularly, complement (Papamichael *et al.*, 1975) and may be associated with dendritic-type cells studied ultrastructurally by Nossal's laboratory (see our past review, Unanue, 1972; and Nossal and Ada, 1978). The dendritic cells of the follicles have *not* been shown to contain Birbeck granules and appear different from the interdigitating cells (Hoefsmit *et al.*, 1980); their precise identification and role require their isolation (Klaus *et al.*, 1980).

B. ANTIGEN PRESENTATION

In Section II we reviewed the many examples of antigen presentation via the macrophage and the essential role it played in bringing about the stimulation of the lymphocyte, particularly the T cell. It became obvious that the development of all T-cell functions required

an interaction with macrophage-bound antigen regulated by the *I* region. No stimulation of T cells took place by direct interaction with antigen. In general, the regulation of the macrophage-T cell interaction by the MHC was akin to that shown for T-B cell collaboration or for the interactions between cytolytic T cells and their target cells.

1. Ir Gene Function and Determinant Selection

The genetic basis of the MHC "restriction" has been the subject of extensive and fascinating studies, still in progress, that have examined the cellular basis of *Ir* genes, the role of the nonlymphoid cell "environment" prior to the entrance of antigen, the involvement of the thymus and extrathymic cells in regulating the interactions, etc. Hypotheses have been put forward to explain the restrictions, one postulating that the T cell bears two receptors, one for the antigen and a second for the MHC product; another postulating a single T cell receptor that recognizes an antigen-MHC complex. No definitive answers are yet available and will not be, in my opinion, until the immunogenic moiety from the macrophage and the T cell receptors are isolated. I will limit this section to a consideration of important pieces of information that associate the macrophage to *Ir* gene function. Extensive reviews of immune response genes have appeared (Katz, 1977; Benacerraf and Germain, 1978; Zinkernagel, 1978; Berzofsky, 1980).

A number of important points came out from the studies reviewed in Section II. The involvement of the *I* region in the T cell-macrophage interactions applied not only to antigens under *Ir* gene control—as defined by states of responsiveness or nonresponsiveness—but to all proteins, even complex ones with multiple antigenic determinants. Analyzing antigens under *Ir*-gene control, it became evident in several studies that the macrophage was a key cell responsible for the responder or nonresponder status. This was shown with synthetic polypeptides where *Ir* gene status could be easily identified. The first study by Shevach and Rosenthal (1973), indicating responses of T cells only to antigens bound to macrophages from responders, was confirmed, for example, using another synthetic polypeptide like (TG)-AL (Erb and Feldman, 1975b; Hodes *et al.*, 1978; Kappler and Marrack, 1978) or natural proteins, to be discussed below.

Let us first discuss the selection of T cell antigen-reactive cells by the MHC environment in which they mature and then continue with the analysis of the antigen-presenting defect in "nonresponder" macrophages. We can start by questioning why there is such limited, if any, T cell reactivity to antigens bound to allogeneic macrophages. The lack of T cell reactivity to antigen-allogeneic Ia may mean that (a)

such cells are never generated during the normal non-antigen-driven differentiation of the T cell in its syngeneic thymus milieu; or (b) that the T cells did develop and joined the pool of mature T cells but are not found in the usual assays simply because they are a very minor component, overshadowed by the growth of the syngeneic reactive T cells. (It is indeed clear that an antigen-reactive cell, if found in the secondary lymphoid tissues, can be selected for and expanded during the handling of the antigen by macrophages. These were the studies showing that F₁ T cells consisted of two antigen-reactive sets of cells, each of which could be selected *in vivo* or *in vitro* by one or the other parental macrophage.) There are experiments in the literature in support of both explanations.

A role of the MHC of the thymus in generating and selecting the various antigen-reactive T cells was theorized by Jerne in 1971. His hypothesis has served as the basis for a number of experimental studies transplanting bone marrow cells into X-irradiated mice of different haplotypes, thus allowing maturation of stem cells to lymphocytes in different H-2-bearing hosts. Simply stated, the Jerne hypothesis envisions lymphocytes carrying germ line genes that encode for the receptors of the MHC of the species. The diversity of receptors to foreign antigens takes place as a result of mutations in T lymphocytes upon reaction with the thymic accessory cells bearing the self-MHC product. Thus, a lymphocyte would express two sets of receptors, one for the MHC product, the other to the foreign antigen (Von Boehmer *et al.*, 1978). Data particularly on cytolytic T cells but also with helper T cells support the fundamental premise of the hypothesis to a greater or lesser extent. Without entering into detail, the general consensus is that the host MHC environment, be it in the thymus and/or in the extrathymic milieu, has an influence, at least in the phenotypic expression of antigen-reactive T cells (reviewed in Berzofsky, 1980). The phagocytes, by virtue of their Ia content, their capacity to present antigen, their widespread tissue distribution, and their migratory patterns, may well be a major cell, not only handling and presenting antigen, but also regulating the non-antigen-driven maturation or "selection" of T cells (Section II,G).

Experiments examining helper cells support the statement that a T cell reactivity, not previously apparent, can be generated if the T cell develops in the tissues of a host bearing the appropriate MHC haplotype. Kappler and Marrack (1978), for example, produced bone marrow chimeras by transplanting bone marrow cells from one mouse strain (i.e., parent *a*) into lethally X-irradiated F₁ mice (from the same parent strain *a* and a different one, i.e., *a* × *b*) and, weeks later, studied

the reactivity of the T cells. The T cells from such chimeras cooperated especially well with B cells and macrophages of either parental H-2 type (*a* or *b*) in bringing about a response to SRBC or hemocyanin. This basic result was confirmed by others (Singer *et al.*, 1979; Erb *et al.*, 1979, 1980a,b). However, T cells from chimeras made of bone marrow from an F₁ into a parent cooperated only with B cells and macrophages of the parental host (this result was also supported by other studies, i.e., Sprent, 1978c; Katz *et al.*, 1978). Thus, in their situation, the potential antigen reactivity of T cells could be expanded or decreased by their differentiation in an appropriate host. This same experimentation also applied to antigens under *Ir*-gene control showing state of responsiveness or unresponsiveness. For example, chimeras were made by transplanting bone marrow stem cells from a "nonresponder" mouse into X-irradiated recipients from an F₁ cross between a responder and a nonresponder; the T cells were subsequently examined. Kappler and Marrack (1978) found that such T cells provided helper cells to (TG)-AL if now tested in cultures with B cells and macrophages from responder, but not from nonresponder, mice [the basic conclusions were also confirmed by Hodes *et al.* (1979)]. The studies of Erb *et al.* (1980a,b), used the *in vitro* system described in Section II,C and confirmed the previous results using hemocyanin, adding experiments with insulin, an antigen under strict MHC control.

A different approach was taken by Thomas and Shevach (1977). They explored the possibility that mature T cells reactive to antigen in allogeneic macrophages could be found if appropriately selected for. They examined a system of *in vitro* generation of hapten-specific T cells. The system consisted of three sequential cultures: (*a*) T cells from strain 13 guinea pigs were cultured with strain 2 macrophages for 3 days, then exposed to bromodeoxyuridine and light to inactivate the anti-strain 2 alloreactive T cells; (*b*) such treated cells were then incubated for 5 days with strain 2 macrophages that contained surface TNP groups; after which (*c*) the T cells were cultured with fresh TNP-macrophages from strain 2 and assayed for proliferation. The strain 13 T cells still reacted with strain 2 macrophages (not derivatized) about 80% less than the T cells not treated with the drug. Significantly, the T cells proliferated vigorously to the TNP-macrophages of strain 2, and this reaction was inhibited by antibodies to the Ia antigens of strain 2 (Thomas *et al.*, 1977b). Not killing the antigen-alloreactive T-cell clone did not allow for the demonstration of the TNP-specific clone. These results, if confirmed, imply that the cells recognizing antigen in the context of allogeneic Ia are present but require to expand in order to become operative. Another situation where T cells react with anti-

gen in an allogeneic macrophage was discussed before for the GAT polymer. In essence, the experimental evidence suggests that the interactions of T cells with the MHC products expressed by the individual is important in the expression of different antigen-reactive T cells. Some experiments suggest that the selection of T cells is a developmental event that takes place prior to contact with antigen, but others have shown it following antigen exposure of mature T cells. Overall, the chimera experiments are difficult and may contain complicating features, and whether the selection of T cells is more apparent than real requires further studies. The way in which the MHC "environment" regulates antigen selection prior to antigen still remains unexplained.

How does one explain the nonresponder status of the macrophage? Alan Rosenthal's laboratory provided evidence that macrophages, through their handling of antigen, would select the appropriate determinant for the T cell. This concept of "determinant selection" became evident during the analysis of the response to insulin by guinea pig T cells (Rosenthal *et al.*, 1977; Barcinski and Rosenthal, 1977; Rosenthal, 1978). *Ir* genes controlled the immune response to the two chains of insulin: T cells from strain 13 proliferated upon challenge with oxidized B chain of pork insulin, but T cells from strain 2 did not. In contrast, T cells from strain 2 proliferated upon interaction with A-chain determinants. With this information at hand, Rosenthal and associates examined the T cell response of F₁ guinea pigs to insulin or its chains presented on macrophages from either strain 2 or strain 13. T cells from guinea pigs immunized with pork insulin proliferated upon interaction with insulin on either macrophage. Using cross-reactive insulin or isolated chains, it was possible to show that the F₁ T cells proliferated to the B chain only when insulin was presented on strain 13 macrophages and to a sequence of three amino acids of the A chain when presented to strain 2 macrophages. Furthermore, using manipulations with bromodeoxyuridine and light, it was possible to identify two distinct sets of cells, the selection of which depended on the macrophage. Similar selection was found in *in vivo* experiments using F₁ guinea pigs transplanted with strain 2 or strain 13 macrophages (Yokomuro and Rosenthal, 1979). Rosenthal concluded that "a selected amino acid sequence and/or conformation within the antigen itself is seen by the T cell receptor and that generation or display of such antigenic determinants is a function of immune response genes operating at the level of the antigen-presenting cell." Perhaps the *Ir* genes "are, or regulate the activity of, families of enzymes which modify or metabolize polypeptide antigens" (Rosenthal, 1978).

The determinant selection phenomenon was found for several proteins. Thomas' laboratory reported it for the response to human fibrinopeptide B, a 14 amino acid fragment derived from the $B\beta$ chain of fibrinogen (Thomas *et al.*, 1979a,b; Thomas and Wilmer, 1980). Macrophages from immune strain 2 guinea pigs, but not from strain 13, presented the antigen or synthetic analogs that contained 10 critical amino acids (positions 5 through 14). The terminal arginine was essential, and its absence resulted in a lack of immunogenicity. A further illustration of this phenomenon involving the *I-C* region in the mouse was shown for myoglobin (Richman *et al.*, 1980).

A final point to analyze is the relationship between *Ir* genes and the Ia antigens. Are they the same? The conclusion, based on the genetics of *Ir* genes and of Ia antigens, is that this is the case. Strong experiments to support this claim are those showing *Ir* gene complementation between the *I-A* and *I-E/C* regions for the response to the peptide poly(Glu-Lys-Phe), GLPhe. The response to this peptide required two complementing *Ir* genes, one mapped at the *I-A* to *I-B* subregion, the other to the *I-E/C* subregion. The responder haplotypes were *I-A^b* and *I-E/C^d* (i.e., *H2^b* and *H2^d* mice did not respond, but their *F₁* offspring or appropriate intra-H2 recombinants mounted a strong immunity (Dorf *et al.*, 1975; Dorf and Benacerraf, 1975). In order to obtain T cell proliferation, the two gene products had to be expressed in the same antigen-presenting cells, which had to come from a responder or from the recombinants bearing the appropriate crossovers at *I-A* and *I-E/C* (Schwartz *et al.*, 1979). In subsequent studies, Jones *et al.* (1978) and Cook *et al.* (1979) brought structural and serological evidence of two complementing gene products in the same strain combinations coding for the *I-E/C* antigens. One gene product mapped at *I-A* and coded for the β chain, and the second mapped at *I-E/C* and coded for the α chain. The finding of a hybrid molecule provides a biochemical explanation to the complementation studies using GLPhe and also brings out a molecular mechanism for generating multiple *I*-region products on the cell surface.

2. Correlation with Antigen Handling

Several approaches have been taken to study the relationship between antigen handling and the immunogenic moiety recognized by lymphocytes. These include (*a*) correlating metabolism of labeled antigen with immunogenicity; (*b*) attempting to modulate the macrophage presentation of antigen with antibodies to Ia or to the antigen, or by treatment with proteases; (*c*) analyzing macrophage-released products; and (*d*) studying the binding of lymphocytes to mac-

rophages in the presence or absence of antigen. These last studies will be described in Section III,B,3.

Metabolism of Labeled Antigens—Use of Antibodies and Trypsin. The two major attempts so far to correlate the handling of a radioactive antigen with immunogenicity were carried out in my laboratory and in Alan Rosenthal's. The results of many of the experiments were remarkably similar, yet there were important differences, too, that indicate two pathways of antigen presentation.

In our first experiments initiated in the laboratory of Dr. B. A. Askonas, macrophages were pulsed with ^{125}I -labeled hemocyanin; at various times thereafter, the macrophages were transferred live into syngeneic recipients and assayed for their capacity to induce an antibody response. Macrophages were also cultured, and the amounts of ^{125}I -labeled antigen remaining in the cell or released into the medium was determined at various times. Three main results were obtained:

1. Most protein antigens were bound initially to the cell surface, then interiorized in vesicles and rapidly catabolized; the bulk of the catabolized antigen was found in the form of ^{125}I bound to amino acids; however, a small percentage of antigen invariably escaped catabolism (approximately 20%) and remained cell associated or was released (Unanue and Askonas, 1968a,b; Cruchaud and Unanue, 1971; Schmidtko and Unanue, 1971a; Calderon and Unanue, 1974). The antigen that escaped rapid catabolism was identified as (a) a released soluble product; (b) a small component on the macrophage surface, solubilized by trypsin; (c) an internal pool of antigen that was slowly degraded.

2. Culturing macrophages for several periods of time established that there was no correlation between the amount of antigen degraded and its immunogenicity; for example, with hemocyanins, the immunogenicity of the macrophage-bound antigen was relatively stable for a few days, while the bulk of the protein was catabolized (Unanue and Askonas, 1968b); with albumins, the life of the immunogenic moiety was shorter, lasting about a day.

3. Incubating the macrophages with antibody or trypsin prior to *in vivo* cell transfer markedly reduced their immunogenicity (Unanue and Cerottini, 1970); these results were confirmed by testing for antibody formation *in vitro*. The immunogenicity of macrophage-bound hemocyanin did not decay after 28 hours of culture and was sensitive to trypsin treatment of macrophages pulsed with antigen 24 hours earlier (Unanue, 1978). It is noteworthy that, in this latter case, the presentation of antigen to T and B cells did *not* require genetic iden-

tity at H-2 (Unanue, 1978). The lack of MHC involvement was explained by a transfer of the macrophage-associated antigen to an antigen-presenting cell syngeneic with the T cell (Section II,C).

The major point in the catabolism studies was that antigen from phagocytes was available for some periods of time, albeit in decreasing amounts. The antigen molecules were found secreted and on the macrophage surface. The antigens secreted from macrophages included all those tested so far: hemocyanin (Unanue and Askonas, 1968b; also, Askonas and Jaroskova, 1970), albumin (Schmidtke and Unanue, 1971a), iodinated SRBC (Cruchaud and Unanue, 1971; also, Cruchaud *et al.*, 1975), and products from *Listeria monocytogenes* (Unanue, 1980). Calderon did a detailed analysis of this released antigen trying to determine its source, pathway of release, and immunogenicity (Calderon and Unanue, 1974). The released hemocyanin derived from active, live macrophages, not from dead cells, and was represented by 3–7% of the protein initially bound to the macrophages. The released product was not affected by trypsin treatment of the macrophage indicating that it was not derived from any hemocyanin on the cell surface; furthermore, it was of heterogeneous size, indicating changes in the molecule, although a substantial amount was still reactive with antibody. The antigen was secreted mainly during the first 2 hours following uptake, but a small amount was slowly released during later times of culture. [Using γ -globulins, the released antigen was much smaller than the native molecule (Cruchaud and Unanue, 1971; Cruchaud *et al.*, 1975).] The hemocyanin released from macrophages was as immunogenic as the native molecule (our unpublished studies). Products from *Listeria* were also found to be released by macrophages and to be immunogenic without any H-2 restrictions (Unanue, 1980). The released antigen, therefore, appeared to derive from the intracellular compartment and probably represented a few molecules in phagosomes that reverted to the surface and were exocytosed.

The surface-bound molecules, released by trypsin (Unanue *et al.*, 1969; Unanue and Cerottini, 1970) and by EDTA (Unanue and Cerottini, 1970; Askonas and Jaroskova, 1970), included a few molecules that remained membrane bound after the internalization of the bulk of antigen molecules. The amount of protein that persisted on the surface was small, no more than 1%, and this amount decayed with time. Why a small number of molecules was kept on the surface is not known. The surface-bound molecules, at least with hemocyanin, retained their basic native structure: the protein was visualized by electron microscopy in unpublished studies with J. Rosenblith and M. Karnovsky

(mentioned in Unanue, 1975); also, antibody reacted with the trypsin-released hemocyanin (Unanue and Cerottini, 1970). Surface-bound molecules were also found after the binding and internalization of particulate antigens (Cruchaud and Unanue, 1970; Cruchaud *et al.*, 1975). These molecules might have derived from either the action of ectoenzymes on the antigen prior to its interiorization or following the endocytosis of the antigen by reversal to the surface of endocytic vesicles containing the antigen. Limited experiments were done in which macrophages were trypsinized twice, first to remove the bulk of the initial membrane-bound antigen, followed by a latent period to allow for "reexpression," then by a second trypsinization to determine whether new labeled protein could be released. The results indicated that the bulk of the surface-bound molecules was found early after the initial period of antigen binding and that very little was reexpressed.

The studies from Rosenthal's laboratory are important inasmuch as they focused on the relationship between the antigen handling and antigen presentation in a strictly MHC-dependent system, that of the proliferation of guinea pig T cells to syngeneic macrophage-bound antigen. Radioiodinated DNP-guinea pig albumin was rapidly catabolized, but approximately 20% remained cell associated for at least 3 days of culture (Ellner and Rosenthal, 1975). Some of the cell-bound protein was surface bound and releasable by trypsin. Also, a small amount of protein was secreted into the culture supernatant. These results are, in essence, identical to the ones described above. The major differences between the two studies concerned the functional effects of treating the antigen-pulsed macrophages with trypsin or antibodies. First, the immunogenicity of macrophage-bound albumin was lost progressively with time. By 24 hours, there was a 40% drop, and this increased further to 80% by the next day. It is noteworthy that the function of macrophages not pulsed until 48 hours of culture also dropped somewhat (by 40%). This can be explained now by the loss of Ia biosynthesis (Section III,A).

Treatment of the macrophages pulsed with DNP-albumin with trypsin removed cell surface molecules so that antibodies to DNP-guinea pig albumin would no longer bind to macrophages. Notably, the immunogenicity was *not* affected by the treatment after 24 or 48 hours of pulsing with antigen. Trypsinization affected the immunogenicity only if applied immediately after a fresh pulse with antigen at 4°C, at a time when the bulk of the molecules had not been interiorized. This result also applied to the protein PPD (Waldron *et al.*, 1974). Furthermore, exposure of the macrophages to antibody did not have an effect on their immunological function. Ellner and Rosenthal (1975) concluded

that "a trypsin-resistant, antibody-inaccessible component of macrophage-associated antigen, presumably at an intracellular site, is of major relevance in macrophage-dependent T cell recognition." Further analysis assaying for lymphokine production instead of T-cell proliferation and examining for clusters of T cells with macrophages (next section) confirmed that antibodies were not inhibitory (Ellner *et al.*, 1977).

Other attempts to block the responses of T cell-macrophage interactions with antibodies also failed. Werdelin and Shevach (1979) did not block the binding of T cells to macrophages pulsed with DNP-albumin, using a functional assay. Ben-Sasson *et al.* (1977) found no effect assaying morphologically the clustering of T cells to macrophages. In our system, antibodies had no effect on macrophage-bound *Listeria monocytogenes* (Farr *et al.*, 1979a).

The point can be made that T cells recognize "sequential," linear amino acid sequences of the protein, not antigen in its native configuration, whereas B cells recognize conformational determinants (Sela, 1969). Therefore, antibodies would not be expected to block a T-cell reaction with antigen. Loblay *et al.* (1980) prepared monoclonal antibodies to the antigenic determinant of the A chain of insulin (amino acids 8 to 10), which is the sequence recognized by T cells. These antibodies did not inhibit the T cell proliferative response to macrophage-bound insulin. Chestnut *et al.* (1980) prepared antibodies to denatured determinants of albumin and also failed to inhibit T-cell proliferation.

Another major attempt to localize the macrophage-bound immunogen was carried out by Shevach and Thomas. They developed an assay system in which immune guinea pig T cells were generated upon culture with trinitrophenylated live macrophages (TNP-macrophages). The TNP groups were bound to most surface proteins of the macrophages, including the transplantation antigens (Forman *et al.*, 1977). Subsequently, the T cells were assayed by their proliferative response to fresh TNP-macrophages (Thomas and Shevach, 1978a,b). Their main findings were as follows:

1. TNP-macrophages were immunogenic if added to the primed T cells immediately after conjugation of TNP or 24 hours later ("aged" macrophages, to use their term).
2. Addition of anti-TNP antibodies to fresh macrophages blocked the response by about 75%.
3. The TNP presentation by "aged" macrophages (i.e., 24 hours after pulsing with TNP) was not affected by anti-TNP antibodies.

Thus, the immunogen was available early, but not late. Shevach (1980) went on to report that the presentation of antigen by aged TNP-macrophages could be inhibited by anti-TNP antibodies if the cells were derivatized again after 24 hours: (a) TNP-macrophages → 24-hour culture → anti-TNP antibody: T cell proliferation; while in (b) TNP-macrophages → 24-hour culture → TNP → anti-TNP antibody: no T cell proliferation. Thomas (1978), on the other hand, examined the effects of glutaraldehyde fixation on this system. He noted that very light fixation affected the mixed leukocyte reaction and the response to PPD bound to macrophages but had a small effect on the response to TNP-macrophages. This finding allowed him to manipulate the system. Presentation by TNP-macrophages was not inhibited by antibodies after 24 hours of culture, but was blocked if the macrophages were lightly fixed with glutaraldehyde immediately after TNP conjugation, and then cultured, i.e., TNP macrophages → 24 hours → antibody: no blocking; TNP-macrophages → glutaraldehyde → 24 hours → antibody: T cell response is blocked. Furthermore, macrophages that were cultured 24 hours, then derivatized, were immunogenic if fixed in glutaraldehyde, whereas TNP-macrophages cultured for 24 hours, then fixed, lost all their function (macrophages → 24 hours → TNP → glutaraldehyde: immunogenic; TNP-macrophages → 24 hours → glutaraldehyde: not immunogenic).

Shevach interpreted his results to mean that a few molecules of TNP were available on the membrane but, being so few, could not be cross-linked by antibody and were not modulated; hence, the need to add fresh antigen. Thomas interpreted his experiments to mean that during culture TNP became inaccessible but was expressed upon contact with T cells. Glutaraldehyde "locked" the antigen in place and, therefore, affected the aged TNP-macrophage but not the fresh TNP cell. In other experiments, Thomas and Shevach found that anti-Ia antibody, even for a short period of 1 hour before or after derivatizing the macrophages, blocked very effectively; they argued, therefore, that TNP must be associated with Ia antigens.

One can argue that TNP conjugation of the macrophage is a drastic manipulation that complicates the issue inasmuch as it brings into operation many surface proteins of the macrophage. It is obvious that hapteneation does not simplify an already complex system, yet some of Thomas and Shevach's results are striking and do suggest the early availability of a pool of surface antigen for a period of time; the experiments of Shevach with antibody are provocative and so are those of Thomas with fixation and must be further explored with better defined antigens.

Kirk Ziegler and I have a series of yet unpublished experiments using *Listeria monocytogenes* that address the issue of whether the antigen recognized by T cells required a step of internalization and digestion. The system consisted of a brief, 15–30 minute interaction of *Listeria*-immune T cells with macrophages; this resulted in the firm adherence of the T cells with concomitant loss of functional cells from the nonadherent population (Section III). Radiolabeled *Listeria* was added to macrophages and followed cytologically and biochemically as it bound to the macrophage membranes and was then internalized and catabolized. The two major findings were (a) that the T cells did not bind to the macrophages until about 30 minutes after the interiorization of bacteria; and (b) that following this lag period, macrophages fixed lightly in paraformaldehyde could interact with the T cell. The implications are that the bacteria had to be taken in to be digested, after which some of the immunogenic products reverted to the cell surface. Of interest is that iodinated *Listeria* peptides could be released by trypsin, yet this treatment did not affect immunogenicity.

A summary of the main findings and my interpretation of them are given in Table VII, taken from a previous discussion (Unanue, 1978). I conclude that antigen is available from the macrophage in two major forms: (a) "unrestricted" by an MHC product and thus free to interact with any cells; and (b) linked—at least functionally—with an *I*-region product.

The "unrestricted" antigen is represented by a small number of molecules that are secreted and by a few that remain membrane bound and easily releasable by trypsin, both of which retain, at least in part, their native structure and can be recognized by specific antibodies. The population of "unrestricted" molecules are found with every antigen so far tested. The macrophage, therefore, serves as a source—or "reservoir"—of this antigen for finite periods that vary from antigen to antigen. With hemocyanin, for example, it was long to the point where its immunogenicity *in vivo* could be shown, up to 2–3 weeks (Unanue and Askonas, 1968). Indeed, this available antigen is an *unavoidable* complication in every system that examines antigen presentation and MHC restrictions. All the indications are that the free "unrestricted" antigen can enter into the Ia-restricted pathway by having another antigen-presenting cell interact with it. This is surmised by observations that allogeneic macrophages can present the antigen to T cells (that contain syngeneic macrophages) or to T and B cells that are in themselves *I*-region restricted. Ellner *et al.* (1977) and Pierce and Maleck (1980) (Section II,C) have presented clear evidence in support of this conclusion. Conceivably, as discussed in Section II,C, the B cell in T-B cell mixtures might interact also with this macrophage-

TABLE VII
TWO FORMS OF ANTIGEN PRESENTATION

	<i>I</i> -region restricted	MHC-independent ^a
Assay	Antigen-pulsed macrophages are added to purified T cells	Antigen-pulsed macrophages are added to unfractionated T cells or to mixtures of T and B cells (with macrophages)
Effects of antibody	Usually does not block the interaction	Effective in blocking interaction
Effects of trypsin	Not sensitive	Removes membrane-bound molecules important in the interaction
Effects of anti-Ia antibodies	Blocks the interaction	Not tested
Presentation by allogeneic macrophages	Present poorly	Can present
Presentation by Ia-bearing macrophages	Strictly required	Probably not required
Localization in macrophages	Not known. Intracellular?	Membrane-bound and soluble secreted molecules
Major role	MHC-restrictive interactions with T cells	Source of antigen available for B cells and/or Ia-bearing macrophages

^a MHC, major histocompatibility complex.

associated antigen and, in turn, present it to T cells in an MHC-regulated interaction (Unanue, 1978).

The antigenic moiety involved in the Ia-restricted interaction has not been characterized: its chemical forms, its location in the macrophage, its association with Ia need to be fully explored. In favor of a processing step for the antigen are the observations that T cells recognize small sequences of amino acids, and that proteins that are not digestible do not stimulate T-cell reactivity. The lack of accessibility of the antigen to proteases (Ellner and Rosenthal, 1975); the requirement for an intracellular handling stage, as suggested by Ziegler's experiment; the lack of inhibition by antibodies to the T cell determinant in the case of the experiments of Thomas and Shevach, Loblay *et al.*, and Chestnut *et al.*, all argue for an intracellular depot. Yet it is clear that the T cell specifically contacts, and adheres tenaciously in a specific way to, macrophages containing antigen. The next section discusses this specific interaction in detail. Rosenthal has argued that the T cell

may first contact macrophages in an antigen-independent manner and that this contact then triggers the flow of specific antigenic molecules from an intracellular site to the membrane, making them available to the T-cell antigen receptor. This argument is echoed in Thomas's experiments with glutaraldehyde described above. Against it, at face value, is Shevach's experiment blocking "aged" macrophages with anti-TNP antibodies or the experiments of Ziegler showing that paraformaldehyde-fixed macrophages are immunogenic. Both situations may not be contradictory: it may well be that the flow of immunogenic material from an intracellular site to the membrane is highly dynamic and that there is a continuous recycling; this recycling could be regulated by a membrane interaction. Depending on how much immunogen is at a given time on the outside—which may vary from antigen to antigen—the T cells will bind to it with different degrees of avidity. Once the T cell binds to it, the process of recycling may augment, making available more immunogen. In this situation, it may be difficult for antibodies to block this close cell-to-cell contact interaction, particularly if the determinants are sparse. Along the lines discussed above, in unpublished studies reproduced in Fig. 9, I have found that the intracellular vesicles which contain antigen also have Ia antigens.

In all the studies on antigen presentation, one puzzling and unexplained fact still remains, and that is the ease of suppression of the interaction by anti-Ia antibodies in contrast to the lack of effects of antibodies to the antigen. One wonders if Ia may have two distinct functions in the macrophage, one to regulate the antigen-handling and antigen-presentation step, and the other to modulate the contact be-

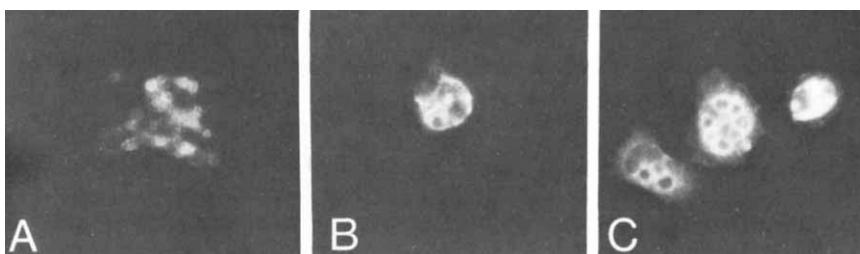


FIG. 9. Macrophages were incubated with heat-killed *Listeria monocytogenes* (panel A) or with opsonized red cells (panels B and C), after which the cells were fixed in acetone and stained with a monoclonal antibody anti-I-A (clone 10-2.16). The endocytic residues with the phagocytized particles are very rich in I-A molecules. Macrophages that have not phagocytized show few very weak punctate fluorescence. [Unpublished studies of E. R. Unanue (1979).]

tween the T cell and the macrophage. To this effect, two observations discussed in Section II showed T cell-macrophage interactions in the response to polyclonal stimuli, involving the macrophage Ia, but not restricted by the *I*-region haplotype—these were the interactions of T cells with macrophages in the response to Con A and to aldehyde. Particularly, the response to aldehydes was thoroughly examined and shown to be blocked with the specific anti-Ia antibody (Greineder *et al.*, 1976) (Section II,B). The results imply a surface-to-surface effect where allogenic Ia modulates the interaction. Ia proteins, therefore, may be versatile proteins capable of influencing the key and specific antigen-handling event at the same time that they can also interact with surface structures of the T cells in a way not involving antigen.

The association of antigen with Ia has been thought to be the immunogenic complex, although this has not been easy to demonstrate directly by biochemical techniques. Perhaps Ia is a protein that can bind sequences of amino acids and form a complex to which the T-cell receptor(s) binds with high affinity. Determinant selection may then involve the specific attachment of a piece of the antigen, resulting from lysosomal digestion to Ia—the degree of binding will vary depending on both the antigen and the structure of the Ia protein, which eventually translates into responder or nonresponder status. Or, perhaps, as Rosenthal speculated, genes code for an enzyme that directs the cleavage of the proteins at the same time that the Ia antigens function also as cellular interaction molecules.

Two experiments have been published in support of a soluble antigen-Ia complex. Erb and Feldmann's studies, described in Section II,C, indicated that a soluble material released from 4-day cultures of macrophages with antigen was effective in inducing helper T cells in an *I*-region-restricted way. The material was specifically removed by its passage through an anti-Ia column. Puri and Lonai (1980) recently reported on a similar phenomenon. Their first approach was to study the binding of ¹²⁵I-labeled (TG)-A-L to T cell suspensions. The binding, using autoradiography, took place if the T cells were first incubated for 2 hours with a 48-hour conditioned medium from macrophages. Furthermore, the binding required the presence of adherent cells in the T cell suspensions. A culture of T cells (containing adherent cells) with labeled antigen for 40 minutes contained a radiolabeled material that would rapidly bind to T cells treated with the macrophage-conditioned medium. The "processed" antigen did not bind to T cells bearing a different *I* region and was removed from solution by an anti-Ia immunoabsorbent. In a second series of experiments, the processed antigen was immunogenic *in vivo* and could, if

highly radioactive, kill the specific T cells (i.e., T cells incubated with "processed" antigen for 40 minutes would no longer show carrier function in a T-B cell collaboration protocol). [Some evidence of Ia released from macrophages and attaching to T cells was also brought up by Lee and Paraskevas (1979).] The Erb-Feldmann and Puri-Lonai observations are at an early stage in development but imply the release of an immunogenic antigen-Ia-bearing product as one pathway of antigen presentation. The results are of obvious importance and need to be further explained and characterized as well as integrated with other phenomena showing direct T cell-macrophage associations.

3. *Lymphocyte-to-Macrophage Interactions*

One of the critical issues relating to the mechanisms of lymphocytes interacting with antigen bound to macrophages is that of cell-to-cell contact. The early anatomical studies of macrophage-lymphocyte association in lymph nodes and spleen were reviewed previously (Unanue, 1972). Few histological studies have appeared in the past few years, and these, in general, have contributed little beyond a descriptive analysis of frequent and intimate associations between phagocytes and lymphocytes in the deep cortex and medulla of stimulated nodes. The early reports on macrophage-lymphocyte association in antigen and lectin-driven cultures were also reviewed. These indicated the frequent finding of multicellular aggregates between phagocytes and lymphocytes (for example, Mosier, 1969; Salvin and Nishio, 1969; Pierce and Benacerraf, 1969; Schechter and MacFarland, 1970; Seeger and Oppenheim, 1970; Salvin *et al.*, 1971). Several recent reports, particularly from five laboratories, have analyzed the issue of cell contact using cell cultures. These reports have indicated the specific and intimate anatomical and functional association between antigen-committed T cells and antigen-containing Ia-positive phagocytes.

The general approach taken was to produce a monolayer of macrophages, to which immune lymphocytes were added, in the presence or the absence of antigen. The interaction was quantitated by counting either the percentage of macrophages binding lymphocytes or the number of lymphocytes per 100 macrophages; or, functionally, by testing in a bioassay the removal of the antigen-specific lymphocytes from the cell suspension.

The studies of Lipsky and Rosenthal (1973, 1975b) using the guinea pig confirmed the observations that *thymocytes* would avidly bind to macrophages in the absence of antigens (Siegel, 1970a,b). About 90% of the macrophages in a preparation would bind the thymocytes; 5% of them went on to ingest the thymocytes. Serum had no effect on the

interaction. Thymocytes attached to cultured macrophages, even better to macrophages from oil-induced exudates, but not to neutrophils or fibroblasts. Lipsky and Rosenthal considered this binding an example of *antigen-independent* lymphocyte-macrophage interaction. The binding required live, active macrophages, took place optimally at 37°C, and by way of a trypsin-sensitive structure of the macrophage (Siegel, 1970b; Lipsky and Rosenthal, 1975b; Lopez *et al.*, 1977). The binding was species specific (Siegel, 1970a, 1970b; Lopez *et al.*, 1974), but not strain specific (Lopez *et al.*, 1977). In contrast to thymocytes, the degree of antigen-independent binding of mature T cells obtained from lymph nodes, although present, was much less pronounced (three- to fivefold less). The antigen-independent binding of either thymocytes or T cells took place *briefly* following culture, reaching maximal values after 1 hour, and disappeared progressively with time, the cells leaving their substrate (Lipsky and Rosenthal, 1973, 1975b; Lopez *et al.*, 1977). The thymocytes that dissociated from the macrophages lost their capacity to bind again (Lopez *et al.*, 1977).

In our own experience using mouse cells, this binding of thymocytes was very marked both in terms of number of thymocytes adhering to the macrophage and percentage of macrophages binding thymocytes (Beller and Unanue, 1978). Interestingly, thymocytes would also bind to macrophages isolated from the thymus (Beller and Unanue, 1978). I am not convinced, however, that the molecular basis for antigen-independent binding of thymocytes to macrophages is the same as that of mature T cells. It is possible that the much greater binding of immature thymocytes is because of a unique surface structure or one common with the mature T cell but at a much higher concentration.

The *antigen-dependent* binding has been tested most frequently by adding immune T lymphocytes to the monolayers of antigen-pulsed macrophages and quantitating the binding morphologically (Fig. 10). Depending on the culture conditions, maximal binding took place between 4 and 8 hours after the initiation of the culture and was maintained for a few days, decaying slowly; the binding was clearly antigen-specific (Werdelin *et al.*, 1974; Lipsky and Rosenthal, 1975a; Lipscomb *et al.*, 1977; Ben-Sasson *et al.*, 1977, 1978; Braendstrup and Werdelin, 1977). The lymphocyte that remained attached to the macrophages exhibited morphological signs of transformation and synthesized DNA (Lipsky and Rosenthal, 1975b; Braendstrup *et al.*, 1976; Ben-Sasson *et al.*, 1978). The lymphocyte that dissociated from the macrophages could bind *de novo* to fresh antigen-pulsed macrophages, suggesting that their release from the macrophage might be because of the loss of immunogenic antigen (Ben-Sasson *et al.*, 1978).

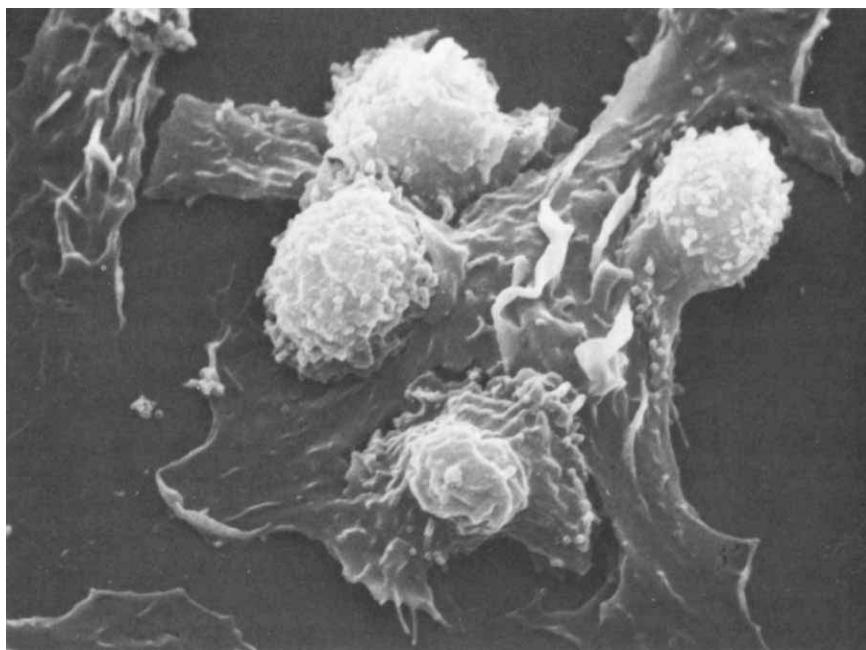


FIG. 10. Clustering of immune T cells to macrophages. *Listeria monocytogenes*-immune T cells were planted onto macrophages previously pulsed with *Listeria* for 1 hour, and then the loosely adherent cells were removed. Note the attachment of four lymphocytes to the macrophage. The attachment required phagocytosis of *Listeria* and Ia molecules on the macrophage surface. (Experiment of K. Ziegler, R. S. Cotran, and E. R. Unanue.)

It was clear that the binding of T cells to antigen-pulsed macrophages showed the same MHC restrictions as found in functional assays. Immune T cells from strain 2 guinea pigs preferentially clustered on antigen-pulsed macrophages of strain 2 rather than on macrophages of strain 13 (Lipsky and Rosenthal, 1975a; Braendstrup *et al.*, 1979; Lyon *et al.*, 1979). Anti-Ia inhibited cluster formation (Braendstrup *et al.*, 1979; Lyon *et al.*, 1979). In their study, Lyon *et al.* (1979) inhibited the clustering of T cells from $(2 \times 13)F_1$ guinea pigs to macrophages of strain 2 pulsed with DNP-GL (an antigen to which strain 2 responds, but not strain 13) by addition of an anti-strain-2-Ia, but not by an anti-strain-13-Ia, suggesting that the macrophages were the target. Functional assays established that this was indeed the case (Ziegler and Unanue, 1979).

Not all antigen-pulsed macrophages bind T cells; the number, although not studied in detail, was about 20–40% (Werdelin *et al.*, 1974;

Ben-Sasson *et al.*, 1974). This could well be explained by the different content of Ia-bearing macrophages inasmuch as the T cells bind preferentially to them. This point was studied in our laboratory by K. Ziegler, who, after allowing the lymphocytes to layer and cluster on the macrophage, fixed the preparation and stained for the Ia antigens of the macrophage by fluorescence. Clearly, the antigen-specific binding was taking place on Ia-bearing macrophages and could be enhanced severalfold using macrophage monolayers with a high content of Ia-positive macrophages.

Detailed morphological and cinematographic studies of the macrophage-lymphocyte clustering were made by Werdelin's laboratory using guinea pig cells (Nielsen *et al.*, 1974; Braendstrup and Werdelin, 1977; Petri *et al.*, 1978, 1979). They found that the lymphocyte-macrophage clusters were formed by a phagocyte to which a "central" lymphocyte was attached to by long areas of intimate membrane-to-membrane contact; interestingly, various other lymphocytes surrounded and attached to the central lymphocyte by way of long uropods (Fig. 11). The "peripheral" lymphocytes interacted sometimes briefly with the central lymphocytes, left, and reattached to other macrophages. Stopping cell motility by drugs, as expected, reduced cluster formation (Braendstrup *et al.*, 1977). The antigen specificity and kinetics of binding of central and peripheral lymphocytes was quite different. The central T lymphocyte bound optimally by 4 hours of culture, whereas the interaction of the peripheral lymphocytes took several hours longer. More important, the binding of the central lymphocyte to the macrophage was antigen specific, whereas the binding of the peripheral lymphocyte was not. Thus, a suspension of lymphocytes could be specifically depleted of their capacity to cluster on antigen-pulsed macrophages by a first culture on macrophage monolayers; i.e., after 4 hours, the cells that were not adherent did not form clusters on a fresh antigen-pulsed macrophage monolayer (Werdelin and Braendstrup, 1979). The peripheral lymphocyte, on the other hand, from immune or nonimmune, syngeneic, or allogeneic guinea pigs, associated and clustered on the cultures of macrophages with a central lymphocyte (Braendstrup *et al.*, 1979). Peripheral lymphocytes were represented by both T and B cells (Petri *et al.*, 1978). The interpretation was that the binding of the peripheral lymphocytes could be an example of lymphocyte-lymphocyte collaboration.

Particularly significant results that addressed the issue of T cell recognition of antigen were obtained using the binding of T cells to macrophages as a means to deplete T cells in a functional T cell-

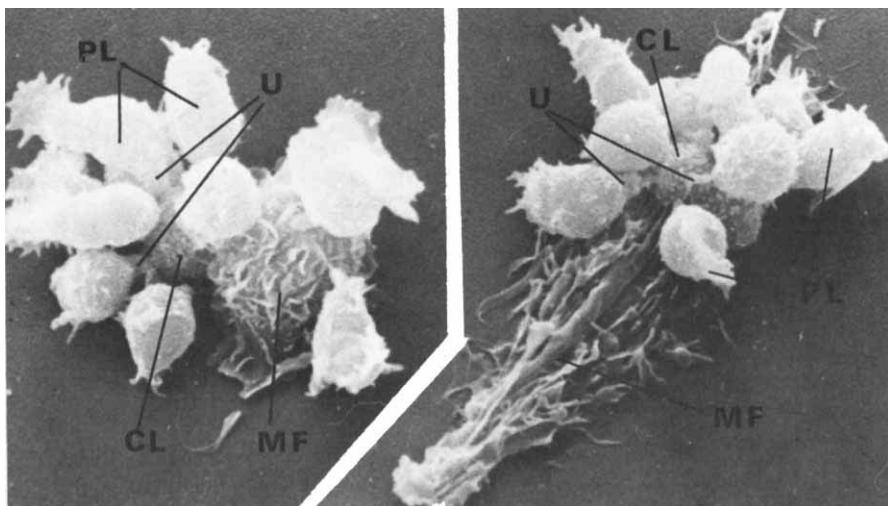


FIG. 11. Clustering of peripheral (PL) and central (CL) lymphocytes to macrophages (MF). In this study from Werdelin's laboratory, guinea pig purified protein derivative (PPD)-primed T lymphocytes were incubated with PPD-pulsed macrophages. The peripheral lymphocytes attached to the central lymphocyte by slender uropods (U). [From Nielsen *et al.* (1974).]

macrophage assay (Swierkosz *et al.*, 1978, 1979; Werdelin and Shevach, 1979; Werdelin *et al.*, 1979; Ziegler and Unanue, 1979; Werdelin, 1980).

The experiments reported by Werdelin and Shevach used guinea pig T cells immune to ovalbumin added to monolayers for 4 hours, after which the nonadherent cells were recovered and again added to a fresh macrophage monolayer and tested for DNA synthesis. The optimal removal of the antigen-specific T cells—74–98%—occurred after three such cycles of binding. In the mouse, we technically modified the system and obtained excellent depletion using *Listeria monocytogenes* as the antigen, after one or two 1-hour cycles of binding (Ziegler and Unanue, 1979). One important manipulation was to lightly centrifuge the immune T cells onto the macrophage monolayer. Werdelin's studies assayed T-cell function by DNA synthesis; Ziegler's did it by DNA synthesis and also by measuring mediator production or macrophage activation.

The main results were that T cells interacted only with antigen molecules associated with the appropriate Ia-bearing macrophages. In contrast, T cells did not bind to soluble antigen. Werdelin *et al.* (1979), were unable to show competition for the T-cell binding to mac-

rophages by an excess of soluble antigen, and neither did Ben-Sasson *et al.* (1977), who also did not observe binding to insolubilized antigen. We did not find binding of immune T cells to *Listeria* attached by poly-L-lysine to a culture dish.

Using F₁ mice, it was possible to find a specific interaction of two clones of T cells, each binding to the antigen in the context of one of the parental macrophages. The experiments of Swierkosz *et al.* (1978, 1979), assayed for helper T cells to SRBC (Section II,C). The F₁ T cells, isolated directly from the macrophage monolayer, were highly enriched in helper cells when tested for an antibody response in a mixture of B cell and macrophages from either parent. Similar results assaying for depletion of T cells rather than enrichment were obtained by Werdelin *et al.* (1979) and Ziegler and Unanue (1979).

We used the F₁ mouse with its two clones of T cells to explore the blocking effect of the anti-Ia antibodies. T cells from (BL6 × A)F₁ mice were bound specifically to the *Listeria*-pulsed macrophages from A or BL/6 and then tested functionally on each parental macrophage. Each reactive clone would be depleted by the specific macrophages. The depletion of the A-reactive clone did not take place when specific anti-Ia antibodies were added during the binding reaction. In contrast to the effects of antibodies to Ia, antibodies to the antigen did not result in abrogation of T cell-macrophage binding (Ellner *et al.*, 1977; Ben-Sasson *et al.*, 1977; Werdelin and Shevach, 1979).

In contrast to the many studies described above showing the specific binding of T cells to macrophages bearing Ia and antigen, there are few reports on the binding of B cells to macrophages. One of our early studies with Schmidtke showed binding of B cells to macrophage monolayers (in the absence of antigen) in a process competed by excess Ig (Schmidtke and Unanue, 1971b). Lipsky and Rosenthal (1975b) also observed antigen-independent binding of guinea pig B cells to macrophages but found no inhibition by excess immunoglobulin. Petri *et al.* (1978) observed binding of B cells to the central lymphocytes bound to macrophages (see above). O'Toole and Wortis (1980) reported antigen-independent binding of B cells to macrophages, particularly of the B cells bearing the Lyb3 marker. The binding of B cells was limited to the first 2 hours of culture if T cells were also present, but persisted in the absence of T cells.

C. SECRETORY FUNCTION OF MACROPHAGES

The mononuclear phagocytes can decisively affect their surrounding environment through the release of a number of biologically active products. These products influence the function of lymphocytes, con-

nective tissue cells, and extracellular proteins. Table VIII summarizes the major secretory products grouped into four major categories. The secretory products include enzymes that affect connective tissue and extracellular proteins—collagenase, elastase, plasminogen activator; a number of molecules critical for “defense” processes—complement proteins, interferon; molecules that affect cells—growth factors such as the lymphostimulatory molecules; and a variety of small molecular weight active compounds—prostaglandins, thymidine, oxygen intermediates. Several reviews of secretory processes have been published (Unanue, 1976; Unanue *et al.*, 1976a; Page *et al.*, 1978; Gordon, 1980). Many of the products secreted by macrophages are molecules destined exclusively for export, plasminogen activator for example, whereas others are intracellular products that are partially released under appropriate stimuli, such as the lysosomal enzymes. Some molecules are secreted continuously, but others only in response to external stimuli.

TABLE VIII
SECRETORY PRODUCTS OF MACROPHAGES

<i>Products That Affect Extracellular and Connective Tissue Proteins</i>	
Lysosomal enzymes (1) ^a	Various other neutral proteases (5, 6)
Plasminogen activator (2)	Esterases (7)
Collagenase (3)	Fibronectin (8)
Elastase (4)	Procoagulant (9)
<i>Products Involved in Defense Processes</i>	
Complement proteins (C1, C2, C3, C4, factor B) (10)	Interferon (12)
Lysozyme (11)	
<i>Biologically Active Proteins</i>	
Lymphostimulatory molecules (LAF) (this review)	Mesenchymal growth factor (14)
Colony-stimulating factor (13)	Angiogenesis factor (15)
<i>Small Molecular Weight Compounds</i>	
Prostaglandins (this review)	Thymidine (this review)
Cyclic nucleotides (16)	Oxygen-derived products (this review)

^a Numbers in parentheses indicate references. This is a partial list that includes the main molecules studied so far. References are not complete and refer to either key studies or review papers. More extensive review of the various secretory products can be found in Unanue, 1976; Page *et al.*, 1978; and Gordon, 1980. Key to numbers: (1) Davies *et al.*, 1974; (2) Unkeless *et al.*, 1974; (3) Werb and Gordon, 1975a; (4) Werb and Gordon, 1975b; (5) Hauser and Voes, 1978; (6) Gordon, 1980; (7) Wiener and Levanon, 1968; (8) Alitalo *et al.*, 1980; (9) Edward and Rickles, 1980; (10) Colten, 1976; (11) Gordon *et al.*, 1974; (12) Smith and Wagner, 1967; (13) Chervenick and LoBuglio, 1972; (14) Leibovich and Ross, 1976; (15) Polverini *et al.*, 1977; (16) Gemsa *et al.*, 1975.

The secretory function of the macrophages is important in the relationship of the macrophage with the lymphocyte for three reasons.

1. Some of the molecules secreted by the phagocytes are powerful stimulants of T and B cells and, moreover, are released following macrophage-lymphocyte interactions regulated by the MHC of the species (Unanue *et al.*, 1976b; Farr *et al.*, 1977, 1979a). Thus, the lymphostimulatory molecules may explain part of the molecular events taking place between the phagocytes and the lymphocytes, each cell regulating the other.
2. The macrophage-lymphocyte interaction, or products derived thereof, may also modify the secretion of many of the molecules listed in Table VIII.
3. The negative side of the macrophage, its inhibition of lymphocyte function can be explained to a great extent by the release of small biologically active products.

1. Secretion of Lymphostimulatory Molecules

It is now well established that phagocytes release proteins that decisively control the function of T and B lymphocytes. The biosynthesis and secretion of lymphostimulatory molecules may, therefore, be considered one of the important molecular events that follow macrophage-lymphocyte interaction.

a. Lymphocyte-Activating Factor. One of the earliest demonstrations of an active soluble material released by the phagocytes came from the studies of the human mixed leukocyte reaction (Bach *et al.*, 1970). No reaction developed between responder and stimulatory lymphocytes depleted of adherent cells (Alter and Bach, 1970). However, a culture medium conditioned by adherent, monocyte-rich cells promoted the development of the allogeneic interaction. That macrophage-conditioned medium might also affect *in vitro* antibody formation was suggested by experiments indicating that such medium could substitute for the macrophage (Hoffman and Dutton, 1971). (The nature of the product in this study was never elucidated. The fact that it was absorbed by the red cells, which were then strongly immunogenic, suggests that it was antibody rather than a true macrophage product.)

The first clear demonstration of a mitogenic activity secreted by macrophages came from the studies of I. Gery, B. Waksman, and associates at Yale (Gery *et al.*, 1972; Gery and Waksman, 1972). In their study, the adherent cells from human peripheral blood released, after 24 hours of culture, a "factor" that promoted the response of murine

thymocytes to PHA. Addition of the factor without PHA also promoted proliferation, but to a lesser extent. The factor was termed lymphocyte-activating factor and became known by the acronym LAF. We are defining LAF, therefore, as an activity in macrophage cultures that, added to thymocytes together with or in the absence of PHA, increases, after 3–4 days of culture, their uptake of tritiated thymidine by severalfold. Gery *et al.* found that LAF was best secreted following stimulation of the human monocytes or murine adherent spleen cells with endotoxin. LAF had a weak mitogenic effect on spleen cells but was active on lymph node lymphocytes, particularly in cultures of high cell density. Gery *et al.* concluded their study by speculating that LAF might well explain part of the helper role of macrophages in immune induction.

These observations were rapidly confirmed and extended. LAF is now considered the most important, perhaps the only, lympho-stimulatory molecule released by phagocytes with a wide range of biological effects that extend from the original effects described by Gery, to promotion of T cell secretion of mediators, to stimulation of B cell differentiation.

After Gery's observations, our laboratory reported some important observations of particular relevance for our understanding of the biological effects of LAF. First, it became apparent that macrophage-conditioned medium was a mixture of both stimulatory and inhibitory molecules, the latter being represented by dialyzable low-molecular-weight molecules frequently at concentrations that masked the stimulatory effects. This "inhibitory" factor became more evident in high-density cultures of macrophages (for example, at approximately 5 to 10×10^6 per milliliter in a round dish 35 mm in diameter). Dialyzing the inhibitor resulted in the clear demonstration of a "mitogenic protein" that turned out to be identical to LAF (Calderon and Unanue, 1975; see also Hoessli *et al.*, 1977). The inhibitors in many instances were represented by thymidine molecules, in others by prostaglandins [another study stressing the dual effect of macrophage-conditioned medium, in this case on hematopoietic cell growth, is that of Kurland *et al.* (1977), discussed below]. Of particular importance are the observations that LAF secretion was under strict regulation and that the key regulating event involved the interactions of macrophages with antigen and with immune T cells (Unanue *et al.*, 1976b; Farr *et al.*, 1977, 1979a; Beller *et al.*, 1978; Ziegler and Unanue, 1979). These studies involving the regulation of LAF secretion are now described in detail.

b. Regulation of LAF Secretion. Under basal conditions, peritoneal

macrophages from unstimulated mice secreted a small and variable amount of thymocyte mitogenic activity. Addition of various stimulants, such as an antigen-antibody complex, a bacterium like *Listeria monocytogenes*, latex beads, or an adjuvant like beryllium sulfate, increased secretion approximately from 2- to 20-fold (Calderon *et al.*, 1975). The increase in secretion, however, depended very critically on the life history of the macrophage: although peptone-induced macrophages were also triggered to release LAF, the macrophages from thioglycolate-injected mice were poor secretors (Calderon *et al.*, 1975; Unanue *et al.*, 1976b; Unanue and Kiely, 1977). Human monocytes also increased their basal secretion of LAF after interaction with latex spheres and antigen-antibody complexes (Blyden and Handschumacher, 1977). It should be noted that the most potent stimulant to secretion—second only to T cells and antigen—was endotoxin. Endotoxin strongly stimulated most macrophages (Meltzer and Oppenheim, 1977), spleen macrophages (Gery *et al.*, 1972), and human monocytes (Gery *et al.*, 1972; Blyden and Handschumacher, 1977; Lachman *et al.*, 1977). The basal or stimulated secretion of LAF usually was optimal during the first 24–48 hours of culture (Fig. 12).

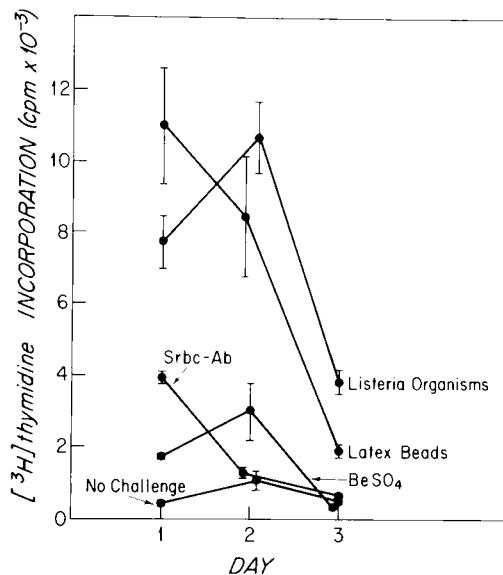


FIG. 12. This figure shows the increase in thymocyte mitogenic (lymphocyte-activating factor) activity secreted from macrophages after exposure to various materials. Culture fluids were tested on their effects on thymocyte proliferation at 25% volume per volume. [From Unanue *et al.* (1976).]

The release of the thymocyte mitogen was regulated by immune T cells upon interaction with macrophage-bound antigen. In our studies, peripheral macrophages from *Listeria*-immune or peptone-injected mice were cultured with heat-killed *Listeria* organisms, without, or together with, *Listeria*-immune T cells. The basal level of secretion by macrophages of LAF was low, but the uptake of *Listeria* increased secretion to a small extent. The addition of immune T cells produced a remarkable increase in secretion (immune T cells plus *Listeria* did not result in any production of mitogen). The secretion of thymocyte mitogenic LAF activity by T cell-macrophage interaction was found to (a) require intimate contact between the macrophage and the T cell—their separation by a filter did not result in secretion (Farr *et al.*, 1977); (b) be antigen specific; and (c) be under control of the *I* region of the MHC (Farr *et al.*, 1977, 1979a; Beller *et al.*, 1978; Ziegler and Unanue, 1980). Thus, the release of the mitogen involved an initial recognition of antigen that followed all the parameters discussed in the preceding sections. Indeed, the secretion of LAF required not only the presence of Ia-bearing phagocytes, but that macrophages and T cells share the *I-A* region of *H-2*. The last experiment was done by culturing macrophages from congenic strains of mice together with immune T cells and *Listeria* organisms for 24 hours, after which the culture fluid was assayed for thymocyte mitogenic activity. A representative result is shown in Table IX. It should be stressed that the secretion of LAF was best induced after cell contact, as described above; addition to macrophages of lymphokine-containing medium resulted in relatively weak stimulation of LAF secretion. The release of LAF following T cell-macrophage interaction was also found with an antigen like hemocyanin (Unanue *et al.*, 1976b) or with antigens in fetal calf serum (Farr *et al.*, 1977, 1979a). Thioglycolate macrophages were also poor secretors of LAF following macrophage-T cell interaction.

The macrophage line P388D1 (Mizel *et al.*, 1978a,b) was found to release LAF. This release was increased by addition of PHA and T cells, even xenogenic, requiring also cell contact; in this unphysiological situation, there was no MHC involvement of the secretory process (Mizel *et al.*, 1978b). The P388D1 line also increased the secretion of LAF following exposure to phorbol myristate acetate (Mizel *et al.*, 1978c) and endotoxin (Mizel *et al.*, 1978b).

There are other important characteristics of LAF secretion that bear on the question of why the very activated macrophages were poor secretors of this protein. The basal or particle-induced secretion of LAF showed a superinduction phenomenon (Unanue and Kiely, 1977). Superinduction refers to the paradoxical increase in the production of a

TABLE IX
ABILITY OF B10.A *Listeria*-IMMUNE T CELLS TO INTERACT WITH MACROPHAGES SHARING DIFFERENT REGIONS OF THE H2 GENE COMPLEX^{a,b}

Macro-phage strain	H-2 haplo-type	H-2 formulas						H-2 homology with T Cell	Mitogenic activity	
		K	I-A	I-B	I-J	I-C	S		No antigen	With <i>Listeria</i>
B10.A	<i>a</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	All	1,164 ± 120	11,395 ± 1,069
B10	<i>a</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	None	1,259 ± 209	1,348 ± 137
B10.A (4R)	<i>h4</i>	<i>k</i>	<i>k</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>K + I-A</i>	689 ± 46	11,163 ± 1,434
B10.A (5R)	<i>i5</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>I-J, I-C, S, D</i>	501 ± 7	1,190 ± 405
B10.A (15R)	<i>h15</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>K, I-A, I-B</i> <i>I-J, I-C, S</i>	1,745 ± 238	13,188 ± 1,313
B10.A	<i>a</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	All	1,164 ± 120	11,185 ± 1,069
B10.G	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	None	1,693 ± 68	1,050 ± 20
B10.A (6R)	<i>y2</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>d</i>	<i>D</i>	1,687 ± 269	1,587 ± 113
B10.AQR	<i>y1</i>	<i>q</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>d</i>	<i>I-A, I-B, I-J,</i> <i>I-C, S, D</i>	1,035 ± 68	12,297 ± 925

^a From Farr *et al.* (1979a).

^b The experiment involved culturing B10.A *Listeria*-immune T cells with macrophages in the presence or the absence of 10⁶ *Listeria* for 24 hours and then assaying the culture fluid for its content of mitogen. Mitogenic activity generated in cultures of T cells and *Listeria* antigen was 1068 ± 23. Background proliferation of thymocytes was 530 ± 10. Previous experiments have established that the lack of response in the combinations where macrophages and T cells did not share *I-A* could not be attributed to a suppressor mechanism.

cell product following inhibition of RNA or protein synthesis (McAuslan, 1963). It was observed, for example, for the secretion of tyrosine aminotransferase (EC 2.6.1.5) by liver cells (Steinberg *et al.*, 1975) or for the release of interferon by fibroblasts (Vilcek and Ng, 1971). One interpretation of superinduction is that the inhibitors of protein synthesis stop the production of a control protein that regulates the synthesis and/or metabolism of the product. In our studies, we found that freshly harvested macrophages did not contain any preformed LAF (freeze-thawed extracts had no thymocyte mitogenic activity); however, a few minutes after planting, LAF could be extracted from the microsomal fraction, reaching peak levels by 1–2 hours and then steadily declining so to disappear altogether by 24 hours. The loss of internal LAF did not correlate with its appearance in the culture fluid. In other words, contact with the dish stimulated the appearance of cell LAF, but not its secretion. The inhibitors of protein synthesis had two contrasting effects, depending on the time at which they were added to the culture. Cycloheximide added to cells immediately after planting stopped all production of internal LAF; however, the addition of cycloheximide after 1 hour of culture when LAF had already been made resulted in a marked *increase* in the secretion of LAF; in this case, the internal LAF did not decline and remained in the cell for long periods of culture (Fig. 13). We postulated that LAF secretion involved an internal control protein that regulated the expression and/or degradation of the cellular LAF; shutting off the secretion of the putative control proteins allowed for the persistence and maintenance of LAF secretion. The fact that highly activated macrophages secreted less LAF argued for an increased content of this inhibitor. In this regard, thioglycolate macrophages treated with cycloheximide 1 hour after planting released the mitogen.

c. *Biochemistry of LAF.* Lymphocyte-activating factor is a protein with a molecular weight of about 13,000–16,000 (Gery and Handschumacher, 1974; Calderon *et al.*, 1975; Blyden and Handschumacher, 1977; Lachman *et al.*, 1977; Economou and Shin, 1978; Mizel, 1979). The protein is sensitive to protease and papain digestion but is relatively resistant to trypsin (Calderon *et al.*, 1976; Blyden and Handschumacher, 1977). It is not inhibited by diisopropyl fluorophosphate (Calderon *et al.*, 1975) nor by phenylmethane sulfonyl-fluoride (Blyden and Handschumacher, 1977), indicating that it is not a serine protease. LAF does not bind to columns of Con A-Sepharose (Mizel, 1979), nor does it contain antigen products encoded in the H-2 of the species (Calderon *et al.*, 1975). LAF shows charge heterogeneity with isoelectric points ranging from 4.5 to 6.5. Economou and Shin's

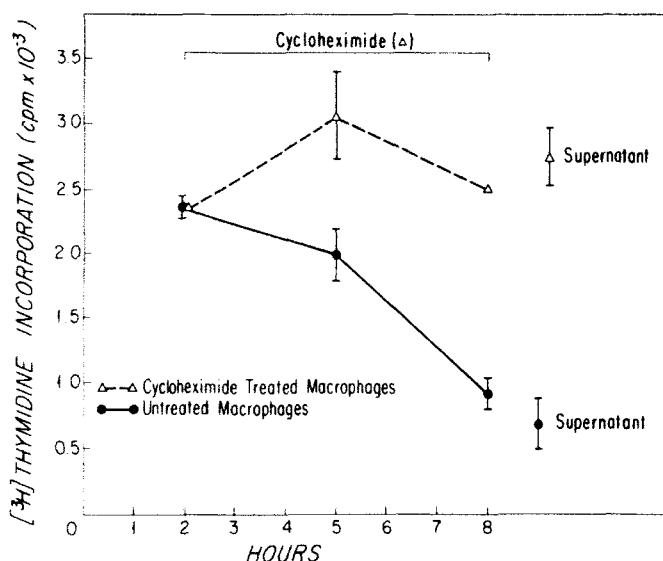


FIG. 13. Superinduction of the release of thymocyte mitogenic protein (lymphocyte-activating factor). The figure depicts the amounts of activity extracted from untreated macrophages or macrophages treated with 10 µg of cycloheximide per milliliter starting 2 hours after planting and terminating at 6 hours. The amount of cell-bound mitogen in untreated macrophages decreases with time, and little is released into the supernatant (after 8 hours of culture). After cycloheximide, mitogenic activity persists longer and is released. [From Unanue and Kiely (1977).]

(1978) excellent study indicated that the charge heterogeneity may result from some proteolytic changes during the isolation. In their studies, analysis of LAF, after a brief period of culture and with enzyme inhibitors in the processing steps, resulted in more homogeneous preparations with an isoelectric point of 4.8. Other reports indicated isoelectric points of 6 to 8 (Lachman *et al.*, 1977) and 5.0 to 5.4 (Mizel, 1979). Economou and Shin's study indicated that LAF had a Stokes' radius of 20 Å, a sedimentation coefficient (SE_{20w}) of 2.0, a buoyant density of 1.30 gm/cm³, and a calculated molecular weight of 16,400–19,600. There are reports of size heterogeneity of LAF-type molecules having apparent molecular weights of 30,000, sometimes 80,000, besides the 15,000 product. The reasons for this size heterogeneity have not been adequately resolved. In fact, whether all these molecular weight products are the same as the major 15,000 MW protein is far from clear. There is some indication that some—but not all—of the size heterogeneity may be caused by protein-protein aggregates (Togawa *et al.*, 1979); thus, some of the isolated 15,000 MW product added to serum rechromatographed in the 50,000–70,000 size

range. In our studies, we have found similar effects, indicating that LAF may easily complex to serum proteins. To be noted is that the intracellular LAF of peritoneal macrophages was highly homogeneous in size, approximately 15,000 MW (Unanue and Kiely, 1977), whereas that found in the macrophage P388 line showed marked size heterogeneity (Mizel and Rosenstreich, 1979).

Lymphocyte-activating factor has been purified extensively by combinations of size and gel chromatography (Lachman *et al.*, 1977; Blyden and Handschumacher, 1977; Economou and Shin, 1978; Mizel, 1979). Not enough material has been isolated for detailed amino acid analysis.

d. Mode of Action. In this section, I will discuss the mitogenic effect of LAF on thymocytes and will also examine the relationship between LAF and other growth-promoting molecules released by cultured lymphoid cells.

Various growth-promoting molecules have been found not only in mixed cultures of macrophages and lymphocytes, but in cultures of purified macrophages. The biological studies of LAF analyzed above disclosed some size and charge heterogeneity that is not entirely explained. Therefore, I will focus the analysis of the action of LAF to situations where the 15,000 MW product has been the major component.

There is no question that the main target cell of the mitogenic effect of LAF is the thymocyte. Directly assaying proliferative activity—without the addition of PHA—established that T cells obtained from spleens or lymph nodes responded poorly, if at all, whereas thymocytes were stimulated by the mitogen, maintaining their level of DNA synthesis (Gery *et al.*, 1972; Calderon *et al.*, 1975; Beller and Unanue, 1979). The thymocytes also responded to LAF by an augmented response to PHA; lymph node T cells also responded to both stimuli (LAF alone or LAF plus PHA), but very much less than the thymocytes (Gery *et al.*, 1972). Within the thymocytes, is there a difference in response? Mature and immature subsets of thymocytes—separated in albumin gradients—responded to LAF; the mature subset, which normally proliferated to PHA, showed a higher DNA synthesis upon culture with LAF (Gery *et al.*, 1972; Beller and Unanue, 1979). Thymocytes, separated on the basis of their agglutinability to peanut agglutinin, were stimulated to LAF, but the “peanut-agglutinin-negative” subset exhibited a greater response (Oppenheim *et al.*, 1980). Attempts to identify a LAF receptor by determining whether it could be removed from solution by thymocytes have so far failed (Beller and Unanue, unpublished observation).

Experiments of David Beller indicated a relationship between the

spontaneous growth of the thymocyte and its response to LAF (Beller and Unanue, 1979). Many of the thymocytes freshly harvested were in cell cycle and responded to the continuous presence of LAF in culture by maintaining their proliferative activity for several days. In the absence of the growth factor, there was a rapid decline in their DNA synthesis. Addition of LAF at a time when spontaneous growth had stopped (by 24 or 48 hours of culture) did not reactivate the cells to go into DNA synthesis. However, such resting thymocytes were stimulated if pulsed briefly with the lectins Con A or wheat-germ agglutinin, followed by removal of the lectins and then addition of LAF. It should be noted that wheat-germ agglutinin is a nonmitogenic lectin and, moreover, is highly inhibitory when added to cultures. However, a brief, 2-hour interaction with the thymocytes was effective in inducing a first "signal" to the cell. Reversing the two stimuli, i.e., LAF first, followed by the lectins, was ineffective. Our results, therefore, indicated that the direct mitogenic action of LAF required a cell in an "activated" state that was inherent in the population of spontaneously cycling thymocytes. In the resting thymocyte, the activated state had to be induced by an initial interaction with a nonmitogenic ligand. Precedent for two growth-promoting molecules in the regulation of cell growth could be found in studies on fibroblasts. In these studies, one growth-promoting molecule had to take the cell to the state where it was able to "receive" the second factor (for example, Pledger *et al.*, 1977; Vogel *et al.*, 1978). Our opinion is that LAF may be an important endogenous regulator of thymocyte proliferation *in situ*. To this effect, we have found a definite basal level of secretion by macrophages directly isolated by the thymus. Thymic macrophages, therefore, by virtue of their content of Ia and their secretion of LAF and differentiation molecules, are a likely candidate for exercising a powerful role in the intrathymic maturation of T cells (Section II,G).

How LAF interacts with antigen or Con A to bring about proliferation of *mature* T cells and the relationship between LAF and other growth-promoting molecules found in lectin-stimulated lymphoid cells, is the subject of intense study at the present time. Cultures of unfractionated lymphocytes with Con A contained a growth-promoting molecule for T cells (Chen and DiSabato, 1976). The T-cell growth factor (TCGF) or "costimulator" has been studied by several groups, especially Smith and Gillis (Gillis and Smith, 1977; Gillis *et al.*, 1978; Smith, 1980) and by Paetkau and associates (Paetkau *et al.*, 1976; Mills *et al.*, 1976; Paetkau, 1980). The TCGF was shown to be a molecule required to maintain proliferation of Con A-activated T cells as well as lines of cytolytic T cells; TCGF appears to be different from LAF,

although similarities are abundant. Biologically, TCGF did not promote DNA synthesis of thymocytes unless Con A was added (Mills *et al.*, 1976; Smith, 1980), whereas LAF had a direct mitogenic action. In contrast, LAF did not stimulate proliferation of cytolytic T cells selected to grow in TCGF (Oppenheim *et al.*, 1980); nor did macrophage-conditioned medium stimulate the growth of Con A-activated blast cells (Larsson *et al.*, 1980). In the mouse, TCGF showed a molecular weight of 30,000 (Paetkau *et al.*, 1976; Smith, 1980), higher than that of LAF.

Evidence obtained from several laboratories indicates that the stimulation by Con A of immune T cells is highly complex, involving the participation of macrophages, producing a factor, and T cells of the Lyl subclass, producing TCGF. The evidence is that the addition of Con A to cultures of T cells (with macrophages) resulted in the production of TCGF (measured by its effect in promoting growth either of clones of cytolytic T cells or of Con A blasts). This production of TCGF correlated with the mitogenic response of the T cells. Removal of adherent cells ablated the Con A response, as we discussed in Section II,B but also resulted in the lack of production of TCGF (Smith *et al.*, 1980; Smith, 1980; Larsson *et al.*, 1980; Paetkau, 1980; Gronvik and Andersson, 1980). More important, addition of a macrophage factor, together with Con A, generated TCGF (Larsson *et al.*, 1980; Smith *et al.*, 1980), and with it, the recovery of the proliferative activity. Thus, Con A induced proliferation of mature T cells (*a*) in the presence of macrophages; (*b*) in the absence of macrophages if a macrophage factor was added; (*c*) in the absence of macrophages if TCGF, a T-cell product, was added; furthermore, (*d*) production of TCGF by Con A required macrophages or a factor derived from them. The nature of the macrophage factor that stimulates growth of Con A, or production of TCGF, is not altogether clear, although the assumption has been made that it is LAF. The first "factor" reported by Rosenstreich and Mizel (1978), produced by guinea pig macrophages, had a molecular weight of about 30,000; that studied by Larsson *et al.* (1980), produced by a macrophage cell line, was close to 25,000; the factor studied by Paetkau (1980) and by Smith *et al.* (1980) had the size characteristic of LAF. Thus, LAF may indeed be responsible for TCGF production, but perhaps other macrophage products may also do it.

Is the effect of LAF on Con A-induced T-cell proliferation mediated entirely by the production of TCGF? Smith *et al.* (1980), suggested that this was the case, based, in part, on the correlation between TCGF production and growth. They also brought evidence that dexamethasone affected the release of TCGF, but not its mitogenic effect

on mature T cells. Also, the drug inhibited the mitogenic effect of LAF (plus Con A) on thymocytes, but not the growth induced by TCGF; thus, the assumption was made that LAF acted only by inducing TCGF production. I find this interpretation to be on weak grounds; it is based mainly on the single experiment described above. The LAF and TCGF molecules clearly acted on different T-cell populations. While LAF had a direct mitogenic effect on thymocytes, TCGF required the action of Con A. Thus, the former was acting, as discussed before, on cells already in cycle, including immature cells, whereas the latter was acting on cells that required Con A stimulation. Thymocytes have different sensitivities to dexamethasone, and this may explain Smith's results.

Regardless of the final explanation, the point is clear: there is great cooperation between two major mediators, which in their production and mode of action are highly interrelated. A comparison of both has been summarized in a recent Letter to the Editor in the *Journal of Immunology* (Aarden *et al.*, 1979).

Some of the effects of macrophage-conditioned medium on antigen-driven T-cell responses were evaluated in the preceding sections and will only briefly be restated here. In Section II,A, we discussed the studies of Rosenwasser and Rosenthal, which indicated that a macrophage factor, most likely LAF (Rosenwasser *et al.*, 1980), allowed for the proliferative response of T cells to hemocyanin. This response required a small number of antigen-presenting cells (Kammer and Unanue, 1980) and, therefore, implies an amplification of a T-cell response by the macrophage product that still requires, in my opinion, antigen presentation. This is the same way in which I interpret the effect of the nonspecific factor studied by Erb and Feldman (Section II,C) in the response to particulate antigens and the activity reported by Lee *et al.* (1976) (Section II,A).

e. *Effects on B Cells.* In culture, macrophage-conditioned medium exerts effects on the antigen-driven B cell differentiation to antibody-forming cells. All the indications are that one of the molecules responsible may be similar, if not identical, to LAF. Experiments on the action of macrophage culture fluids on antibody formation in culture are now summarized.

Schrader reported (1973) that spleen cells from athymic mice developed an antibody response if cultured with peritoneal macrophages or their conditioned medium. Detailed studies were not done of the phenomenon, nor of the activity of the macrophage culture fluid. The activity was generated only by peptone-activated macrophages, particularly if cultured with medium containing fetal calf serum, and was sensitive to trypsinization.

Our laboratory examined the effects of macrophage-conditioned medium using a hapten-carrier system, with spleen cells from mice immune to fluorescein-bound hemocyanin (Calderon *et al.*, 1975; Unanue *et al.*, 1976b). Macrophage-conditioned medium induced a definite, albeit modest, differentiation of spleen cells in the absence of antigen. Challenging with fluorescein on a heterologous carrier (rabbit IgG) in the presence of the macrophage-conditioned medium also induced a mild response, not much over that induced by the conditioned medium without the antigen. The response to a challenge with the homologous antigen was marked and depended very critically on the immunological status of the primed mice donating the spleen cells. Cells from mice primed several months earlier, which responded with a modest antibody response, increased their activity by as much as 100-fold when macrophage-conditioned medium was added, in a dose-related response. Spleen cells from mice primed recently were also stimulated by macrophage-conditioned medium but only at low concentrations; at high concentration, a suppressive effect was induced. Two other experiments indicated that the non-antigen-driven differentiation did not require T cells; and nude mice, in confirmation of Schrader's experiment, responded to SRBC in the presence of macrophage-conditioned medium.

Wood's laboratory examined the response of SRBC of either murine spleen cells depleted of T cells (by killing them with anti-Thy.1 and serum as a complement source) or of spleen cells from athymic nude mice (Wood and Gaul, 1974; Wood *et al.*, 1976; Wood and Cameron, 1975, 1976). The macrophage-conditioned medium was obtained from human monocytes. The experiments indicated a substantial response of the B cells, in the presumed absence of T cells. The degree of reconstitution was about 50% when compared to that produced by T cells. A similar degree of reconstitution was obtained by medium conditioned by a mixed leukocyte reaction. The macrophage culture fluid also increased to a small extent the non-antigen-dependent "basal," plaque-forming cells and clearly augmented the response of whole spleen (i.e., with T cells).

The system used by Hoffman to study the effect of macrophage-conditioned medium was the response to TNP bound to autologous red cells (Schmidtke and Dixon, 1972b). The addition of endotoxin with the antigen resulted in antibody response to TNP that was macrophage dependent (Hoffman *et al.*, 1976a,b); this was taken as an example of a thymus-independent effect modulated by the polyclonal effect of the endotoxin on macrophages. A major point was that spleen cells responded to TNP-mouse red cells in the presence of conditioned medium from macrophages cultured with LPS (Hoffman *et al.*, 1979).

Because the response was found in C3H/He mice that are not responsive to LPS, the logical argument was made that the effect was only mediated by the macrophage products. The response observed by Hoffman was dose-dependent and was claimed not to be impaired by anti-Thy.1 treatment of the spleen cells, although the data did show some effect of the treatment. Addition of T cells with the macrophage-conditioned medium increased the response to a noticeable extent. Thus, as in Calderon and Wood's experiments, a clear potentiation of an antibody response was also taking place as a result of a macrophage product, plus the T cell "stimulus."

What are the conditions resulting in the production of the B cell stimulatory molecule(s)? What is their relationship with LAF? How do they act?

A major stimulus for the production of the stimulatory molecules was the endotoxin activation of the monocytes as found for the case of human cells (Wood and Gaul, 1974; Wood and Cameron, 1976). Human monocytes, unstimulated, or after phagocytosis of antigen-antibody complexes or latex did not release the B cell-activating factor (Wood and Cameron, 1976). We compared the release of B-cell stimulatory molecules and the thymocyte mitogen by the mouse peritoneal macrophages and found about the same results in most cases. As reported for LAF, increased B-cell stimulatory activity was found after uptake of macrophages of *Listeria monocytogenes*, latex beads, or antigen-antibody complexes, or following T cell-antigen interaction (Unanue *et al.*, 1976b). Also, as with LAF, highly activated macrophages released less material in basal conditions or after phagocytosis.

The two studies examining the B-cell stimulatory activity indicated that a substantial part of it separated in Sephadex columns with an apparent size of about 15,000 MW (Calderon *et al.*, 1975; Wood *et al.*, 1976; Wood, 1979a). In our studies, LAF and the B-cell stimulatory molecules usually chromatographed in the same position (Calderon *et al.*, 1976), but this was not the case in Wood's studies using human material (Wood *et al.*, 1976). His last report (1976) did conclude that similar size material contained both thymocyte mitogenicity as well as B-cell stimulatory activity. Although it may well be that the LAF molecule contains the B-cell activity, it has not been conclusively proved. We have also found some B-cell stimulatory molecules in the range of 110,000 and 35,000 that may or may not relate to LAF (Unanue, 1978).

Concerning the mode of action of the B cell-activating molecule, there is general agreement on the following points.

1. It induces to a small extent some differentiation to antibody secretion in the absence of antigen; whether this target cell is the imma-

ture, small B cell or B cells in already an activated state has not been critically examined and deserves consideration.

2. It increases the response of B cells in the presence of T cells in a situation where there is clear synergy between the macrophage-derived molecule and the helper function of T cells.

3. To some extent, it stimulates B cells in the apparent absence of T cells. The few studies that have critically compared the response of B cells to antigen and macrophage-conditioned media in the presence or absence of T cells have shown much better responses in the former situation, i.e., with T cells (Calderon *et al.*, 1975; Hoffman *et al.*, 1979). Unquestionably, the macrophage product exerted a good effect on T cell-deprived spleen cells, particularly noticeable with the SRBC response. Certainly with hapten-carrier we could not state that the macrophage product "substituted" for T cells.

The important issue of how the macrophage products act on B cells, if at all, and its synergy with T cell helper activity still requires analysis. Hoffman's studies indicated an effect on the expression of Ia antigens by bone marrow cells incubated for just 2–5 hours with the macrophage-conditioned medium. This was taken to mean that the macrophage product, which in his case was not characterized, might influence B-cell maturation. It is also possible that the macrophage products in cultures of B cells with antigen were acting as maturation molecules on T-cell precursors remaining in the culture. This has not been critically ruled out. Regardless, a striking feature is the interaction between the macrophage product and the T cell that results in heightened B-cell differentiation. Early studies of Farrar and associates (1977) had shown a number of "activities" in a mixed leukocyte supernatant that, in part, "replaced" T cells in the anti-sheep response *in vitro*. These activities were resolved by gel filtration in molecules of apparent sizes of 15,000 and 40,000 MW that *synergized* when added together. The 15,000 MW material was shown to have thymocyte mitogenic activity compatible with LAF (Koopman *et al.*, 1977). Indeed, in support of this cooperativity are recent studies where the macrophage product, together with a T cell-replacing factor (induced by Con A-activated T cells), induced strong B-cell responses in culture (Hoffman and Watson, 1979; Hoffman, 1980).

Finally, there are few studies on the effects of macrophage-conditioned medium on the B-cell responses in man. One analysis indicated that macrophage-conditioned medium potentiated the response to pokeweed mitogen (Dimitri and Fauci, 1978).

f. Summary: Other LAF Activities. There is no question that macrophages secrete powerful lymphostimulatory molecules that exert effects on T and B cells and that the major principle is the 15,000 MW

LAF. Still to be resolved are two questions: (*a*) whether all the molecules of larger size found with some biological activities represent aggregates of LAF; and (*b*) whether all the activities of the 15,000 MW protein can be attributed to a single protein or to a family of them. The action of LAF, if indeed a single molecule, is extensive as we just analyzed.

Two other activities have been associated with a 15,000 MW molecule. Endogenous pyrogen has been partially purified and found to be a 15,000 MW protein (Dinarello *et al.*, 1977; Dinarello and Wolff, 1977). This purified material was found to potentiate the antigen-driven T cell response in the system developed by Rosenwasser and Rosenthal (Rosenwasser *et al.*, 1980). Another preparation of rabbit endogenous pyrogen (from peritoneal exudates stimulated with endotoxin) was shown to contain thymocyte mitogenic activity; furthermore, a purified rabbit LAF had pyrogenicity (Murphy *et al.*, 1980). The results, therefore, strongly indicate that the LAF molecule may be the major leukocyte protein that mediates fever.

A human monocyte product has been identified, again of low molecular weight, that when added to cultured synovial cells markedly increased their secretion of collagenase and prostaglandins. This secretion could possibly be involved in the connective tissue changes associated with the inflammatory response of the joints (Dayer *et al.*, 1977a,b). Obviously, the role of LAF and LAF-like products in the inflammatory response involving macrophages needs more analysis, but these two studies strongly indicate the protean role that this special molecule(s) may play.

Last, it is worthy of note that macrophage-conditioned medium may contain other lymphostimulatory molecules aside from LAF. A 40,000 MW protein has been identified in macrophage-conditioned medium, distinct from LAF, that induced marked maturational changes in early thymocytes (Beller and Unanue, 1977) (Section II,G).

2. Modulation of the Macrophage Secretory Process by the Lymphocyte

The addition of T cells to macrophages with antigen or a polyclonal stimulus, or the addition of T cell-derived products—a lymphokine-containing culture fluid—may change the pattern of secretion of the macrophage. We discussed before our own studies showing that the secretion of lymphostimulatory molecules by the macrophages was regulated by the T cell when it responded to antigen under an MHC-regulated interaction. Addition of lymphocytes with antigens also stimulated the macrophage to secrete the C2 protein, a key early com-

ponent in the complement cascade (Littman and Ruddy, 1977). In this study, an eightfold increase in hemolytically active C2 was found by human peripheral blood leukocytes stimulated with SK-SD; addition of a lymphokine to the monocytes resulted in less stimulation, about fivefold.

Lymphokines stimulated macrophages to secrete lysosomal enzymes (Pantalone and Page, 1975), interferon (Neumann and Sorg, 1977), collagenase (Wahl *et al.*, 1975a), and plasminogen activator (Vassali and Reich, 1977; Klimetzek and Sorg, 1976, 1977; Nogueira, *et al.*, 1977; Greineder, *et al.*, 1979). The study of Vassali and Reich (1977) is one of several that documented in detail the stimulation of secretion by a lymphokine-rich medium. They demonstrated a dose-response relationship between the amount of lymphokine added to the culture and the secretion of plasminogen activator: the unstimulated macrophage did not release the enzyme until after the addition of the conditioned medium, as little as 0.3% volume per volume. Thioglycolate-stimulated macrophages secreted the enzyme but increased their rate of synthesis and secretion about 10-fold after addition of lymphokine. In these studies, it was possible to document that most macrophages secreted the plasminogen activator. Macrophages also released a procoagulant activity following interaction with lymphocytes stimulated by endotoxin (Edwards *et al.*, 1979; Edwards and Rickles, 1980; Levy and Edgington, 1980); in a comparable situation, macrophages also released a molecule that stimulated lymphocytes to secrete colony-stimulating factor (Apte *et al.*, 1980).

3. Molecules Involved in Suppressive Effects

Macrophages release a number of compounds that are directly responsible for inhibitory effects. These compounds include prostaglandins, oxygen intermediates, thymidine, and some macromolecular inhibitors. The suppressive role of macrophages depends on their number and degree of activation, their response to a stimulus, and on the nature of the target cell. This will become evident as we analyze the various observations.

a. Prostaglandins. Prostaglandins are important metabolites derived from the cyclooxygenation of arachidonic acid following its release from phospholipids by phospholipases (Fig. 14). Prostaglandins are believed to be important mediators of inflammation, inducing vasodilatation and edema. There is very good evidence that prostaglandins mediate many of the inhibitory effects produced by macrophages in cell cultures. It is likely that these highly active metabolites may mediate effects *in vivo* under appropriate circumstances. Prostaglandins

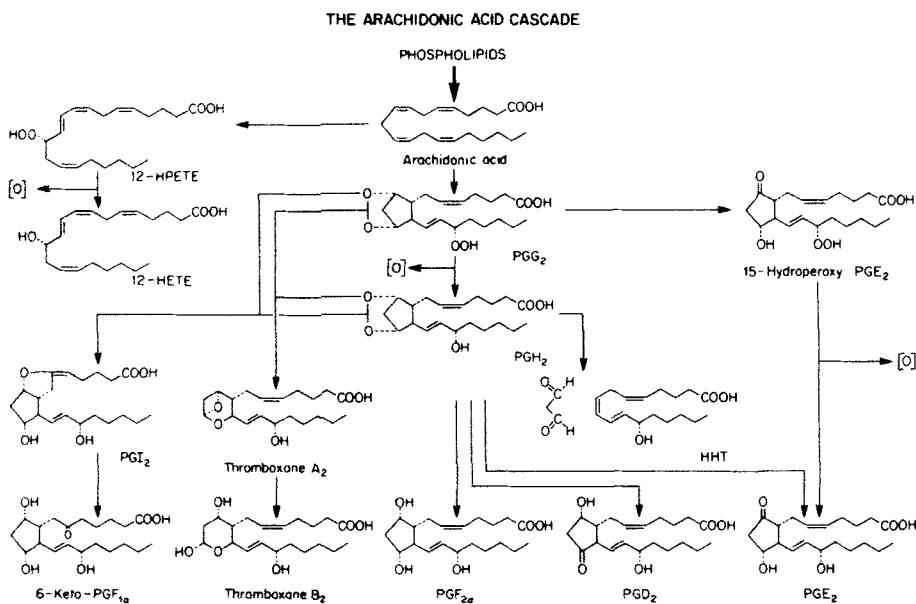


FIG. 14. The prostaglandin cascade taken from a diagram by courtesy of Dr. Philip Davies.

increase cellular cyclic AMP, which may be responsible for many of the modulatory effects on lymphocytes. The interest in the role of prostaglandins in macrophage-lymphocyte interactions came about from several observations. First, macrophages definitely synthesize prostaglandins, and the synthesis increases quite dramatically after their stimulation. Second, prostaglandins of the E series, added to cultures of lymphocytes, inhibit a number of functions. Third, the inhibitory effects of macrophages in macrophage-lymphocyte interactions can be reversed by the addition of drugs that inhibit the cyclooxygenase pathway, such as indomethacin.

Several studies indicated that prostaglandins were synthesized and released by cultured macrophages (Gordon *et al.*, 1976; Goodwin *et al.*, 1977; Humes *et al.*, 1977; Gemsa *et al.*, 1978; Kurland and Bockman, 1978; Brune *et al.*, 1978; Davies *et al.*, 1980). The prostaglandins were measured by radioimmunoassays or directly analyzed biochemically in cultures of macrophages pulsed with [³H]arachidonic acid. The major biosynthetic products were PGE₂ and the 6-keto prostaglandin F_{1α}, the stable product of prostaglandin I₂ (Hume *et al.*, 1977; Bonney *et al.*, 1978; Davies *et al.*, 1980). Small amounts of

PGE_{2α} and thromboxane B₂ were found in one study (Brune *et al.*, 1978). The synthesis and release of prostaglandins took place continuously, but increased markedly after stimulation of the macrophage. For example, the studies of Humes *et al.* (1977), showed that the addition to resident macrophages of zymosan, antigen-antibody complexes, or phorbol myristate acetate resulted in a marked increase in synthesis—up to 60-fold—by 30 minutes and continuing for at least a day of culture. Phagocytosis of latex did not have an effect. Essentially similar results were obtained in other studies (Gemsma *et al.*, 1978; Bonney *et al.*, 1979; Scott *et al.*, 1980). Increases in prostaglandins were reported following addition of endotoxin to murine and human phagocytes (Kurland and Bockman, 1972), colony-stimulating factor to murine macrophages (Kurland *et al.*, 1978), and lymphokines to guinea pig macrophages (Gordon *et al.*, 1976). Human monocytes increased their secretion by severalfold after addition of the Fc fragment of IgG, soluble aggregated human IgG, or Con A, but not by phagocytosis of latex particles (Paswell *et al.*, 1979).

The life history of the macrophage has an important effect on the synthesis of prostaglandins. In the studies of Hume *et al.* (1977), summarized by Davies (Davies *et al.*, 1980), normal, resident murine peritoneal macrophages and peritoneal macrophages, following injection of thioglycolate broth or *Corynebacterium parvum*, released equal amounts under basal conditions; yet, while the unstimulated macrophages responded to zymosan with a 20- to 30-fold increase in synthesis, the response of the stimulated macrophages was small, about 5-fold. Four papers reported an increase in basal stimulation of activated macrophages *in vivo*, one from tumor-bearing mice (Pelus and Bockman, 1979), two from mice that received *C. parvum* (Farzad *et al.*, 1977; Grimm *et al.*, 1978), and one from mice infected with BCG (Tracey and Adkinson, 1980); these last studies are of interest in view of the inhibitory effects of *C. parvum*- or BCG-stimulated macrophages in cultures (Scott, 1972; Klimpel and Henney, 1978). The issue of basal secretion by activated macrophages is, on the whole, not completely settled, with some discrepant results; the study of Farzad studied the synthesis for a brief period in comparison to Davies' study, and the study of Grimm was made from whole spleen and did not characterize critically the adherent cells responsible for biosynthesis. Further analyses of macrophage populations are warranted. In most reports, the addition of indomethacin inhibited the synthesis of prostaglandins.

Addition of prostaglandins inhibited various functions of lympho-

cytes in culture. Not all the studies, however, used prostaglandins at physiological concentrations. A list of the inhibitory effects is shown in Table X. Noteworthy is the study of Goodwin *et al.* (1977). They found that stimulation of human peripheral blood lymphocytes with lectins resulted in the production of prostaglandins in amounts similar to those inducing a significant suppression of the proliferative response when directly added to the culture. Addition to the culture of indomethacin stopped prostaglandin biosynthesis and also resulted in an increase in proliferation. Indomethacin had no effect on the action of suppressor T cells induced by the lectin. These investigators went on to show that patients with Hodgkins' disease contained adherent cells (monocytes?) that produced large amounts of prostaglandins; the poor responsiveness of these patients' cells to lectins resulted in marked improvement following addition of indomethacin.

The addition of indomethacin has been found to improve the response of lymphocytes in a number of situations, reducing to a great extent the inhibitory effects of excess numbers of macrophages (Webb and Jamieson, 1976; Webb and Osheroff, 1976; Webb and Nowowiejski, 1977; Demenkoff *et al.*, 1980; Metzger *et al.*, 1980).

It is noteworthy that not all the effects of prostaglandins are inhibitory. Some secretory functions, in fact, are mediated by them, such as the secretion of osteoclast-activating factor by T cells stimulated by lectins (Yoneda and Mundy, 1979) and collagenase by endotoxin-stimulated macrophages (Wahl *et al.*, 1977).

There are some studies that are worth discussing in relation to the

TABLE X
INHIBITORY EFFECTS OF PROSTAGLANDINS

Mitogen-induced proliferation (1-6) ^a
Anti-red cell responses (7, 8)
Lymphokine production (9, 10)
Killing by cytolytic T cells (11)
Mixed leukocyte reaction (12)
Antibody-dependent cell cytotoxicity (13)
Hematopoietic colony formation (14-17)
Natural killer cell generation and activity (13, 18)

^a Numbers in parentheses indicate the following references: (1) Bourne *et al.*, 1974; (2) Goodwin *et al.*, 1977; (3) Webb and Jamieson, 1976; (4) De Rupertis *et al.*, 1974; (5) Berenbaum *et al.*, 1976; (6) Smith *et al.*, 1971; (7) Webb and Nowowiejski, 1977; (8) Plescia *et al.*, 1975; (9) Gordon *et al.*, 1976; (10) Lomnitzer *et al.*, 1976; (11) Henney *et al.*, 1972; (12) Webb and Osheroff, 1976; (13) Droller *et al.*, 1978; (14) Kurland *et al.*, 1977; (15) Kurland *et al.*, 1978; (16) Williams 1979; (17) Pelus *et al.*, 1979; (18) Tracey and Adkinson, 1980.

regulation of prostaglandin synthesis by macrophages and its significance. The study of Goodwin *et al.* (1977), mentioned above, showed the critical relationship between the accessory function of the monocyte, on the one hand, and its suppressive effect on the other: addition of indomethacin improved the proliferative response to lectins, yet the removal of the monocyte resulted in a drop in the response. The message is clear, therefore, that the number and state of activation of the macrophage becomes critical in closed cell culture systems where the active metabolites accumulate.

The study of Gordon *et al.* (1976) called attention to a possible feedback loop involving the T cells and the macrophages. Indeed, lymphokines stimulated prostaglandin secretion, but these, in turn, stopped the biosynthesis of lymphokines produced by antigen in T cell-macrophage mixtures. A whole loop of macrophage-T cells → lymphokines → macrophages → prostaglandins → lymphocytes can be discerned. A similar kind of situation was reported by Kurland and associates: a critical number of macrophages by way of a growth-promoting molecule stimulated formation of bone marrow-derived hematopoietic colonies; yet an excess amount of macrophages suppressed colony formation through the release of prostaglandins. Colony-stimulating factor in itself enhanced prostaglandin secretion by the macrophage (Kurland *et al.*, 1977, 1978).

Finally, some studies have addressed the issue of synthesis by macrophages of arachidonic acid products produced by the lipoxygenase pathway. Macrophages have been shown to secrete 12-hydroxyeicosatetraenoic acids (Rigaud *et al.*, 1979; Scott *et al.*, 1980) and the leukotrienes (slow reactive substance of anaphylaxis) (reviewed by Stenson and Parker, 1980).

b. Oxygen-Derived Products. A recent study of Metzger *et al.* (1980) is of importance in that it adds oxygen metabolites to the list of inhibitors of lymphocyte function in culture, together with the prostaglandins. Leukocytes, neutrophils, and macrophages, particularly if stimulated with various particles and agents, can release a number of highly reactive oxygen intermediates, such as the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and possibly hydroxyl radical ($OH\cdot$) and oxygen in its excited state, singlet oxygen ($'O_2'$). These very active radicals are believed to be of particular importance for the microbicidal action of the cell and can also damage cells. Good evidence has been presented for a role of H_2O_2 in the tumoricidal effect of activated macrophages (Nathan *et al.*, 1978). Release of oxygen intermediates best takes place following phagocytosis by macrophages, particularly if previously activated by a cellular immune reaction (for a review of

these studies, see the papers of Nathan, 1980; Johnston, Jr. *et al.*, 1980; Klebanoff, 1975, 1980; Karnovsky *et al.*, 1975; Babior, 1978). In their study, Metzger *et al.* confirmed that indomethacin added to cultures of Con A-activated T cells stopped the suppression produced by phagocytes, except when these were in high numbers; they also added to their cultures catalase, an enzyme that rapidly converts H_2O_2 to H_2 (plus O_2). Addition of catalase resulted in an improvement in DNA synthesis, particularly in cultures strongly inhibited by an excess of macrophages; moreover, addition of indomethacin and catalase had a significant synergistic effect. The indications from their results is that part of the inhibitory effect of macrophages in culture could be due to H_2O_2 . This is an important observation that needs to be extended, but which points out the existence of oxygen products as powerful inhibitors. Clearly, situations that result in stimulation of the phagocytes and thus increase their release of prostaglandins or H_2O_2 result in adverse effects in cultures.

c. *Thymidine.* Thymidine is released continuously from the cultured macrophages. Depending upon the concentration of macrophages, the amount of thymidine that accumulates in the medium can be significant. We calculated that a 24-hour culture of 10^7 macrophages in 1 ml of medium planted in a culture plate of 35 mm diameter contained thymidine at a concentration of $4 \times 10^{-6} M$ (Stadecker *et al.*, 1977). The thymidine in the macrophage-conditioned medium may compete for radiolabeled thymidine used for pulsing cells growing in such medium; the thymidine may also directly block DNA synthesis (Stadecker *et al.*, 1977) by preventing the conversion of cytidilate to deoxycytidilate (Morris and Fischer, 1963).

A number of reports indicated the presence of an inhibitor of DNA synthesis in macrophage-conditioned medium (Nelson, 1973; Waldman and Gottlieb, 1973; Calderon *et al.*, 1974; Calderon and Unanue, 1975; Opitz *et al.*, 1975a). Our studies indicated that a dialyzable molecule was clearly masking the presence of lymphostimulatory molecules in macrophage-conditioned medium (Calderon and Unanue, 1975). Opitz and associates showed that the inhibitor was dialyzable and affecting only the incorporation of 3H -labeled thymidine by the lymphocyte, not its growth. The inhibitor was behaving, in a limited number of biochemical analyses, as thymidine (Opitz *et al.*, 1975b; Kasahara and Shioiri-Nakano, 1976). In our laboratory we found a dialyzable inhibitor that truly stopped DNA synthesis of the tumor cell line EL-4 (Calderon *et al.*, 1974). Biochemical characterization of the inhibitor also revealed a molecule identical to

thymidine (Stadecker *et al.*, 1977). The observations that the EL-4 tumor line was truly inhibited in its growth was explained by an unusual sensitivity of this cell to thymidine. Most tumor lines or growing lymphocytes were blocked by concentrations of thymidine ranging from 10^{-3} to 10^{-4} , while EL-4 was blocked at $10^{-6} M$. This is a clear example of the importance of the target cell in determining the effects of macrophage-secretory products.

The release of thymidine by the macrophage is quite unique. The possibility was raised by Opitz *et al.* (1975b) that the molecule could derive from the degradation of nuclear material phagocytized by macrophages. The bulk of the released thymidine, however, was synthesized by the macrophage, as was evident from biochemical studies using [^{14}C]formate (Stadecker *et al.*, 1977). The release of thymidine was explained by the absence of thymidine kinase, the enzyme that phosphorylates thymidine and rescues it from being released (Stadecker and Unanue, 1979). We found that macrophages lacked thymidine kinase and that addition to them of the growth-promoting molecule from L cell-conditioned medium resulted in *de novo* appearance of the kinase and the expected drop in thymidine release. Previous studies in fibroblasts had shown that mutant lines lacking thymidine kinase released thymidine into the culture (Chan *et al.*, 1974).

d. Macromolecular Inhibitor. One of the most interesting inhibitor molecules produced in culture is the soluble immune response suppressor (SIRS) identified in C. Pierce's laboratory (Tadakuma and Pierce, 1976, 1978; Tadakuma *et al.*, 1976). SIRS is a molecule found in culture of Con A-activated T cells that suppresses immune responses *in vitro*. When added to a culture of spleen cells, it abruptly stopped the plaque-forming cell response at day 4. The molecule was made by Ly2-positive T cells but required macrophages for the suppressive effect. Recent studies showed that the T cell product isolated from Con A-stimulated T cells was in a precursor, inactive form that could be converted into the active moiety upon direct incubation with macrophages. The macrophages did not require synthesis of new protein, nor was the generation of the active inhibitor blocked by indomethacin. Addition of catalase, however, stopped the production by the macrophage. To this effect, addition of H_2O_2 to the precursor molecule in the absence of macrophages resulted in generation of the active molecule. The inhibitory effects of SIRS was rapid, probably during G_2 or mitosis, a unique situation for a cell cycle inhibitor, and was reversed by 2-mercaptoethanol. Both the precursor and the active product had a molecular weight of approximately 55,000 (Pierce and Aune,

1981). Thus, all the indications are that an Ly2-positive suppressor T cell releases a molecule that, upon oxidation by H_2O_2 released by macrophages (probably only upon activation), converts into a strong and rapid inhibitor of cell proliferation.

Cooperativity of two cells by way of their released products has also been found between malignant plasma cells and macrophages. Plasmacytomas are known to inhibit normal Ig production. In an attempt to investigate the basis for this suppressor, it was found that cultured plasma cells released a large-molecular-weight protein that, upon interaction with macrophages, resulted in the release of a small-molecular-weight product of about 6000 MW. The product inhibited antibody formation *in vitro* in a noncytotoxic reaction and did not affect lymphocyte proliferation (Kennard and Zolla-Pazner, 1980).

Macromolecular "toxins" have been identified in high-density cultures of macrophages. Whether all are derived from macrophages, and their mode of action on lymphocytes, is not clear. A discussion of these groups of molecules can be found in a study of Chen *et al.*, who, in their own work, found that high-density cultures of macrophages released a 110,000 MW protein that inhibited DNA synthesis and antibody formation *in vitro* (Chen *et al.*, 1977).

IV. Conclusions

Historically, the participation of the macrophage in immunobiology was first studied in the context of the resistance to intracellular pathogenic bacteria. The analysis of the activated macrophage started with Koch's early description of the tuberculous granuloma and has continued up to the present time. We accept the notion that resistance to many infectious agents requires the mobilization of bone marrow-derived phagocytes to infectious foci, where they are activated, acquiring potent cytocidal functions; this process of macrophage recruitment and activation is brought about by soluble mediators secreted by antigen-stimulated T cells. Our information on the role of the macrophage in regulating immune induction is more recent, dating from the past 20 years. The intensive work of this period has demonstrated that the phagocytes exert a fine control on the early events that lead to antigen stimulation of T and B cells. Thus, the phagocytes participate at two very distinct stages in the immune process: first, as a cell regulating the extent to which lymphocytes are stimulated; second, as an effector cells responding to the signals from stimulated lymphocytes. Both processes are obviously deeply interrelated. It is indeed fascinating to note how the specific cells of the immune system—the

lymphocytes—and the nonspecific element—the macrophage—are so deeply dependent upon each other to the point of symbiosis.

The immunological cellular network is disturbed by the entrance of antigen, an event that sets in motion a complex number of cellular interactions. The phagocytes play a central role at this stage essentially because of the inability of the specifically reactive components of the immune system to respond to soluble antigen molecules. The T cells, or at least the helper subset, respond primarily to antigen that has undergone a handling process in which the products of the *I* region of the MHC play a critical and essential modulatory role (Sections II and III,B). The macrophages thus come into action at an early stage of the immune process by virtue of their capacity to take up and handle antigen, to express Ia, and to secrete lymphostimulatory molecules. It is noteworthy that two of these key functions, Ia expression and secretion of active factors, are relatively brief events (Sections III,B and III,C) in the life of the macrophage and that both are controlled by antigen and T cells in positive and negative ways. Thus, we reviewed how phagocytes displaying Ia antigen are able, after their uptake of antigen, to establish physical and functional interactions with T cells, the result of which is a burst of synthesis of lymphostimulatory molecules and Ia antigens, on the part of the macrophage, and of secretion of mediators and DNA synthesis on the part of the T cell. In closed cell cultures, the interactions are shut off when the macrophage, as it becomes stimulated, synthesizes inhibitory molecules (Section III,C). This negative control function appears likely to apply to *in vivo* situations. It is also likely from some of the evidence brought out in Sections II,H and III,C that suppressor cells may intervene in the T cell-macrophage interaction, even using macrophages as an intermediary. Other reciprocal control mechanisms that are now emerging are those regulating the percentage and number of Ia-bearing macrophages in tissues. This is particularly evident in the response to various inflammatory stimuli and in the ontogeny of the macrophage in the mouse (Section III,C). The ontogeny studies are indicating the need to limit the number of Ia-bearing phagocytes in the various tissues at a very critical stage of development. Overall, it appears that the macrophage antigen-presenting function is controlled internally as well as by external signals.

A major advancement in our understanding of the role of the macrophage came from the discovery of its participation in *I*-region-regulated events (Section II,A). The essential role of the *I*-region products in controlling all macrophage-T cell interactions is now an accepted fact. The antigen-presenting function of the mac-

rophage extends beyond a simple focusing of antigen to a more sophisticated and elaborate process. Such antigen-presenting function is essential in promoting growth of the selective T cells that recognize antigen in the context of the proper *I*-region molecules of the macrophage. The importance of the antigen-presenting function can be surmised from the many experiments mentioned in Section II. It becomes especially pointed in the case of the response to peptide antigens under *Ir*-gene control. The *Ir* gene effects indicate a very specific interaction between a fragment of antigen and the various *Ir* gene products, most likely the Ia antigens. The selective interaction between *I*-region products and antigen imparts specificity to the antigen-handling step. It is, in my view, a device by which the system ensures that there is no reaction against soluble or tissue self-molecules, but only against those antigens whose intrinsic properties allow them to bind to phagocytes (i.e., microorganisms, for example).

The macrophage regulates the interaction with antigen, in part, by selecting the key antigenic determinants, a point clearly brought out in the experiments using the insulin molecule in the guinea pig (Section III,B). Determinant selection appears to take place at the same time that antigen molecules are degraded, an apparent paradoxical situation. The macrophage destroys most of the antigen but conserves a few key determinants for the lymphocytes to interact. The molecular and biochemical events in determinant selection and antigen presentation are not entirely known and represent key areas of future research, together with the issues of why and how the helper T cells interact with macrophage-bound antigen, the molecular nature of the T cell receptor, and the development of the various T cell subsets. With regard to the latter point, there are indications that the macrophages in tissues may also play a role in non-antigen-driven maturational events (Section III,B).

Several cells aside from lymphocytes bear Ia antigens, the key modulatory proteins in antigen-driven and non-antigen-driven events. The question that comes to mind is whether all such cells are capable of antigen presentation or other forms of immune modulation. We discussed the Langerhans cell and a number of related dendritic cells, abundant in skin and lymphoid tissues, some of which are capable of expressing Ia and presenting antigen (Section III,A). The developmental and functional relationship between the Langerhans cells and the classical phagocyte require more analysis, yet the distinctive features of the Langerhans cell indicates the presence of a novel accessory cell performing in conjunction with the phagocytes. Aside from the Langerhans cells, stromal cells in the thymus have been found to bear Ia and, more recently, so were human endothelial cells in culture.

(Hirschberg *et al.*, 1980). Will all these present antigen? Or are they involved in non-antigen-driven events? Aschoff coined the term reticuloendothelial system to encompass a number of connective tissue cells all having the common feature of taking up vital dyes and particles to a major or lesser extent. The term lost its use when each cell type—fibroblasts, macrophages, endothelial cells, etc.—was identified into their proper lineage. Perhaps the time has come to reintegrate these cells into a system with a unifying function, that of regulation of cellular interactions by the Ia antigens.

The developments in macrophage biology over the past few years were not predicted at the time of my 1972 review in this Series. It is safe to assume that the discoveries in the next few years will prove equally surprising, with the study of the macrophage continuing to provide unique, illuminating insights into the complexities of immune cellular interactions.

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REFERENCES

- Aarden, L. A., *et al.* (1979). *J. Immunol.* **123**, 2928.
Ahman, G. B., Sachs, D. H., and Hodes, R. J. (1978a). *J. Immunol.* **121**, 159.
Ahman, G. B., Sachs, D. H., and Hodes, R. J. (1978b). *J. Immunol.* **121**, 1981.
Ahman, G. B., Nadler, P. I., Birnkrant, A., and Hodes, R. J. (1979). *J. Immunol.* **123**, 903.
Alblas, A. B., and van Furth, R. (1979). *J. Exp. Med.* **149**, 1504.
Albrechtsen, D. (1977). *Scand. J. Immunol.* **6**, 907.
Alitalo, C., Itovi, T., and Vaheri, A. (1980). *J. Exp. Med.* **151**, 602.
Alter, B. J., and Bach, F. H. (1970). *Cell. Immunol.* **1**, 207.
Andersson, J., Gronvik, K., Larsson, E. L., and Coutinho, A. (1979). *Eur. J. Immunol.* **9**, 581.
Andersson, J., Schrier, M. H., and Melchers, F. (1980). *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1612.
Apte, R. N., Hertogs, Ch. F., and Pluznik, D. H. (1980). *J. Immunol.* **124**, 1223.
Arala-Chaves, M. P., Hope, L., Korn, J. H., and Fudenberg, H. (1978). *Eur. J. Immunol.* **8**, 77.
Araneo, B. A., and Kapp, J. A. (1980). *J. Immunol.* **124**, 1492.
Argyris, B. A. (1968). *J. Exp. Med.* **128**, 459.
Asherson, G. L., and Zembala, M. (1974). *Eur. J. Immunol.* **4**, 804.
Askonas, B. A., and Jaroskova, L. (1970). In "Mononuclear Phagocytes" (R. van Furth, ed.), p. 595.
Askonas, B. A., and Roelants, G. E. (1974). *Eur. J. Immunol.* **4**, 1.
Babior, B. M. (1978). *N. Engl. J. Med.* **298**, 659.
Bach, F. H., Alter, B. J., Solliday, S., Zoschke, D. C., and Janis, M. (1970). *Cell. Immunol.* **1**, 219.

- Barcinski, M. A., and Rosenthal, A. S. (1977). *J. Exp. Med.* **134**, 1529.
- Behbehani, K., Pan, S., and Unanue, E. R. (1981). *Clin. Immunol. Immunopathol.* **19**, 190.
- Beller, D. I., and Unanue, E. R. (1977). *J. Immunol.* **118**, 1780.
- Beller, D. I., and Unanue, E. R. (1978). *J. Immunol.* **121**, 1861.
- Beller, D. I., and Unanue, E. R. (1979). *J. Immunol.* **123**, 2890.
- Beller, D. I., and Unanue, E. R. (1980). *J. Immunol.* **124**, 1433.
- Beller, D. I., and Unanue, E. R. (1981). *J. Immunol.* **126**, 263.
- Beller, D. I., Farr, A. G., and Unanue, E. R. (1978). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 91.
- Beller, D. I., Kiely, J.-M., and Unanue, E. R. (1980). *J. Immunol.* **124**, 1426.
- Benacerraf, B., and Germain, R. N. (1978). *Immunol. Rev.* **38**, 70.
- Benacerraf, B., Green, I., Bluestein, H., and Ellman, L. (1971). *Transplant. Rev.* **3**, 1327.
- Ben-Sasson, S. Z., Shevach, E. M., Green, I., and Paul, W. E. (1974). *J. Exp. Med.* **140**, 383.
- Ben-Sasson, S. Z., Paul, W. E., and Shevach, E. M. (1975a). *J. Exp. Med.* **142**, 90.
- Ben-Sasson, S. Z., Paul, W. E., and Shevach, E. M. (1975b). *J. Immunol.* **115**, 1723.
- Ben-Sasson, S. Z., Lipscomb, M. F., Tucker, T. F., and Uhr, J. W. (1977). *J. Immunol.* **119**, 1493.
- Ben-Sasson, S. Z., Lipscomb, M. F., Tucker, T. F., and Uhr, J. W. (1978). *J. Immunol.* **120**, 1902.
- Berenbaum, M. C., Cope, W. A., and Bundick, R. V. (1976). *Clin. Exp. Immunol.* **26**, 534.
- Bergholtz, B. O., and Thorsby, E. (1977). *Scand. J. Immunol.* **6**, 779.
- Bergholtz, B. O., and Thorsby, E. (1978). *Scand. J. Immunol.* **8**, 63.
- Bergholtz, B. O., and Thorsby, E. (1979). *Scand. J. Immunol.* **9**, 511.
- Berman, M. A., and Weigle, W. O. (1977). *J. Exp. Med.* **146**, 241.
- Bevan, M. J., Epstein, R., and Cohn, M. (1974). *J. Exp. Med.* **139**, 1025.
- Berzofsky, J. A. (1980). In "Biological Regulation and Development" (R. F. Goldberger, ed.), Vol. 2, in press.
- Birbeck, M. S., Breathnach, A. S., and Everall, J. D. (1961). *J. Invest. Dermatol.* **37**, 51.
- Blaese, R. M., and Lawrence, E. C. (1977). In "Development of Host Defense" (M. D. Cooper and D. H. Dayton, eds), p. 207.
- Bluestein, H., and Pierce, C. W. (1973a). *J. Immunol.* **111**, 130.
- Bluestein, H., and Pierce, C. W. (1973b). *J. Immunol.* **111**, 137.
- Bluestein, H., Ellman, L., Green, I., and Benacerraf, B. (1971). *J. Exp. Med.* **134**, 1529.
- Blyden, G., and Handschumacher, R. E. (1977). *J. Immunol.* **118**, 1631.
- Bona, C., Broder, S., Dimitriu, A., and Waldmann, T. A. (1979). *Immunol. Rev.* **45**, 69.
- Bonney, R. J., Wightman, P. D., Sadowski, S., Kuehl, F. A., Jr., and Humes, J. L. (1978). *Biochem. J.* **176**, 433.
- Bonney, R. J., Naruns, P., Davies, P., and Humes, J. L. (1979). *Prostaglandins* **18**, 605.
- Boswell, H. S., Sharow, S. O., and Singer, A. (1980). *J. Immunol.* **124**, 989.
- Bourne, H. R., Lichtenstein, L. M., Melman, K. L., Henney, C. S., Weinstein, Y., and Shearer, G. M. (1974). *Science* **184**, 19.
- Braendstrup, O., and Werdelin, O. (1977). *Cell. Immunol.* **32**, 263.
- Braendstrup, O., Andersen, V., and Werdelin, O. (1976). *Cell. Immunol.* **25**, 207.
- Braendstrup, O., Andersen, P., Jensen, H., and Werdelin, O. (1977). *Cell. Immunol.* **32**, 274.
- Braendstrup, O., Werdelin, O., Shevach, E. M., and Rosenthal, A. S. (1979). *J. Immunol.* **122**, 1608.
- Braun, J., and Unanue, E. R. (1980). *Immunol. Rev.* **52**, 58.

- Breard, J., Fuks, A., Friedman, S. M., Schlossman, S. F., and Chess, L. (1979). *Cell. Immunol.* **45**, 108.
- Breathnach, A. S. (1965). *Int. Rev. Cytol.* **18**, 1.
- Broome, J. D., and Jeng, M. W. (1973). *J. Exp. Med.* **138**, 574.
- Brune, K., Glatt, M., Kalin, H., and Peskar, H. (1978). *Nature (London)* **274**, 261.
- Calamai, E. G., and Unanue, E. R. (1980). Experiments to be published.
- Calderon, J., and Unanue, E. R. (1974). *J. Immunol.* **112**, 1804.
- Calderon, J., and Unanue, E. R. (1975). *Nature (London)* **253**, 359.
- Calderon, J., Williams, R. T., and Unanue, E. R. (1974). *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4273.
- Calderon, J., Kiely, J.-M., Lefko, J. L., and Unanue, E. R. (1975). *J. Exp. Med.* **142**, 151.
- Cantor, H., and Boyse, E. A. (1977). *Contemp. Top. Immunobiol.* **7**, 47.
- Cerottini, J. C., Engers, H. D., MacDonald, A. R., and Brunner, K. T. (1974). *J. Exp. Med.* **140**, 703.
- Chan, T. S., Meuth, M., and Green, H. (1974). *J. Cell. Physiol.* **83**, 263.
- Chase, M. W. (1945). *Proc. Soc. Exp. Biol. Med.* **59**, 134.
- Chen, C., and Hirsch, J. G. (1972a). *Science* **176**, 60.
- Chen, C., and Hirsch, J. G. (1972b). *J. Exp. Med.* **136**, 604.
- Chen, D. M., and DiSabato, G. (1976). *Cell. Immunol.* **22**, 211.
- Chen, P. C., Gaetjens, E., and Broome, J. D. (1977). *Immunology* **33**, 391.
- Chervenick, P. A., and LoBuglio, A. F. (1972). *Science* **178**, 164.
- Chestnut, R. W., and Grey, H. M. (1981). *J. Immunol.* **126**, 1075.
- Chestnut, R. W., Endres, R. O., and Grey, H. M. (1980). *Clin. Immunol. Immunopathol.* **15**, 397.
- Chused, T. M., Kassan, S. S., and Mosier, D. E. (1976). *J. Immunol.* **116**, 1579.
- Click, R. E., Benck, L., and Alter B. J. (1972). *Cell. Immunol.* **3**, 156.
- Cline, M. J., and Moore, M. A. S. (1972). *Blood* **39**, 842.
- Cline, M. J., and Sweet, V. C. (1968). *J. Exp. Med.* **128**, 1309.
- Cline, M. J., and Summer, M. A. (1972). *Blood* **40**, 62.
- Cohn, Z. A. (1978). *J. Immunol.* **121**, 813.
- Colten, H. R. (1976). *Adv. Immunol.* **22**, 67.
- Cook, R. G., Vitetta, E. S., Uhr, J. W., and Capra, J. D. (1979). *J. Exp. Med.* **149**, 981.
- Cosenza, H., and Leserman, L. D. (1971). *J. Immunol.* **108**, 418.
- Coutinho, A., and Moller, G. (1975). *Adv. Immunol.* **21**, 113.
- Cowing, C., Schwartz, B. D., and Dickler, H. B. (1978a). *J. Immunol.* **120**, 378.
- Cowing, C., Pincus, S. H., Sachs, D. H., and Dickler, H. B. (1978b). *J. Immunol.* **121**, 1680.
- Cruchaud, A., and Unanue, E. R. (1971). *J. Immunol.* **107**, 1329.
- Cruchaud, A., Berney, M., and Balant, L. (1975). *J. Immunol.* **114**, 102.
- Cudcowicz, G., and Hochman, P. S. (1979). *Immunol. Rev.* **44**, 13.
- Cullen, S. E., David, C. S., Shreffler, D. S., and Nathenson, S. G. (1974). *Proc. Natl. Acad. Sci. U.S.A.* **71**, 648.
- Cullen, S. E., Freed, J. H., and Nathenson, S. G. (1976). *Transplant. Rev.* **30**, 236.
- Dannenberg, A. M., Jr. (1968). *Bacteriol. Rev.* **32**, 85.
- David, C. S., Shreffler, D. C., and Frelinger, J. A. (1973). *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2509.
- David, J. R., and David, R. R. (1972). *Prog. Allergy* **16**, 300.
- Davidson, W. F. (1977). *Immunol. Rev.* **35**, 263.
- Davies, P., Page, R. C., and Allison, A. C. (1974). *J. Exp. Med.* **139**, 1262.
- Davies, P., Bonney, R. J., Humes, J. L., and Kuehl, F. A., Jr. (1980). In "Mononuclear Phagocytes" (R. van Furth, ed.), p. 1317. Nijhoff, The Hague.
- Dayer, J. M., Robinson, D. R., and Krane, S. M. (1977a). *Science* **195**, 181.

- Dayer, J. M., Robinson, D. R., and Krane, S. M. (1977b). *J. Exp. Med.* **145**, 1399.
- Delfraissy, J. F., Galanaud, P., Dormont, J., and Wallon, C. (1977). *J. Immunol.* **118**, 630.
- Demenkoff, J. H., Ansfield, M. J., Kaltreider, H. B., and Adams, E. (1980). *J. Immunol.* **124**, 1365.
- DeRupertis, F. R., Zenser, T. V., Adler, W. H., and Hudson, T. (1974). *J. Immunol.* **113**, 151.
- Desaymard, C., and Feldmann, M. (1975). *Cell. Immunol.* **16**, 106.
- DeVries, J. E., Caviles, A. P., Jr., Bont, W. S., and Mendelsohn, J. (1979). *J. Immunol.* **122**, 1099.
- Diamantstein, T., Vogt, W., Ruhl, H., and Buchert, G. (1973). *Eur. J. Immunol.* **3**, 488.
- Dickler, H. B., Cowing, C., Ahman, G. B., Hathcock, D. L., Sharro, S. O., Hodes, R. J., and Singer A. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 265. Academic Press, New York.
- Dimitro, A., and Fauci, A. S. (1978). *J. Immunol.* **120**, 1818.
- Dinarello, C. S., and Wolff, S. M. (1977). *Inflammation* **2**, 179.
- Dinarello, C. A., Renfer, L., and Wolff, S. M. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4624.
- Djeu, J. Y., Heinbaugh, J. A., Holden, H. T., and Herberman, R. B. (1979a). *J. Immunol.* **122**, 175.
- Djeu, J. Y., Heinbaugh, J. A., Holden, H. T., and Herberman, R. B. (1979b). *J. Immunol.* **122**, 182.
- Dorf, M. E., and Benacerraf, B. (1975). *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3671.
- Dorf, M. E., and Unanue, E. R. (1978). In "Ir Genes and Ia Antigens" (H. O. McDevitt, ed.), p. 171. Academic Press, New York.
- Dorf, M. E., Stimpfling, J. H., and Benacerraf, B. (1975). *J. Exp. Med.* **141**, 1459.
- Drexhage, H. A., Lens, J. W., Dvetanou, J., Kamperdijk, E. W. A., Mullink, R., and Balfour, B. M. (1980). In "Mononuclear Phagocytes" (R. van Furth, ed.), p. 235. Nijhoff, The Hague.
- Droller, M. J., Schneider, M. U., and Perlmann, P. (1978). *Cell. Immunol.* **39**, 165.
- Duclos, H., Galanaud, P., Maillot, M. C., Drevon, M. C., and Dormont, J. (1979). *Scand. J. Immunol.* **9**, 159.
- Eardley, D., Huggenberger, J., McVay-Boudreau, L., Shen, F. W., Gershon, R. K., and Cantor, H. (1978). *J. Exp. Med.* **147**, 1106.
- Economu, J. S., and Shin, H. S. (1978). *J. Immunol.* **121**, 1446.
- Edwards, R. L., and Rickles, F. R. (1980). *Lymphokine Rep.* **1**, 181.
- Edwards, R. L., Rickles, F. R., and Bobrove, A. (1979). *Blood* **54**, 359.
- Ellman, L., Green, I., Martin, W. J., and Benacerraf, B. (1970). *Proc. Natl. Acad. Sci. U.S.A.* **66**, 322.
- Ellner, J. J., and Rosenthal, A. S. (1975). *J. Immunol.* **114**, 1563.
- Ellner, J. J., Lipsky, P. E., and Rosenthal, A. S. (1976). *J. Immunol.* **116**, 876.
- Ellner, J. J., Lipsky, P. E., and Rosenthal, A. S. (1977). *J. Immunol.* **118**, 2053.
- Engers, H. D., and Unanue, E. R. (1973). *J. Immunol.* **110**, 465.
- Erb, P., and Feldmann, M. (1975a). *Nature (London)* **254**, 352.
- Erb, P., and Feldmann, M. (1975b). *Cell. Immunol.* **19**, 356.
- Erb, P., and Feldmann, M. (1975c). *J. Exp. Med.* **142**, 460.
- Erb, P., and Feldmann, M. (1975d). *Eur. J. Immunol.* **5**, 759.
- Erb, P., Feldmann, M., and Hoggs, N. (1976). *Eur. J. Immunol.* **6**, 365.
- Erb, P., Meier, B., and Feldmann, M. (1979a). *J. Immunol.* **122**, 1916.
- Erb, P., Meier, B., Matsunaga, T., and Feldmann, M. (1979b). *J. Exp. Med.* **149**, 686.
- Erb, P., Vogt, P., Matsunaga, T., Rosenthal, A. S., Rees, A., and Feldmann, M. (1980a). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 175. Academic Press, New York.

- Erb, P., Vogt, P., Matsunaga, T., Rosenthal, A. S., and Feldmann, M. (1980b). *J. Immunol.* **124**, 2656.
- Erb, P., Stern, A. C., Alkan, S. S., Studer, S., Zoumbou, E., and Gisler, R. H. (1980c). *J. Immunol.* **125**, 2504.
- Fanger, M. W., Hart, D. A., and Wells, J. V. (1970). *J. Immunol.* **105**, 1043.
- Farr, A. G., Dorf, M. E., and Unanue, E. R. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3542.
- Farr, A. G., Kiely, J.-M., and Unanue, E. R. (1979a). *J. Immunol.* **122**, 2413.
- Farr, A. G., Wechter, W. J., Kiely, J.-M., and Unanue, E. R. (1979b). *J. Immunol.* **122**, 2405.
- Farrar, J. J., Koopman, W. J., and Fuller-Bonar, J. (1977). *J. Immunol.* **119**, 47.
- Farzad, A., Penneys, N. S., Ghaftar, A., Ziboh, V. A., and Schlossberg, J. (1977). *Prostaglandins* **14**, 829.
- Feldman, J. D., and Unanue, E. R. (1971). *Cell. Immunol.* **2**, 275.
- Feldmann, M. (1972). *J. Exp. Med.* **135**, 1049.
- Feldmann, M., and Kontiainen, S. (1976). *Eur. J. Immunol.* **6**, 302.
- Feldmann, M., and Palmer, J. (1971). *Immunology* **21**, 685.
- Fidler, I. J. (1975). *J. Natl. Cancer Inst.* **55**, 1159.
- Fidler, J. M., Chiscon, M. O., and Golub, E. S. (1972). *J. Immunol.* **109**, 136.
- Forman, J., Vitetta, E. S., Hart, D. A., and Klein, J. (1977). *J. Immunol.* **118**, 797.
- Forsum, U., Klareskog, L., and Person, P. A. (1979a). *Scand. J. Immunol.* **9**, 343.
- Forsum, U., Tjernlund, U. M., Rask, L., and Peterson, P. A. (1979b). *Invest. Ophthalmol.* **18**, 310.
- Frelinger, J. A. (1977). *Eur. J. Immunol.* **7**, 447.
- Frelinger, J. G., Hood, L., Hill, S., and Frelinger, J. A. (1979). *Nature (London)* **282**, 321.
- Friedman, S. M., Kuhns, J., Irigoyen, O., and Chess, L. (1979). *J. Immunol.* **123**, 43.
- Gallily, R., and Feldman, M. (1967). *Immunology* **12**, 197.
- Garland, J. B., and Owen, J. J. T. (1978). *Immunology* **34**, 707.
- Geczy, A., de Weck, A. L., Schwartz, B. D., and Shevach, E. M. (1975). *J. Immunol.* **115**, 704.
- Geha, R., Milfrom, H., Broff, M., Alpert, S., Martin, S., and Yunis, E. J. (1979). *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4030.
- Gems, D., Steggermann, L., Menzel, J., and Till, G. (1975). *J. Immunol.* **114**, 1422.
- Gems, D., Seitz, M., Kramer, W., Till, G., and Resch, K. (1978). *J. Immunol.* **120**, 1187.
- Germain, R. N., Pierres, M., and Benacerraf, B. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds), p. 15. Academic Press, New York.
- Gery, I., and Handschumacher, R. E. (1974). *Cell. Immunol.* **11**, 162.
- Gery, I., and Waksman, B. H. (1972). *J. Exp. Med.* **135**, 143.
- Gery, I., Gershon, R. K., and Waksman, B. H. (1972). *J. Exp. Med.* **136**, 128.
- Gidlund, M. A., Orn, A., Wigzell, H., Senik, A., and Gresser, I. (1978). *Nature (London)* **273**, 759.
- Gillis, S., and Smith, K. A. (1977). *Nature (London)* **268**, 154.
- Gillis, S., Germ, M., Ou, W., and Smith, K. A. (1978). *J. Immunol.* **120**, 2027.
- Gmelig-Meyling, F., and Waldmann, T. A. (1981). *J. Immunol.* **126**, 529.
- Goodman, M. G., and Weigle, W. O. (1977). *J. Exp. Med.* **145**, 473.
- Goodwin, J. S., Bankhurst, A. D., and Messner, R. P. (1977). *J. Exp. Med.* **146**, 1719.
- Gordon, S. (1980). In "Mononuclear Phagocytes" (R. van Furth, ed), p. 1273. Nijhoff, The Hague.
- Gordon, S., Todd, J., and Cohn, Z. A. (1974). *J. Exp. Med.* **139**, 1228.
- Gordon, D., Bray, M. A., and Morley, J. (1976). *Nature (London)* **262**, 401.
- Gotze, D., Reisfeld, R. A., and Klein, J. (1973). *J. Exp. Med.* **138**, 1003.
- Green, I., Paul, W. E., and Benacerraf, B. (1967). *J. Exp. Med.* **126**, 959.

- Greineder, D. K., and Rosenthal, A. S. (1975a). *J. Immunol.* **114**, 1541.
Greineder, D. K., and Rosenthal, A. S. (1975b). *J. Immunol.* **115**, 932.
Greineder, D. K., Shevach, E. M., and Rosenthal, A. S. (1976). *J. Immunol.* **117**, 1261.
Greineder, D. K., Connerton, K. J., and David, J. R. (1979). *J. Immunol.* **123**, 2808.
Grimm, W., Seitz, M., Kirchner, H., and Gemsa, D. (1978). *Cell. Immunol.* **40**, 419.
Gronvik, K. O., and Andersson, J. (1980). *Immunol. Rev.* **57**, 35.
Habu, S., and Raff, M. C. (1977). *Eur. J. Immunol.* **7**, 451.
Hammarstrom, L., Smith, C. I. E., and Persson, U. (1978). *Scand. J. Immunol.* **8**, 263.
Hammerling, G. L., Deak, B. D., Mauve, G., Hammerling, U., and McDevitt, H. O. (1974). *Immunogenetics* **1**, 68.
Hammerling, G. L., Mauve, G., Goldberg, E., and McDevitt, H. O. (1975). *Immunogenetics* **1**, 428.
Hardy, B., Globerson, A., and Danon, D. (1973). *Cell. Immunol.* **9**, 282.
Harris, J., MacDonald, W., Engers, H. D., Fitch, F. W., and Cerottini, J.-C. (1976). *J. Immunol.* **116**, 1071.
Hartmann, K. U., Dutton, R. W., McCarthy, M. M., and Mishell, R. E. (1970). *Cell. Immunol.* **1**, 183.
Haskill, J. S., Byrt, P. N., and Marbrook, J. (1970). *J. Exp. Med.* **131**, 57.
Hauptfeld, V., Klein, D., and Klein, J. (1973). *Science* **181**, 167.
Hauser, P., and Vaes, G. (1978). *Biochem. J.* **172**, 275.
Heber-Katz, E., and Click, R. E. (1972). *Cell. Immunol.* **5**, 410.
Hedfords, E., Holm, G., and Pettersson, D. (1975). *Clin. Exp. Immunol.* **22**, 223.
Henney, C. S., Bourne, H. R., and Lichtenstein, L. M., Jr. (1972). *J. Immunol.* **108**, 1256.
Hersch, E. M., and Harris, J. E. (1968). *J. Immunol.* **100**, 1184.
Hewlett, G., Opitz, H. G., Schlumberger, H. D., and Lemke, H. (1977). *Eur. J. Immunol.* **7**, 781.
Hewlett, G., Opitz, H. G., Flad, H. D., and Schlumberger, H. D. (1979). *J. Immunol.* **123**, 2265.
Hirschberg, H., Bergh, O. J., and Thorsby, E. (1980). *J. Exp. Med.* **152**, 249s.
Hodes, R. J., and Singer, A. (1977). *Eur. J. Immunol.* **7**, 892.
Hodes, R. J., Ahmann, G. B., Hathcock, K. S., Dickler, H. B., and Singer, A. (1978). *J. Immunol.* **121**, 1501.
Hodes, R. J., Hathcock, K. S., and Singer, A. (1979). *J. Immunol.* **123**, 2823.
Hoefsmit, E. Ch. M., Kamperdijk, E. W. A., and Balfour, B. M. (1980). In "Mononuclear Phagocytes" (R. van Furth, ed.), p. 1809. Nijhoff, The Hague.
Hoessli, D. C., Jones, A. P., and Waksman, B. H. (1977). *Cell. Immunol.* **30**, 310.
Hoffman-Feizer, G., Gotze, D., Rodt, H., and Thierfelder, S. (1978). *Immunogenetics* **6**, 367.
Hoffman, M. K. (1980). *J. Immunol.* **125**, 2076.
Hoffman, M. K., and Dutton, R. W. (1971). *Science* **172**, 1047.
Hoffman, M. K., and Watson, J. (1979). *J. Immunol.* **122**, 7371.
Hoffman, M. K., Green, S., Old, L. J., and Oettgen, H. F. (1976a). *Nature (London)* **263**, 416.
Hoffman, M. K., Galanos, C., Koenig, S., and Oettgen, H. F. (1976b). *J. Exp. Med.* **146**, 1640.
Hoffman, M. K., Koenig, S., Mittler, R. S., Oettgen, H. F., Ralph, P., Galanos, C., and Hammerling, U. (1979). *J. Exp. Med.* **122**, 497.
Horton, J. E., Oppenheim, J. J., Mergenhagen, S. E., and Raisz, L. G. (1974). *J. Immunol.* **113**, 1278.
Houli, T., Mosher, D., and Vaheri, A. (1977). *J. Exp. Med.* **145**, 1580.
Howie, S., and Feldmann, M. (1978). *Nature (London)* **273**, 664.

- Humes, J. L., Bonney, J. R., Pelus, L., Dahlgren, M. E., Sadowski, S. J., Kuehl, F. A., Jr., and Davies, P. (1977). *Nature (London)* **269**, 149.
- Igarashi, T., Okada, M., Kishimoto, T., and Yamamura, Y. (1977). *J. Immunol.* **118**, 1697.
- Ishizaka, K., and Adachi, T. (1976). *J. Immunol.* **117**, 40.
- Janossy, G., Thomas, J. A., Bollum, F. J., Granger, S., Pizzolo, G., Bradstock, K. F., Wong, L., McMichael, A., Ganeshhaguru, K., and Hoffbrand, A. V. (1980). *J. Immunol.* **125**, 202.
- Jenkinson, E. J., Owen, J. J. T., and Aspinwall, R. (1980). *Nature (London)* **284**, 1771.
- Jerne, N. K. (1971). *Eur. J. Immunol.* **1**, 1.
- Johnston, R. B., Jr., Chadwick, D. A., and Pabst, M. J. (1980). In "Mononuclear Phagocytes" (R. van Furth, ed.), p. 1143. Nijhoff, The Hague.
- Jones, P. P., Murphy, D. B., and McDevitt, H. O. (1978). *J. Exp. Med.* **148**, 925.
- Julius, M. H., Simpson, E., and Herzenberg, L. A. (1973). *Eur. J. Immunol.* **3**, 645.
- Kammer, G. M., and Unanue, E. R. (1980). *Clin. Immunol. Immunopathol.* **15**, 434.
- Kamperdijk, E. W. A., and Hoefsmit, E. (1978). *Ultramicroscopy* **3**, 137 (cited by Thorbecke *et al.*, 1980).
- Kamperdijk, E. W. A., Raaymakers, E. M., de Leeuw, J. H. S., and Hoefsmit, E. (1978). *Cell. Tissue Res.* **192**, 1.
- Kantor, F. S., Ojeda, A., and Benacerraf, B. (1963). *J. Exp. Med.* **117**, 55.
- Kappler, J. W., and Marrack, P. (1976). *Nature (London)* **262**, 797.
- Kappler, J. W., and Marrack, P. (1978). *J. Exp. Med.* **148**, 1510.
- Karnovsky, M. L., and Lazdins, J. K. (1978). *J. Immunol.* **121**, 809.
- Karnovsky, M. L., Lazdins, J. K., Drath, D., and Harper, A. (1975). *Ann. N. Y. Acad. Sci.* **256**, 266.
- Kasahara, T., and Shiori-Nakano, K. (1976). *J. Immunol.* **116**, 1251.
- Katz, D. H. (1977). In "Lymphocyte Differentiation, Recognition, and Regulation." Academic Press, New York.
- Katz, D. H., and Unanue, E. R. (1973). *J. Exp. Med.* **137**, 967.
- Katz, D. H., Hamaoka, T., Dorf, M. E., Maurer, P. H., and Benacerraf, B. (1973). *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2624.
- Katz, D. H., Skidmore, B. J., Katz, L. R., and Bogowitz, C. A. (1978). *J. Exp. Med.* **148**, 727.
- Katz, S. I., Tamaki, K., and Sachs, D. H. (1979). *Nature (London)* **282**, 334.
- Kedar, E., Schwartzbach, M., Gaanan, Z., and Hefetz, S. (1977). *J. Immunol. Methods* **16**, 39.
- Keightley, R. G., Lawton, A. R., and Cooper, M. D. (1976). *J. Immunol.* **117**, 1538.
- Kelly, R. H., Balfour, B. M., Armstrong, J. A., and Griffiths, S. (1978). *Anat. Rec.* **190**, 5.
- Kennard, J., and Zolla-Panzer, S. (1980). *J. Immunol.* **124**, 268.
- Kincade, P. W., Paige, C. J., Parkhouse, M. E., and Lee, G. (1978). *J. Immunol.* **120**, 1289.
- Kindred, B., and Shreffler, D. C. (1972). *J. Immunol.* **109**, 940.
- Kirkland, T. N., Sieckmann, D. G., Longo, D. L., and Mosier, D. E. (1980). *J. Immunol.* **124**, 1721.
- Klarskog, L., Malmnas-Tjernlund, U., Forsum, U., and Peterson, P. A. (1977). *Nature (London)* **268**, 248.
- Klaus, G. B. (1974). *Cell. Immunol.* **10**, 483.
- Klaus, G. G. B., Humphrey, J. H., Kunkl, A., and Dongworth, D. W. (1980). *Immunol. Rev.* **53**, 3.
- Klebanoff, S. J. (1975). *Sem. Hematol.* **12**, 117.
- Klebanoff, S. J. (1980). In "Mononuclear Phagocytes" (R. van Furth, ed.), p. 1105. Nijhoff, The Hague.
- Klein, J. (1975). "The Biology of the Mouse Histocompatibility-2 Complex." Springer-Verlag, Berlin and New York.

- Klein, J., and Hauptfeld, V. (1976). *Transplant. Rev.* **30**, 83.
Klimetzeck, V., and Sorg, C. (1976). *Cell. Immunol.* **27**, 350.
Klimetzeck, V., and Sorg, C. (1977). *Eur. J. Immunol.* **7**, 185.
Klimpel, G. R., and Henney, C. S. (1978). *J. Immunol.* **120**, 563.
Klimpel, G. R., Okada, M., and Henney, C. S. (1979). *J. Immunol.* **123**, 350.
Knapp, W., and Baumgartner, G. (1978). *J. Immunol.* **121**, 1177.
Kojima, A., Tamura, S. I., and Egashira, Y. (1979). *J. Immunol.* **109**, 940.
Komura, K., and Boyse, E. A. (1973). *Lancet* **1**, 740.
Konda, S., Stockert, E., and Smith, R. (1973). *Cell. Immunol.* **7**, 275.
Koopman, W. J., Farrar, J. J., Oppenheim, J. J., Fuller-Bonar, J., and Dougherty, S. (1977). *J. Immunol.* **119**, 55.
Koren, H. S., and Hodes, R. J. (1977). *Eur. J. Immunol.* **7**, 394.
Koster, F. T., McGregor, D. D., and MacKaness, G. B. (1971). *J. Exp. Med.* **133**, 400.
Kunin, S., Shearer, G. M., Globerson, A., and Feldman, M. (1972). *Cell. Immunol.* **5**, 288.
Kurland, J. I., and Bockman, R. (1978). *J. Exp. Med.* **147**, 952.
Kurland, J. I., and Moore, M. A. S. (1977). In "Experimental Hematology Today" (S. J. Braun and G. L. Lefney, eds.), p. 51.
Kurland, J. I., Kincade, P. W., and Moore, M. A. S. (1977). *J. Exp. Med.* **146**, 1420.
Kurland, J. I., Bockman, R. S., Broxmeyer, H. E., and Moore, M. A. S. (1978). *Science* **199**, 552.
Lachmann, L. B., Hacker, M. P., and Handschumacher, R. E. (1977). *J. Immunol.* **119**, 2019.
Lamvik, J. O. (1969). *Scand. J. Haematol.* **6**, 149.
Landahl, C. A. (1976). *Eur. J. Immunol.* **6**, 130.
Lane, F. C., and Unanue, E. R. (1972). *J. Exp. Med.* **135**, 1104.
Landolfo, S., Herberman, R. B., and Holden, H. T. (1977). *J. Immunol.* **118**, 1244.
Landsteiner, K., and Chase, M. W. (1942). *Proc. Soc. Exp. Biol. Med.* **49**, 688.
Larsson, E. L. (1978). *Scand. J. Immunol.* **8**, 465.
Larsson, E. L., Coutinho, A., and Martinez, C. (1980a). *Immunol. Rev.* **51**, 61.
Larsson, E. L., Iscove, N. N., and Coutinho, A. (1980b). *Nature (London)* **283**, 664.
Lee, K. C., and Wong, M. (1980). *J. Immunol.* **125**, 86.
Lee, K. C., Shiogawa, C., Shaw, A., and Diener, E. (1976). *Eur. J. Immunol.* **6**, 63.
Lee, K. C., Wilkinson, A., and Wong, M. (1979). *Cell. Immunol.* **48**, 79.
Lee, S. T., and Paraskevas, F. (1979). *Cell. Immunol.* **48**, 1.
Lefford, M. J. (1975). *Annu. Rev. Resp. Dis.* **111**, 243.
Leibovich, S. J., and Ross, R. (1976). *Am. J. Pathol.* **84**, 501.
Lemke, H., and Opitz, H. B. (1976). *J. Immunol.* **117**, 388.
Levis, W. R., and Robbins, J. H. (1970). *Exp. Cell Res.* **61**, 153.
Levy, G. A., and Edgington, T. S. (1980). *J. Exp. Med.* **151**, 1232.
Lipscomb, M. F., Ben-Sasson, S. Z., and Uhr, J. W. (1978). *J. Immunol.* **118**, 1748.
Lipscomb, M. F., Toews, G. B., Lyons, C. R., and Uhr, J. W. (1981). *J. Immunol.* **126**, 286.
Lipsky, P. E., and Rogoff, T. M. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 333. Academic Press, New York.
Lipsky, P. E., and Rosenthal, A. S. (1973). *J. Exp. Med.* **138**, 900.
Lipsky, P. E., and Rosenthal, A. S. (1975a). *J. Exp. Med.* **141**, 138.
Lipsky, P. E., and Rosenthal, A. S. (1975b). *J. Immunol.* **115**, 440.
Lipsky, P. E., Ellner, J. J., and Rosenthal, A. S. (1976). *J. Immunol.* **116**, 868.
Littman, B. H., and Ruddy, S. (1977). *J. Exp. Med.* **145**, 1344.
Loblay, R. H., Schroer, J., and Rosenthal, A. S. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 87. Academic Press, New York.
Lohrman, H., Novikors, L., and Graw, R. G. (1974). *J. Exp. Med.* **139**, 1153.
Lomnitzer, R., Rabson, A. R., and Koornhoff, H. J. (1976). *Cell. Immunol.* **24**, 42.

- Loor, F. (1979). *Immunology* **37**, 157.
- Lopez, L. R., Johansen, K. S., Radovich, J., and Talmage, D. W. (1974). *J. Allergy Clin. Immunol.* **53**, 336.
- Lopez, L. R., Vatter, A. E., and Talmage, D. W. (1977). *J. Immunol.* **119**, 1668.
- Lu, C. Y., Calamai, E. G., and Unanue, E. R. (1979). *Nature (London)* **282**, 327.
- Lu, C. Y., Beller, D. I., and Unanue, E. R. (1980). *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1597.
- Ly, I. A., and Mishell, R. A. (1974). *J. Immunol. Methods* **5**, 239.
- Lyon, C. R., Tucker, T. F., and Uhr, J. W. (1979). *J. Immunol.* **122**, 1598.
- Macher, E., and Chase, M. W. (1969). *J. Exp. Med.* **129**, 103.
- McAuslan, B. R. (1963). *Virology* **21**, 383.
- McDevitt, H. O., and Chinitz, A. (1969). *Science* **163**, 1207.
- McDevitt, H. O., Deak, B. D., Shreffler, D. C., Klein, J., Stimpfling, J. H., and Snell, G. D. (1972). *J. Exp. Med.* **135**, 1259.
- McDougal, J. S., and Cort, S. P. (1978). *J. Immunol.* **120**, 445.
- McDougal, J. S., and Gordon, D. S. (1977). *J. Exp. Med.* **145**, 676.
- McGregor, D. D., Koster, F. T., and MacKaness, G. B. (1971). *J. Exp. Med.* **133**, 389.
- MacKaness, G. B. (1962). *J. Exp. Med.* **116**, 381.
- MacKaness, G. B., and Blanden, R. V. (1967). *Prog. Allergy* **11**, 89.
- Martin, W. J., Ellman, L., Green, I., and Benacerraf, B. (1970). *J. Exp. Med.* **132**, 1259.
- Meltzer, M. S., and Oppenheim, J. J. (1977). *J. Immunol.* **118**, 77.
- Metcalf, D. (1976). *J. Immunol.* **116**, 635.
- Metcalf, D., Nossal, G. V. J., Warner, N. L., Miller, J. F. A. P., Mandel, T. E., Layton, J. E., and Gutman, G. A. (1975). *J. Exp. Med.* **142**, 1534.
- Metzger, Z., Hoffeld, J. T., and Oppenheim, J. J. (1980). *J. Immunol.* **124**, 983.
- Miller, J. F. A. P., Vadas, M. A., Whitelaw, A., and Gamble, J. (1975). *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5095.
- Miller, J. F. A. P., Vadas, M. A., Whitelaw, A., and Gamble, J. (1976). *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2486.
- Miller, J. F. A. P., Vadas, M. A., Whitelaw, A., Gamble, J., and Bernard, C. (1977). *J. Exp. Med.* **145**, 1623.
- Miller, J. F. A. P., Gamble, J., Mottram, P., and Smith, F. I. (1979). *Scand. J. Immunol.* **9**, 29.
- Mills, G., Monticone, V., and Paetkau, V. (1976). *J. Immunol.* **117**, 1325.
- Minami, M., Shreffler, D. C., and Cowing, C. (1980). *J. Immunol.* **124**, 1314.
- Mizel, S. B. (1979). *J. Immunol.* **122**, 2167.
- Mizel, S. B., and Rosenstreich, D. L. (1979). *J. Immunol.* **122**, 2173.
- Mizel, S. B., Oppenheim, J. J., and Rosenstreich, D. L. (1978a). *J. Immunol.* **120**, 1497.
- Mizel, S. B., Oppenheim, J. J., and Rosenstreich, D. L. (1978b). *J. Immunol.* **120**, 1504.
- Mizel, S. B., Rosenstreich, D. L., and Oppenheim, J. J. (1978c). *Cell. Immunol.* **40**, 230.
- Moller, G., Lemke, H., and Opitz, H. G. (1976). *Scand. J. Immunol.* **5**, 269.
- Morgan, E. L., and Weigle, W. O. (1979a). *J. Exp. Med.* **150**, 256.
- Morgan, E. L., and Weigle, W. O. (1979b). *J. Exp. Med.* **151**, 1.
- Morgan, E. L., and Weigle, W. O. (1980). *J. Immunol.* **124**, 1330.
- Morris, N. R., and Fischer, G. A. (1963). *Biochim. Biophys. Acta* **68**, 84.
- Mosier, D. E. (1967). *Science* **158**, 1573.
- Mosier, D. E. (1969). *J. Exp. Med.* **129**, 351.
- Mosier, D. E., and Pierce, C. W. (1972). *J. Exp. Med.* **136**, 1484.
- Mosier, D. E., Johnson, B. M., Paul, W. E., and McMaster, P. R. B. (1974). *J. Exp. Med.* **139**, 1354.
- Mottram, P. L., and Miller, J. F. A. P. (1980). *Eur. J. Immunol.* **10**, 165.
- Murphy, P. A., Simon, P. L., and Willoughby, W. F. (1980). *J. Immunol.* **124**, 2498.
- Nadler, P. I., Klingenstein, R. J., and Hodes, R. J. (1980). *J. Immunol.* **125**, 914.

- Nakano, K., Hosokawa, T., and Muramatsu, S. (1978). *Dev. Comp. Immunol.* **2**, 505.
- Nathan, C. F. (1980). In "Mononuclear Phagocytes" (R. van Furth, ed.), p. 1165. Nijhoff, The Hague.
- Nathan, C. F., Brukner, L. H., Silverstein, S. C., and Cohn, Z. A. (1978). *J. Exp. Med.* **149**, 84.
- Nelson, D. S. (1973). *Nature (London)* **246**, 306.
- Nelson, R. D., and Leu, R. W. (1975). *J. Immunol.* **114**, 606.
- Neumann, C., and Sorg, C. (1977). *Eur. J. Immunol.* **7**, 720.
- Niederhuber, J. E. (1978). *Immunol. Rev.* **40**, 28.
- Niederhuber, J. E. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 197. Academic Press, New York.
- Niederhuber, J. E., and Allen, P. (1980). *J. Exp. Med.* **151**, 1103.
- Niederhuber, J. E., Frelinger, J. A., Dine, M. S., Shoffner, P., Dugan, E., and Shreffler, D. C. (1976). *J. Exp. Med.* **143**, 372.
- Niederhuber, J. E., Allan, P., and Mayo, L. (1979). *J. Immunol.* **122**, 1342.
- Nielsen, M. H., Jensen, H., Braendstrup, O., and Werdelin, O. (1974). *J. Exp. Med.* **140**, 1260.
- Nordin, A. A. (1978). *Eur. J. Immunol.* **8**, 776.
- North, R. J. (1969). *J. Exp. Med.* **130**, 299.
- North, R. J. (1978). *J. Immunol.* **121**, 806.
- Nossal, G. J. V., and Ada, G. L. (1971). In "Antigens, Lymphoid Cells, and the Immune Response." Academic Press, New York.
- Novogrodsky, A., and Katchalski, E. (1972). *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3207.
- Nussenzweig, M. C., and Steinman, R. M. (1980). *J. Exp. Med.* **151**, 1196.
- Oehler, J. R., and Herberman, R. B. (1977). *J. Immunol.* **119**, 1785.
- Ohishi, M., and Onoue, K. (1975). *Cell. Immunol.* **18**, 220.
- Oi, V. T., Jones, P. P., Goding, J. W., Herzenberg, L. A., and Herzenberg, L. A. (1978). In "Lymphocyte Hybridomas" (F. Melchers, M. Potter, and N. Warner, eds.), p. 115. Plenum, New York.
- Opitz, H. G., Niethammer, D., Lemke, H., Flad, H. D., and Huget, R. (1975a). *Cell. Immunol.* **16**, 379.
- Opitz, H. G., Niethammer, D., and Jackson, R. C. (1975b). *Cell. Immunol.* **18**, 70.
- Opitz, H. G., Opitz, U., Lemke, H., Hewlett, G., Schreml, S., and Flad, H. D. (1977a). *J. Exp. Med.* **145**, 1029.
- Opitz, H. H., Opitz, U., Lemke, H., Flad, H. D., Hewlett, G., and Schlumberger, H. D. (1977b). *J. Immunol.* **119**, 2089.
- Opitz, H. G., Lemke, H., and Hewlett, G. (1978). *Immunol. Rev.* **40**, 53.
- Opitz, H. G., Hewlett, G., and Schlumberger, H. D. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 415. Academic Press, New York.
- Oppenheim, J. J., Leventhal, B. G., and Hersch, E. M. (1968). *J. Immunol.* **101**, 262.
- Oppenheim, J. J., Northoff, H., Greenhill, A., Mathieson, B. J., Smith, K., and Gillis, S. (1980). In "Characterization of Lymphokines" (A. de Weck, F. Kristensen, and M. Landy, eds.), p. 399.
- O'Toole, M. M., and Wortis, H. H. (1980). *J. Immunol.* **124**, 2010.
- Paetkau, V. (1980). *Immunol. Rev.* **51**, 157.
- Paetkau, V., Mills, G., Gerhart, S., and Monticone, V. (1976). *J. Immunol.* **117**, 1320.
- Page, R. C., Davies, P., and Allison, A. C. (1978). *Int. Rev. Cytol.* **52**, 119.
- Pantalone, R. M., and Page, R. C. (1975). *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2091.
- Papamichael, M., Gutierrez, C., Embling, P., Johnson, P., Holborow, E., and Pepys, M. (1975). *Scand. J. Immunol.* **4**, 343.
- Paswell, J. H., Dayer, J. M., and Merler, E. (1979). *J. Immunol.* **123**, 115.

- Paul, W. E., Shevach, E. M., Pickeral, S., Thomas, D. W., and Rosenthal, A. S. (1977). *J. Exp. Med.* **145**, 618.
- Pelus, L. M., and Bockman, R. S. (1979). *J. Immunol.* **123**, 2118.
- Pelus, L. M., Broxmeyer, H. E., Kurland, J. I., and Moore, M. A. S. (1979). *J. Exp. Med.* **150**, 277.
- Persson, U., Hammarstrom, L., and Smith, C. I. E. (1977). *J. Immunol.* **119**, 1138.
- Persson, U., Hammarstrom, L., Moller, E., Moller, G., and Smith, C. I. E. (1978). *Immunol. Rev.* **40**, 78.
- Petri, J., Braendstrup, O., and Werdelin, O. (1978). *Cell. Immunol.* **35**, 427.
- Petri, J., Braendstrup, O., and Werdelin, O. (1979). *Scand. J. Immunol.* **10**, 493.
- Pettinelli, C. B., Ahmann, G. B., and Shearer, G. M. (1980). *J. Immunol.* **124**, 1911.
- Pierce, C. W. (1969). *J. Exp. Med.* **130**, 345.
- Pierce, C. W., and Aune, T. M. (1981). In "Advances in Immunopharmacology" (J. Hadden and P. Mullen, eds.), p. 397. Pergamon, Oxford.
- Pierce, C. W., and Benacerraf, B. (1969). *Science* **166**, 1002.
- Pierce, C. W., and Malek, T. R. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 35. Academic Press, New York.
- Pierce, C. W., Kapp, J. A., Wood, D. D., and Benacerraf, B. (1974). *J. Immunol.* **112**, 1181.
- Pierce, C. W., Kapp, J. A., and Benacerraf, B. (1976). *J. Exp. Med.* **144**, 371.
- Pierres, M., and Germain, R. N. (1978). *J. Immunol.* **121**, 1306.
- Pledger, V. J., Stiles, C. D., Antoniades, H. N., and Sher, C. D. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4481.
- Plescia, O. J., Smith, A. H., and Greenwich, K. (1975). *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1848.
- Polverini, P. J., Cotran, R. S., Gimbrone, M. A., Jr., and Unanue, E. R. (1977). *Nature (London)* **269**, 804.
- Ptak, W., Naidorf, K. F., and Gershon, R. K. (1977). *J. Immunol.* **119**, 444.
- Ptak, W., Zembala, M., and Gershon, R. K. (1978a). *J. Exp. Med.* **148**, 424.
- Ptak, W., Zembala, Hanczakowski-Newicka, M., and Ssherson, G. L. (1978b). *Eur. J. Immunol.* **8**, 645.
- Puri, J., and Lonai, P. (1980). *Eur. J. Immunol.* **10**, 273.
- Radcliffe, G. N., and Axelrod, M. A. (1971). *J. Exp. Med.* **133**, 846.
- Raff, H. V., Picker, L. J., and Stobo, J. D. (1980). *J. Exp. Med.* **152**, 581.
- Raviola, E. (1975). In "A Textbook of Histology" (W. Bloom and D. Fawcett, eds.), p. 457. Saunders, Philadelphia.
- Richman, L. K., Klingenstein, R. J., Richman, J. A., Strober, W., and Berzofsky, J. A. (1979). *J. Immunol.* **123**, 2602.
- Richman, L. K., Strober, W., and Berzofsky, J. A. (1980). *J. Immunol.* **124**, 619.
- Rigaud, M., Durane, J., and Breton, J. C. (1978). *Biochim. Biophys. Acta* **573**, 408.
- Riisgaard, S., Rhodes, J. M., and Bennedsen, J. (1978). *Scand. J. Immunol.* **7**, 209.
- Rode, H. N., and Gordon, J. (1974). *J. Immunol.* **111**, 1270.
- Rodey, G. E., Luehrman, L. K., and Thomas, D. W. (1979). *J. Immunol.* **123**, 2250.
- Rogoff, T. M., and Lipsky, P. E. (1979). *J. Immunol.* **123**, 1921.
- Rogoff, T. M., and Lipsky, P. E. (1980). *J. Immunol.* **124**, 1740.
- Roseman, J. (1969). *Science* **165**, 1125.
- Rosenberg, S. A., and Lipsky, P. E. (1979). *J. Immunol.* **122**, 926.
- Rosenstreich, D. L., and Mizel, S. B. (1978). *Immunol. Rev.* **40**, 102.
- Rosenstreich, D. L., and Rosenthal, A. S. (1973). *J. Immunol.* **110**, 934.
- Rosenstreich, D. L., Blake, J. T., and Rosenthal, A. S. (1971). *J. Exp. Med.* **134**, 1170.
- Rosenstreich, D. L., Farrar, J. J., and Dougherty, S. (1976). *J. Immunol.* **116**, 131.
- Rosenthal, A. S. (1978). *Immunol. Rev.* **40**, 136.
- Rosenthal, A. S., and Shevach, E. M. (1973). *J. Exp. Med.* **138**, 1194.

- Rosenthal, A. S., Barcinski, M. A., and Blake, J. T. (1977). *Nature (London)* **267**, 156.
- Rosenwasser, L. J., and Rosenthal, A. S. (1978a). *J. Immunol.* **120**, 1991.
- Rosenwasser, L. J., and Rosenthal, A. S. (1978b). *J. Immunol.* **121**, 2497.
- Rosenwasser, L. J., and Rosenthal, A. S. (1979a). *J. Immunol.* **123**, 471.
- Rosenwasser, L. J., and Rosenthal, A. S. (1979b). *J. Immunol.* **123**, 1141.
- Rosenwasser, L. J., Dinarello, C. A., and Rosenthal, A. S. (1980). *J. Exp. Med.* **150**, 709.
- Rouse, van Ewijk, W., Jones, P. P., and Weissman, I. L. (1979). *J. Immunol.* **122**, 2508.
- Rowden, G., Lewis, M. G., and Sullivan, A. K. (1977). *Nature (London)* **268**, 247.
- Rowden, G., Phillips, T. M., and Delovitch, T. L. (1978). *Immunogenetics* **7**, 465.
- Sachs, D. H., and Cone, J. L. (1973). *J. Exp. Med.* **138**, 1289.
- Salvin, S. B., and Nishio, J. (1969). *J. Immunol.* **103**, 138.
- Salvin, S. B., Sell, S., and Nishio, J. (1971). *J. Immunol.* **107**, 655.
- Schechter, B., Treves, A. J., and Feldman, M. (1976). *J. Natl. Cancer Inst.* **56**, 975.
- Schechter, G. P., and MacFarland, W. (1970). *J. Immunol.* **105**, 661.
- Scher, M. S., Beller, D. I., and Unanue, E. R. (1980). *J. Exp. Med.* **152**, 1684.
- Scher, M. S., Beller, D. I., and Unanue, E. R. (1981). Submitted.
- Schmidtko, J. R., and Dixon, J. F. (1972a). *J. Immunol.* **108**, 1624.
- Schmidtko, J. R., and Dixon, J. F. (1972b). *J. Exp. Med.* **136**, 392.
- Schmidtko, J. R., and Hatfield, S. (1976). *J. Immunol.* **116**, 357.
- Schmidtko, J. R., and Unanue, E. R. (1971a). *J. Immunol.* **107**, 331.
- Schmidtko, J. R., and Unanue, E. R. (1971b). *Nature (London)* **233**, 84.
- Schrader, J. W. (1973). *J. Exp. Med.* **138**, 1466.
- Schultz, R. M., Papamatheakis, J. D., and Chirigos, M. A. (1977). *Science* **197**, 674.
- Schwartz, B. D., Paul, W. R., and Shevach, E. M. (1976). *Transplant. Rev.* **30**, 174.
- Schwartz, R. H., and Paul, W. E. (1976). *J. Exp. Med.* **143**, 529.
- Schwartz, R. H., Dickler, H. B., Sachs, D. H., and Schwartz, B. D. (1976). *Scand. J. Immunol.* **5**, 731.
- Schwartz, R. H., Yano, A., and Paul, W. E. (1978). *Immunol. Rev.* **40**, 153.
- Schwartz, R. H., Yano, A., Stimpfling, J. H., and Paul, W. E. (1979). *J. Exp. Med.* **149**, 40.
- Scott, M. T. (1972). *Cell. Immunol.* **5**, 459.
- Scott, W. A., Zrike, J. M., Hamill, A. L., Kempe, J., and Cohn, Z. A. (1980). *J. Exp. Med.* **152**, 324.
- Seeger, R. C., and Oppenheim, J. J. (1970). *J. Exp. Med.* **132**, 44.
- Seeger, R. C., and Oppenheim, J. J. (1972). *J. Immunol.* **109**, 244.
- Sela, M. (1969). *Science* **166**, 1365.
- Shelley, W. B., and Juhlin, I. (1977). *Arch. Dermatol.* **113**, 187.
- Sherr, D. H., Heghinian, K. M., Benacerraf, B., and Dorf, M. E. (1980). *J. Immunol.* **124**, 1389.
- Shevach, E. M. (1976). *J. Immunol.* **116**, 1482.
- Shevach, E. M. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 59. Academic Press, New York.
- Shevach, E. M., and Rosenthal, A. S. (1973). *J. Exp. Med.* **138**, 1213.
- Shevach, E. M., Paul, W. E., and Green, I. (1972). *J. Exp. Med.* **136**, 1207.
- Shirrmacher, V., Pena-Martinez, J., and Festenstein, H. (1975). *Nature (London)* **255**, 155.
- Shortman, K., and Palmer, J. (1971). *Cell. Immunol.* **2**, 399.
- Shortman, K., Diener, E., Russell, P., and Armstrong, W. D. (1970). *J. Exp. Med.* **131**, 461.
- Shreffler, D. C., and David, C. S. (1975). *Adv. Immunol.* **20**, 125.
- Sidman, C. L., and Unanue, E. R. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2401.
- Sidman, C. L., and Unanue, E. R. (1979). *J. Immunol.* **122**, 406.
- Sieckman, D. G., Aszofsky, R., Mosier, D. E., Zitron, I. M., and Paul, W. E. (1978). *J. Exp. Med.* **147**, 814.

- Siegel, I. (1970a). *J. Allergy* **46**, 190.
- Siegel, I. (1970b). *J. Immunol.* **105**, 879.
- Silberberg, I., Baer, R. L., Rosenthal, S. A., Thorbecke, G. J., and Berezowsky, V. (1975). *Cell. Immunol.* **32**, 400.
- Silberberg, I., Baer, R. L., and Rosenthal, S. A. (1976). *J. Invest. Dermatol.* **66**, 210.
- Silberberg-Sinakin, I., and Thorbecke, G. J. (1980). *J. Invest. Dermatol.* **75**, 61.
- Silberberg-Sinakin, I., Thorbecke, G. J., Baer, R. L., Rosenthal, S. A., and Berezowsky, V. (1976). *Cell. Immunol.* **25**, 137.
- Silberberg-Sinakin, I., Baer, R. L., and Thorbecke, G. J. (1978). *Prog. Allergy* **24**, 268.
- Singer, A., Dickler, H. B., and Hodes, R. J. (1977). *J. Exp. Med.* **146**, 1096.
- Singer, A., Cowing, C., Hatchcock, K. A., Dickler, H. B., and Hodes, J. J. (1978). *J. Exp. Med.* **147**, 1611.
- Singer, A., Hatchcock, K. S., and Hodes, R. J. (1979). *J. Exp. Med.* **149**, 1208.
- Sjoberg, O., Andersson, J., and Moller, G. (1972). *Eur. J. Immunol.* **2**, 123.
- Small, M., and Trainin, N. (1976). *J. Immunol.* **117**, 292.
- Smith, C. I. E., and Hammarstrom, L. (1978). *J. Immunol.* **121**, 823.
- Smith, J. W., Steiner, A. L., and Parker, C. W. (1971). *J. Clin. Invest.* **50**, 442.
- Smith, K. A. (1980). *Immunol. Rev.* **51**, 337.
- Smith, K. A., Lachman, L. A., Oppenheim, J. J., and Favata, M. L. (1980). *J. Exp. Med.* **151**, 1551.
- Smith, T. J., and Wagner, R. R. (1967). *J. Exp. Med.* **125**, 559.
- Snell, G. D. (1978). *Immunol. Rev.* **38**, 3.
- Snyder, D. S., Lu, C. Y., and Unanue, E. R. (1981). *Fed. Proc.* **40**, 4520.
- Sokolowski, J., Jakobsen, E., and Johannessen, J. V. (1978). *Lymphology* **11**, 202.
- Spitznagel, J. K., and Allison, A. C. (1971). *J. Immunol.* **104**, 128.
- Sprent, J. (1978a). *J. Exp. Med.* **147**, 1142.
- Sprent, J. (1978b). *J. Exp. Med.* **147**, 1159.
- Sprent, J. (1978c). *J. Exp. Med.* **147**, 1838.
- Sprent, J. (1980a). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.) p. 97. Academic Press, New York.
- Sprent, J. (1980b). *J. Exp. Med.* **152**, 996.
- Spry, C. J., Pflug, J., Janossy, G., and Humphrey, J. H. (1980). *Clin. Exp. Immunol.* **39**, 750.
- Stadecker, M. J., and Unanue, E. R. (1979). *J. Immunol.* **123**, 568.
- Stadecker, M. J., Calderon, J., Karnovsky, M. L., and Unanue, E. R. (1977). *J. Immunol.* **119**, 1738.
- Steeg, P. S., Moore, R. N., and Oppenheim, J. J. (1980). *J. Exp. Med.* **152**, 1734.
- Steinberg, R. A., Levinson, B. B., and Tomkins, G. M. (1975). *Cell* **5**, 29.
- Steinman, R. M., and Cohn, Z. A. (1973). *J. Exp. Med.* **137**, 1142.
- Steinman, R. M., and Cohn, Z. A. (1974). *J. Exp. Med.* **139**, 380.
- Steinman, R. M., and Witmer, M. G. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5132.
- Steinman, R. M., Lustig, D., and Cohn, Z. A. (1974). *J. Exp. Med.* **139**, 1431.
- Steinman, R. M., Adams, J., and Cohn, Z. A. (1975). *J. Exp. Med.* **141**, 804.
- Steinman, R. M., Kaplan, G., Witmer, M. D., and Cohn, Z. A. (1979). *J. Exp. Med.* **149**, 1.
- Steinman, R. M., Noqueira, N., Witmer, M. D., Tydings, J. D., and Mellman, I. S. (1980). *J. Exp. Med.* **152**, 1248.
- Stenson, W. F., and Parker, C. W. (1980). *J. Immunol.* **125**, 1.
- Stern, A. C., Erb, P., and Gissler, R. H. (1979). *J. Immunol.* **123**, 612.
- Stewart, C. C. (1980). In "Mononuclear Phagocytes—Functional Aspects" (R. van Furth, ed.), p. 377. Nijhoff, The Hague.

- Stingl, G., Wolff-Schreiner, E. C., Pichler, W. J., Schnait, F. G., Knapp, W., and Wolff, K. (1977). *Nature (London)* **268**, 245.
- Stingl, G., Katz, S. I., Clement, L., Green, I., and Shevach, E. M. (1978a). *J. Immunol.* **121**, 2005.
- Stingl, G., Katz, S. I., Shevach, E. M., Rosenthal, A. S., and Green, I. (1978b). *J. Invest. Dermatol.* **71**, 59.
- Stingl, G., Katz, S. I., Shevach, E. M., Wolff-Schreiner, E. C., and Green, I. (1978c). *J. Immunol.* **120**, 570.
- Stingl, G., Katz, S. I., Clement, L., Green, I., and Shevach, E. M. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 285. Academic Press, New York.
- Suzuki, K., Fatham, C. G., and Tomasi, T. B., Jr. (1979). *J. Immunol.* **123**, 1530.
- Swierkosz, J. E., Roch, K., Marrack, P., and Kappler, J. W. (1978). *J. Exp. Med.* **147**, 554.
- Swierkosz, J. E., Marrack, P., and Kappler, J. W. (1979). *J. Immunol.* **123**, 654.
- Tadakuma, T., and Pierce, C. W. (1976). *J. Immunol.* **117**, 967.
- Tadakuma, T., and Pierce, C. W. (1978). *J. Immunol.* **120**, 481.
- Tadakuma, T., Kuhner, A. L., Rich, R. R., David, J. R., and Pierce, C. W. (1976). *J. Immunol.* **117**, 323.
- Talmage, D. W., and Hemmingsen, H. (1975). *J. Allergy Clin. Immunol.* **55**, 442.
- Tamaki, K., Stingl, G., Gullino, M., Sachs, D. H., and Katz, S. I. (1979). *J. Immunol.* **123**, 784.
- Taniguchi, N., Miyawaki, T., Moriya, N., Nagaoki, T., Kato, E., and Kuda, N. O. (1977). *J. Immunol.* **118**, 193.
- Taniyama, T., and Holden, H. T. (1979). *J. Immunol.* **123**, 43.
- Theis, G. A., and Thorbecke, G. J. (1970). *J. Exp. Med.* **131**, 970.
- Thomas, D. W. (1978). *J. Immunol.* **121**, 1760.
- Thomas, D. W., and Shevach, E. M. (1976). *J. Exp. Med.* **144**, 1263.
- Thomas, D. W., and Shevach, E. M. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2104.
- Thomas, D. W., and Shevach, E. M. (1978a). *J. Immunol.* **121**, 1145.
- Thomas, D. W., and Shevach, E. M. (1978b). *J. Immunol.* **121**, 1152.
- Thomas, D. W., and Wilner, G. D. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 47. Academic Press, New York.
- Thomas, D. W., Forni, G., Shevach, E. M., and Green, I. (1977a). *J. Immunol.* **118**, 1677.
- Thomas, D. W., Yamashita, U., and Shevach, E. M. (1977b). *J. Immunol.* **119**, 223.
- Thomas, D. W., Meltz, S. K., and Wilner, G. D. (1979a). *J. Immunol.* **123**, 759.
- Thomas, D. W., Meltz, S. K., and Wilner, G. D. (1979b). *J. Immunol.* **123**, 1299.
- Thorbecke, G. J., Silberberg-Sinakin, I., and Flotte, T. J. (1980). *J. Invest. Dermatol.* **75**, 32.
- Toews, G. B., and Lipscomb, M. E. (1980). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 911.
- Toews, G. B., Bergstresser, P. D., and Streilein, J. W. (1980). *J. Immunol.* **124**, 445.
- Togawa, A., Oppenheim, J. J., and Mizel, S. B. (1979). *J. Immunol.* **122**, 2112.
- Tracey, D. E. (1979). *J. Immunol.* **123**, 840.
- Tracey, D. E., and Adkinson, N. F., Jr. (1980). *J. Immunol.* **125**, 136.
- Tracey, D. E., Wolfe, S. A., Durdik, J. M., and Henney, C. S. (1977). *J. Immunol.* **119**, 1145.
- Treves, A. J. (1978). *Immunol. Rev.* **40**, 205.
- Treves, A. J., Schechter, B., Cohen, I. R., and Feldman, M. (1976). *J. Immunol.* **116**, 1059.
- Unanue, E. R. (1970). *J. Immunol.* **105**, 1339.
- Unanue, E. R. (1972). *Adv. Immunol.* **15**, 95.

- Unanue, E. R. (1975). In "Mononuclear Phagocytes in Immunity, Infection, and Pathology" (R. van Furth, ed.), p. 721. Nijhoff, The Hague.
- Unanue, E. R. (1976). *Am. J. Pathol.* **83**, 396.
- Unanue, E. R. (1978). *Immunol. Rev.* **40**, 227.
- Unanue, E. R., and Askonas, B. A. (1968a). *Immunology* **15**, 287.
- Unanue, E. R., and Askonas, B. A. (1968b). *J. Exp. Med.* **127**, 915.
- Unanue, E. R., and Cerottini, J.-C. (1970). *J. Exp. Med.* **131**, 711.
- Unanue, E. R., and Feldman, J. D. (1971). *Cell. Immunol.* **2**, 269.
- Unanue, E. R., and Katz, D. H. (1973). *Eur. J. Immunol.* **3**, 559.
- Unanue, E. R., and Kiely, J.-M. (1977). *J. Immunol.* **119**, 925.
- Unanue, E. R., Cerottini, J.-C., and Bedford, M. (1969). *Nature (London)* **222**, 1193.
- Unanue, E. R., Dorf, M. E., David, C. S., and Benacerraf, B. (1974). *Proc. Natl. Acad. Sci. U.S.A.* **71**, 5014.
- Unanue, E. R., Beller, D. I., Calderon, J., Kiely, J.-M., and Stadecker, M. J. (1976a). *Am. J. Pathol.* **85**, 465.
- Unanue, E. R., Kiely, J.-M., and Calderon, J. (1976b). *J. Exp. Med.* **144**, 155.
- Unanue, E. R., Farr, A. G., and Kiely, J.-M. (1980). In "Mononuclear Phagocytes" (R. van Furth, ed.), p. 1837. Nijhoff, The Hague.
- Unkeless, J. C., Gordon, S., and Reich, E. (1974). *J. Exp. Med.* **139**, 834.
- Vadas, M. A., Miller, J. F. A. P., Gamble, J., and Whitelaw, A. (1975). *Int. Arch. Allergy Appl. Immunol.* **49**, 670.
- Vadas, M. A., Miller, J. F. A. P., Whitelaw, A., and Gamble, J. (1977). *Immunogenetics* **4**, 137.
- Van den Tweel, J. G., and Walker, W. S. (1977). *Immunology* **33**, 817.
- Van Ewijk, W., Verzijden, J. H. M., Van der Kwast, T. H., and Luijex-Meijer, S. S. M. (1974). *Cell Tissue Res.* **419**, 43.
- Van Furth, R., and Cohn, Z. A. (1968). *J. Exp. Med.* **128**, 415.
- Van Furth, R., and Diesselhoff-Den Dulk, M. M. C. (1970). *J. Exp. Med.* **132**, 813.
- Van Furth, R., Diesselhoff-Den Dulk, M. M. C., and Maltie, H. (1973). *J. Exp. Med.* **138**, 1314.
- Varesio, L., Herberman, R. B., Gerson, J. M., and Holden, H. T. (1979). *Int. J. Cancer* **24**, 97.
- Vassali, J. D., and Reich, E. (1977). *J. Exp. Med.* **145**, 429.
- Veerman, A. J. (1974). *Cell Tissue Res.* **148**, 247.
- Veerman, A. J., and Van Ewijk, W. (1975). *Cell Tissue Res.* **156**, 441.
- Vilcek, J., and Ng, M. H. (1971). *J. Virol.* **7**, 588.
- Vogel, A., Raines, E., Kariya, B., Rivest, M. J., and Ross, R. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2810.
- Volkman, A. (1970). *Ser. Haematol.* **3**, 62.
- Volkman, A. (1976). *J. RES Soc.* **19**, 249.
- Volkman, A., and Collins, F. M. (1974). *J. Exp. Med.* **139**, 264.
- Volkman, A., and Gowans, J. L. (1965a). *Br. J. Exp. Pathol.* **46**, 50.
- Volkman, A., and Gowans, J. L. (1965b). *Br. J. Exp. Pathol.* **46**, 62.
- Von Boehmer, H., Haas, W., and Jerne, N. K. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2439.
- Wagner, H., Feldman, M., Boyle, W., and Schrader, J. W. (1972). *J. Exp. Med.* **136**, 331.
- Wagner, H., Hardt, C., Heeg, K., Pfizenmaier, K., Solbach, W., Bartlett, R., Stockinger, H., and Rollinghof, M. (1980). *Immunol. Rev.* **51**, 215.
- Wahl, L. M., Wahl, S. M., Mergenhagen, S. E., and Martin, G. R. (1975a). *Science* **187**, 261.

- Wahl, L. M., Wilton, J. M., Rosenstreich, D. L., and Oppenheim, J. J. (1975b). *J. Immunol.* **114**, 1296.
- Wahl, L. M., Olsen, C. E., Sandberg, A. L., and Mergenhagen, S. E. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4955.
- Waldman, S. R., and Gottlieb, A. A. (1973). *Cell. Immunol.* **9**, 142.
- Waldron, J. A., Horn, R. G., and Rosenthal, A. S. (1973). *J. Immunol.* **111**, 58.
- Waldron, J. A., Horn, R. G., and Rosenthal, A. S. (1974). *J. Immunol.* **112**, 746.
- Webb, D. R., and Jamieson, A. T. (1976). *Cell. Immunol.* **24**, 45.
- Webb, D. R., and Osheroff, P. L. (1976). *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1300.
- Webb, D. R., and Nowowiejski, I. (1977). *Cell. Immunol.* **33**, 1.
- Weinberg, D. S. (1980). *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 911.
- Weinberg, D. S., and Unanue, E. R. (1981). *J. Immunol.* **126**, 794.
- Wekerle, H., and Ketelsen, U. P. (1980). *Nature (London)* **283**, 402.
- Wekerle, H., Ketelsen, U. P., and Ernst, M. (1980). *J. Exp. Med.* **151**, 925.
- Werb, Z., and Gordon, S. (1975a). *J. Exp. Med.* **142**, 346.
- Werb, Z., and Gordon, S. (1975b). *J. Exp. Med.* **142**, 361.
- Werdelin, O. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 213. Academic Press, New York.
- Werdelin, O., and Braendstrup, O. (1979). *Scand. J. Immunol.* **10**, 499.
- Werdelin, O., and Shevach, E. M. (1979). *J. Immunol.* **123**, 2779.
- Werdelin, O., Braendstrup, O., and Pederson, E. (1974). *J. Exp. Med.* **140**, 1245.
- Werdelin, O., Braendstrup, O., and Shevach, E. M. (1979). *J. Immunol.* **123**, 1755.
- Wiener, E., and Levanon, D. (1968). *Science* **159**, 217.
- Williams, N. (1979). *Blood* **53**, 1089.
- Wong, D. M., and Hersowitz, H. B. (1979). *Immunology* **37**, 765.
- Wood, D. D. (1979a). *J. Immunol.* **123**, 2395.
- Wood, D. D. (1979b). *J. Immunol.* **123**, 2400.
- Wood, D. D., and Cameron, P. M. (1975). *J. Immunol.* **114**, 1094.
- Wood, D. D., and Cameron, P. M. (1976). *Cell. Immunol.* **21**, 133.
- Wood, D. D., and Gaul, S. L. (1974). *J. Immunol.* **113**, 925.
- Wood, D. D., Cameron, P. M., Poe, M. T., and Morris, C. A. (1976). *Cell. Immunol.* **21**, 88.
- Woodward, J. H., and Daynes, R. A. (1979). *J. Immunol.* **123**, 1227.
- Woodward, J. H., Fernandez, P. A., and Daynes, R. A. (1979). *J. Immunol.* **122**, 1196.
- Wolfe, S. A., Tracey, D. E., and Henney, C. S. (1976). *Nature (London)* **262**, 584.
- Yamashita, U., and Hamaoka, T. (1979). *J. Immunol.* **123**, 2637.
- Yamashita, U., and Shevach, E. M. (1977). *J. Immunol.* **119**, 1584.
- Yamashita, U., and Shevach, E. M. (1978). *J. Exp. Med.* **148**, 1171.
- Yano, A., Schwartz, R. H., and Paul, W. E. (1977). *J. Exp. Med.* **146**, 828.
- Yano, A., Schwartz, R. H., and Paul, W. E. (1978). *Eur. J. Immunol.* **8**, 344.
- Yokomuro, K., and Rosenthal, A. S. (1979). *J. Immunol.* **123**, 2019.
- Yoneda, T., and Mundy, G. R. (1979). *J. Exp. Med.* **149**, 279.
- Zembala, M., and Asherson, G. L. (1974). *Eur. J. Immunol.* **4**, 799.
- Ziegler, K., and Unanue, E. R. (1979). *J. Exp. Med.* **150**, 1143.
- Ziegler, K., and Unanue, E. R. (1980). In "Macrophage Regulation of Immunity" (E. R. Unanue and A. S. Rosenthal, eds.), p. 245. Academic Press, New York.
- Zinkernagel, R. M. (1974). *Nature (London)* **251**, 230.
- Zinkernagel, R. M. (1978). *Immunol. Rev.* **42**, 224.
- Zinkernagel, R. M., and Doherty, P. C. (1974). *Nature (London)* **251**, 547.
- Zinkernagel, R. M., Althage, A., Adler, B., Blanden, R. V., Davidson, W. F., Kees, U., Dunlop, M. B. C., and Shreffler, D. C. (1977). *J. Exp. Med.* **145**, 1353.