Immunological Reviews 1989, No. 110

Published by Munksgaard, Copenhagen, Denmark

Peripheral T Cells Select the B-Cell Repertoire in Old Mice

MARC E. WEKSLER, CARLO RUSSO & GREGORY W. SISKIND

INTRODUCTION

Age-associated alterations in humoral immunity have been recognized for a long time. More than 50 years ago, serum anti-sheep erythrocyte antibodies were found to be lower in old as compared to young humans (Paul & Bunnel 1932). Subsequently, the serum levels of another natural antibody (anti-salmonella flagellin antibody) and of anti-DNA antibody were measured in healthy humans of different ages (Roberts-Thomson et al. 1968). Increasing age was associated with both a progressive decline in anti-flagellin antibody and a progressive increase in anti-DNA antibody.

This divergence in the concentration of antibodies to self and to foreign antigens is now recognized as a general characteristic of immune senescence. That is, with age there is a decreased immune response to foreign antigens and an increased immune reactivity against self antigens. Furthermore, it now appears that the increased antibody response to auto-antigens and decreased response to foreign antigens are not merely associated but truly behave as if there is a "cross-wiring" of the immune system. Thus, the antibody response of old mice or of cultured spleen cells from old mice induced by foreign antigens leads to a lower antibody response to the foreign antigens but a greater antibody response to a number of auto-antigens compared to those responses in young adult mice (Naor et al. 1976, Bovbjerg et al. (1989) unpublished observations).

In this review, the evidence that alterations of humoral immunity associated with aging are due to changes in the expressed repertoire of B lymphocytes selected by long-lived peripheral T cells from old mice will be discussed. We have found no evidence that the repertoire of B lymphocytes generated in the bone marrow of old mice is intrinsically altered. This conclusion is in accord with other studies that have found no effect of age on the capacity of bone marrow to reconstitute the hematopoietic and immunologic systems (Harrison et al. 1984).

Department of Medicine, Cornell University Medical College, 1300 York Avenue, New York, NY 10021, U.S.A.

However, while the repertoire of B lymphocytes generated in the bone marrow is not influenced by age, the repertoires of peripheral B cells of old and young mice differ significantly. This, as will be reviewed below, results from the different idiotypic repertoire expressed by peripheral T lymphocytes in old mice. The alteration in the peripheral T lymphocyte repertoire probably results from life-long exposure to foreign and self antigens and from changes in the functional capacity of the involuting thymus gland to positively and negatively select specific T-cell repertoires.

THE INFLUENCE OF AGE ON HUMORAL IMMUNITY

Aged experimental animals and humans in general have a decreased concentration of serum antibodies to a variety of foreign antigens when compared to young controls. Despite this fact, the overall level of serum immunoglobulins does not change significantly with age, presumably as a result of the increasing serum concentration of auto-antibodies. This was probably the first clue to suggest that the expressed repertoire of B lymphocytes is altered during aging.

We investigated the nature of the age-related alterations in the expressed repertoire of B cells using the response to nitrophenylated bovine gamme globulin as the model system (Goidl et al. 1976). We confirmed the results of prior studies by both Makinodan (1971) and Walford (Walford 1969) and their colleagues that, in quantitative terms, the antibody response to foreign antigens is diminished in old as compared to young mice. The decrease with age in specific antibody responses is greater following immunization with thymic-dependent antigens, such as hapten-protein conjugates, as compared to largely thymic-independent antigens, such as hapten-carbohydrate conjugates (Weksler et al. 1978). This appears to result, at least in part, from a preferential reduction of IgG vis-a-vis IgM antibody responses in old mice (Goidl et al. 1976). This is not surprising as the production of IgG antibodies is more T cell-dependent than the production of IgM antibodies (Anderson et al. 1974). Furthermore, some reports suggested that the diminished antibody response of old mice was due to an increased ratio of suppressor-to-helper T cell activity (Miller 1984).

Despite the decreased quantitative response to foreign antigen, as measured by the specific plaque-forming cell (PFC) response or total specific antibody production of old compared to young mice, we have found no difference in the total number of Ig-secreting cells or total amount of immunoglobulin produced following immunization *in vitro* or *in vivo* (Kim et al. unpublished observations). This finding implies that there must be qualitative as well as quantitative differences in the immune response of old and young mice. In order to examine qualitative changes in the immune response with age, we examined the fine specificity of the anti-hapten response.

The heterogeneity of antibody with respect its avidity for hapten is one index

of the fine specificity of the immune response. We found, using hapten inhibition of plaque formation by spleen cells from immunized mice, that old mice have a significantly less heterogeneous repertoire of antibody with respect to affinity (Goidl et al. 1976). This resulted from a preferential loss of the more thymic-dependent high-affinity antibody-dorming cells from old mice. The role of thymic-dependent processes in the preferential loss of IgG and high-affinity PFC was supported by the finding that the administration of T cells or thymopoietin to old mice was associated with an increase in both IgG and higher affinity PFC as compared to untreated old mice (Weksler et al. 1978).

The relative stability of the serum concentration of immunoglobulin over the life span, despite the decrease in the serum concentration of antibody to foreign antigens, strongly implies that much of the immunoglobulin in aged animals is directed against auto-antigens. Therefore, we tested the auto-antibody response of old and young mice induced by xenogeneic erythrocytes and thyroglobulin (Goidl et al. 1981). The apparently paradoxical results were that old mice produced no more and probably less auto-antibodies following immunization with cross-reacting rat erythrocytes or thyroglobulin than did young mice. In contrast, spleen cells from old mice cultured in the presence or absence of lipopolysaccharide produced more anti-bromelain-mouse erythrocyte PFC than did spleen cells from young mice. Thus, the greater production of auto-antibodies by old mice appeared to result, at least in part, from an increased "internal activity" of the immune system. The notion of increased "internal activity" of old mice was supported by the finding that there was an increased number of activated, low-density lymphocytes in the peritoneal cavity and spleen of old as compared to young mice (Weksler et al. 1988, unpublished observations). Additional evidence for an increase in the "internal activity" of the immune system is derived from our studies of the auto-anti-idiotypic antibody response in young and old mice.

EFFECT OF AGE ON THE AUTO-ANTI-IDIOTYPIC ANTIBODY RESPONSE

During studies of the distribution of PFC with respect to affinity, it was observed that the number of PFC frequently was increased in the presence of low concentrations of hapten (Schrater et al. 1979). Hapten-augmentable PFC are antibody-producing cells whose secretion of antibody has been inhibited by the presence of auto-anti-idiotypic antibody bound to cell-surface antibody molecules (Goidl et al. 1979). Hapten competes with auto-anti-idiotypic antibody for available cell-surface antibody molecules, displaces bound auto-anti-idiotypic antibody and, in this manner, reverses its inhibitory effect on antibody secretion. These studies support the concept that auto-anti-idiotypic antibody regulates the immune response in a fashion predicted by Jerne's network theory (Jerne 1974).

Using the hapten-augmentable PFC assay, we found that the auto-anti-idiotypic antibody response increased with age in each of the four mouse strains studied

(C57BL/6, BALB/c, AKR/J and CBF1) following immunization with the relatively T-independent antigen, TNP-Ficoll (Goidl et al. 1980). Szewczuk & Campbell (1980) also reported greater hapten-augmentable PFC in old as compared to young mice immunized with a T-dependent antigen. As other factors, such as soluble immune complexes, in addition to auto-anti-idiotypic antibody, could induce hapten-augmentable PFC, we confirmed the presence of increased auto-anti-idiotypic antibody in serum from aged mice by independent techniques. The binding of enzyme-conjugated idiotype (prepared from the serum of immunized young mice) by serum from old and young immunized mice was compared. As a control, binding of idiotype to serum from unimmunized mice was measured. Twice as much purified idiotype was bound by serum from old as compared to young immunized mice (Goidl et al. 1983). This is a minimal estimate of the auto-anti-idiotypic antibody in serum from old mice, as we knew that there is only a partial sharing of idiotypes between young and old mice (see below). A high auto-anti-idiotypic response by aged mice was also suggested by the studies of Klinman (1981). As will be discussed later, the increased auto-anti-idiotypic antibody response may contribute to the decreased immune response of old mice, in general, and to the loss of high-affinity and IgG PFC in particular. However, even under conditions which eliminate most if not all of the effects of auto-anti-idiotypic antibody on the PFC response, immunized, old mice express fewer PFC than do young mice.

EFFECT OF AGE ON IDIOTYPE EXPRESSION

Hapten-reversible inhibition of PFC was used to investigate the repertoire of the anti-TNP antibody response with respect to idiotype. Sera from immunized, old mice inhibited – in a hapten-reversible manner – plaque formation by immune spleen cells from old mice to a much greater extent than from immunized young

TABLE I

Inhibition of PFC by serum from immunized mice of different ages

	Age of PFC Donors		
	1 month	2 months	21-22 months
Age of immune sera donors	Percentage Inhibition of PFC		
1 month	56%	15%	20%
2 months	0%	67%	50%
21-22 months	10%	7%	81%

Immune spleen cells were incubated with immune serum for 5 minutes at room temperature. The cells were washed and assayed for anti-PFC in the absence or presence of 10^{-9} and 10^{-8} M TNP-EACA. Immune sera were obtained from C57BL/6 mice immunized with TNP-Ficoll 7 to 11 days previously. Serum-induced suppression of PFC was fully reversible by hapten.

adult or immature mice (Table I). Similarly, sera from 1- or 2-month-old mice inhibited to the greatest extent plaque formation by immune spleen cells from mice of the same age and to a lesser extent plaque formation by immune spleen cells from older or younger mice.

These data suggest that the repertoire of anti-TNP idiotypes changes with age. It should be noted that sera from old mice inhibit a greater percentage of PFC in younger mice than sera from young mice inhibit PFC in old mice. This may result from the increased concentration of auto-anti-idiotypic antibody in the sera of old mice, or a reduced heterogeneity of the anti-idiotypic antibody from old mice. Relevant to these findings is the shift in major clonotypes expressed by 1- and 10-week-old mice (Cancro et al. 1979). It appears that such changes may be related to the shift of V region gene utilization observed during development of the immune system (Yancopoulos et al. 1988). While it is possible that V region gene utilization differs in 1-, 2- and 21-month-old mice, it seems equally likely that the repertoire of the immune system is altered as a consequence of lifelong interactions of the immune system with foreign and self antigens.

THE CELLULAR BASIS OF THE INCREASED AUTO-ANTI-IDIOTYPIC ANTIBODY RESPONSE AND ALTERED REPERTOIRE IN AGED MICE

Studies in reconstituted mice

The studies discussed above have documented a number of qualitative differences between the B-cell repertoire of old and young mice. We next turned our attention to identifying, by cell transfer experiments, the lymphocyte population which was responsible for the increased hapten-augmentable PFC response. Two- to 3-month-old, lethally-irradiated, syngeneic recipients of spleen cells from old mice produced a much greater hapten-augmentable PFC response following immunization than did recipients of spleen cells from young mice (Goidl et al. 1983). The ability of spleen cells from old mice to transfer their increased auto-anti-idiotypic antibody response was not diminished when splenic T cells were eliminated prior to cell transfer. This further supported the concept that the peripheral B-cell repertoire expressed by young and old mice was different. In contrast, recipients of bone marrow cells from old and young mice produced comparable and low auto-anti-idiotypic antibody responses, suggesting that the idiotype distribution of B cells newly arising from the bone marrow was similar in old and young mice.

Since the high auto-anti-idiotypic antibody response of old mice was transferable with B cells from the spleen but not from the bone marrow, studies were undertaken to identify the cell type that selected the repertoire of the peripheral B-cell population. Four groups of irradiated transfer recipients were established: the first group received bone marrow and splenic T cells from young donors; the

second group received bone marrow from young donors and splenic T cells from old donors; the third group received bone marrow and splenic T cells from old donors; and the fourth group received bone marrow from old donors and splenic T cells from young donors. All recipients were immunized and hapten-augmentable PFC were measured. Recipients of old splenic T cells had high hapten-augmentable PFC responses like those of old mice, regardless of the age of the bone marrow donor (Table II). In contrast, all recipients of young splenic T cells had a low percentage of hapten-augmentable PFC. Thus, the magnitude of the auto-anti-idiotypic antibody response is regulated by splenic T cells.

The difference between B lymphocytes freshly generated from bone marrow precursors and splenic B cells of old mice, with respect to the generation of an auto-anti-idiotypic antibody response, appears to result from the selection of the peripheral B-cell repertoire by peripheral T lymphocytes resident in old mice. It is reasonable to suppose that the repository of the evolving repertoire of antibody specificities rests within the population of long-lived T lymphocytes. Peripheral T cells alter the distribution of B lymphocytes as they leave the bone marrow as a consequence of the B-cell exposure to the idiotypic specificities expressed by peripheral T cells. The peripheral T cells are thought to stimulate the preferential expansion of B cells which express complementary idiotypes. The shift in the peripheral T-cell repertoire with age presumably results from the continuous exposure during life, not only to self-immunoglobulins and immune complexes, but also to autologous and foreign antigens. This results in the age-associated changes in the idiotype repertoire and increased incidence of auto-reactive B cells including those producing auto-anti-idiotypic antibody.

This view of peripheral B-lymphocyte repertoire selection resulting from a network of B-lymphocyte and T-lymphocyte interactions suggests that depletion of peripheral lymphocytes in old animals followed by autologous bone marrow repopulation of the immunologic system should lead to the re-establishment of

TABLE II

Effect of splenic T cells on hapten-augmentable PFC

Age of Cell Donors		Percentage augmentation of PFC
Bone Marrow	Splenic T cells	by hapten
2–3 mo.	18 mo.	55%
18 mo.	18 mo.	38%
2–3 mo.	2–3 mo.	12%
18 mo.	2–3 mo.	16%

Male C57BL/6 mice were lethally irradiated and reconstituted with 30 million bone marrow cells and 30 million splenic T cells from donors of the indicated ages. All recipients were immunized by the intravenous injection of 10 micrograms of TNP-Ficoll 5 days after cell transfer. PFC were assayed 1 wk after immunization.

a B-cell repertoire in old mice that is young-like with respect to the auto-anti-idiotypic antibody response and B-cell idiotype repertoire.

Studies in auto-reconstituted mice

The studies described above suggest that the changes in idiotype expression and auto-anti-idiotypic antibody production in old animals are due to shifts in clonal distribution among the long-lived peripheral T cells. The shifts in T-cell repertoire are, in turn, imposed upon B lymphocytes after they leave the bone marrow. According to this view, depletion of peripheral T and B cells from old mice followed by their auto-reconstitution from their own bone marrow should result in an old mouse with a young-like auto-anti-idiotype response and idiotype repertoire.

This hypothesis was tested by lethally irradiating mice with their bone marrow partially shielded. Auto-reconstitution of the peripheral immune system occurs within 8 wk of irradiation (Kim et al. 1985). The old, auto-reconstituted mice produced, following immunization, the same level of auto-anti-idiotypic antibody when measured by both hapten-augmentable PFC and by ELISA as did young non-irradiated control mice or young, auto-reconstituted mice (Table III).

Peripheral T cells also regulated auto-anti-idiotypic antibody responses in auto-reconstituted mice. Thus, the auto-anti-idiotypic antibody response of mice irradiated with their bone marrow partially shielded that were given splenic T cells from young or old donors 1 wk later, and immunized 7 wk after T-cell administration, was dependent upon the age of the T-cell donors. Thus, auto-reconstituted old or young mice given splenic T cells from old donors had an average hapten-augmentable PFC response of 33% while recipients of splenic T

TABLE III

Auto-anti-idiotypic antibody response of auto-reconstituted young and old mice

Age	Treatment	Auto Anti-id by Elisa (OD)	Percentage augmentation of PFC by Hapten (%)
Young	None	0.275	19%
Young	Irradiated	0.279	25%
Old	None	0.398	45%
Old	Irradiated	0.294	21%

C57BL/6 mice of ages indicated were or were not exposed to 800 Rads of gamma irradiation with their bone marrow partially shielded. Six weeks later the mice were injected intravenously with 10 micrograms of TNP-Ficoll and the mice bled 6 d later for auto-anti-idiotypic antibody and then sacrificed for splenic PFC response assayed in the presence or absence of TNP-EACA. Sera from old and young mice were used to coat wells. Enzyme-linked idiotype from young mice was used to measure idiotype binding.

cells from young donors had an average hapten-augmentable PFC response of 13%.

In summary, transfer studies as well as the studies in auto-reconstituted mice suggest that the bone marrows of old and young mice are similar with respect to the repertoire of B cells which they can generate. In addition, studies using both models demonstrated that the peripheral T-cell population is responsible for the age-related changes in auto-anti-idiotypic antibody responses and in expressed idiotypic repertoire. Zharhary & Klinman (1984) have also presented evidence that the degree of diversity of the B-cell repertoire, in their studies specific for PR8 influenza virus, does not change with age. They concluded that the limited antibody diversity expressed by old animals results from regulatory influences on the B-cell population.

During the auto-reconstitution studies in young mice it was noted that, soon after irradiation with partial bone marrow shielding, there was a preferential loss of IgG and high-affinity PFC (Tsuda et al. 1988). However, by 8 wk after irradiation, autoreconstituted mice had regained the ratio of IgG to IgM PFC and the heterogeneity of the PFC response with respect to affinity comparable to those of unirradiated young mice. This suggested that auto-reconstitution might also influence other age-associated immune alterations in addition to the auto-anti-idiotypic antibody response. Specifically, we investigated whether the decrease in IgG and high-affinity PFC seen in old mice persisted after auto-reconstitution. We found that the anti-TNP response of 18- to 20-month-old mice, whose peripheral lymphoid system had been reconstituted from their own bone marrow after lethal irradiation with partially shielding of their bone marrow, included many more high-avidity and IgG PFC than unirradiated old mice and was, in fact, at least with respect to avidity, very similar to the response of normal young mice with respect to these parameters (Table IV).

The capacity of auto-reconstituted old mice to generate IgG and high-avidity PFC is of particular interest in as much as these two types of antibodies are highly T cell-dependent (Gershon & Paul 1971, Anderson et al. 1974). As the function of the thymus in 18-month-old mice is markedly reduced when compared to that in young mice, it appears paradoxical that auto-reconstituted old mice produce young-like levels of highly thymic-dependent antibody. These findings raise the possibility that peripheral lymphoid tissues in old mice can take over certain thymic functions. The development of major histocompatibility complex class I-restricted cytolytic cells in adult nude mice (Kruisbeek et al. 1984) and the appearance of Thy-1-positive T cells in the spleen and lymph nodes of adult nude mice (MacDonald et al. 1981) are consistent with this hypothesis. Thus, old mice liberated of the regulatory constraints of peripheral, long-lived T cells might be able to regenerate a young-like repertoire of peripheral B cells. Such mice might respond to foreign antigen similar to young animals, even producing antibodies which, at least in young mice, are highly thymic-dependent.

TABLE IV

Effect of auto-reconstitution on IgG and high avidity PFC production by old mice immunized with TNP-BGG

Age	anti-TNP PFC (PFC/spleen) Direct	Indirect	Average Avidity ($K50 \times 10^{-5}$)
Untreated Mice			
2 mo.	3260	8530	20.6
18–20 mo.	1360	412	1.1
Auto-reconstituted			
18–20 mo.	3520	3830	24.7

Old C57BL/6 mice were lethally irradiated with their bone marrow partially shielded. Eight weeks later these mice, age-matched controls and 2-month-old mice were immunized with TNP-BGG in complete Freund's adjuvant. All mice were sacrificed 2 wk after antigen injection and the number and average avidity (concentration of hapten required for 50% inhibition of PFC, K50) were measured.

Auto-reconstituted mice given peripheral T cells from old or young donors during recovery from irradiation made a PFC response to TNP bovine gamme globulin that reflected the immune response characteristic of the age of the T-cell donor with respect to heterogeneity, average avidity and the ratio of IgG to IgM PFC. These results support the hypothesis presented above that the bone marrows of old and young mice are similar with regard to the spectrum of B-cell clones that they can produce and that the expressed repertoire of peripheral B cells is regulated by the peripheral T-lymphocyte population.

These studies did not distinguish between a direct selection of the expressed B-lymphocyte repertoire by peripheral T cells or an indirect effect of downregulation by auto-anti-idiotypic antibody. To examine this question, the capacity of immunized auto-reconstituted mice – which had been given peripheral T cells from old mice during recovery from radiation – to produce high avidity antibody was measured under conditions which freed the peripheral B lymphocytes of bound auto-anti-idiotypic antibody and allowed them to secrete antibody. If the curves for hapten inhibition of plaque formation (from which the avidity distributions are calcualted) are based upon the maximum number of PFC detected in the presence of low concentrations of hapten, rather than upon the number of PFC in the absence of hapten, the avidity distribution obtained will include those cells whose secretion of antibody had been inhibited *in vivo* by auto-anti-idiotypic antibody (Tsuda et al. 1988). From such a calculation it is clear that immune old mice have more B cells capable of secreting high-avidity antibody than are detected by the conventional plaque inhibition assay. Thus, the loss of expressed high-avidity antibody by B lymphocytes from old mice, is, at least in

part, a consequence of the inhibition of high-avidity antibody secretion by auto-anti-idiotypic antibody.

SELECTION OF THE PERIPHERAL B-LYMPHOCYTE REPERTOIRE BY PERIPHERAL T LYMPHOCYTES IN THE ABSENCE OF ANTIGEN

We have considered two possible mechanisms underlying the capacity of peripheral T lymphocytes from old and young mice to influence the expressed B-cell repertoire. First, it was possible that different repertoires of idiotype-specific helper T cells were present in old and young mice. Such idiotype-specific helper cells, in the presence of antigen, select the expansion of B cells expressing a restricted group of idiotypes. An alternate possibility was that T cells directly modify the idiotype repertoire distribution of B lymphocytes following their release from the bone marrow, in the absence of antigen, through a series of recursive circuits as described by Marcos et al. (1988). The first mechanism would operate during the response to antigen. The second would operate prior to and independently of antigen exposure. We have, therefore, studied the requirement for antigen in the differential selection of the B-cell repertoire by peripheral T cells from old and young mice.

In the studies described above, the differences in the B-cell repertoire were detected in recipients given both T cells and antigen. Thus, it was not possible in those studies to distinguish the role of antigen in the selection of the B-cell repertoire as distinct from the requirement for antigen in the expression of antibody repertoire of the B-cell population. We have used sequential cell transfers to test the capacity of T cells from old and young mice to influence B-cell repertoire in the absence of antigen (Kim et al. 1989).

Peripheral T cells from young or old mice were transferred to young, syngeneic, primary recipients together with bone marrow cells from young syngeneic donors. Primary recipients did not receive antigen. After 8 wk, purified splenic B cells from the primary recipients were transferred, together with T cells from naive, young syngeneic donors, to secondary recipients. The secondary recipients were then immunized and hapten-augmentable PFC and the idiotypic repertoire assayed. Any difference in the responses of the secondary recipients was, therefore, attributable to the history of the peripheral B lymphocytes. In one group the B cells matured in the presence of peripheral T cells from old mice while, in the other group, the B cells matured in the presence of peripheral T cells from young mice. The results showed that the idiotype repertoire and the level of auto-anti-idiotypic antibody expressed in the secondary recipients reflected the age of the donors of the T cells transferred to the primary recipients, suggesting that T cells can directly influence the B-cell repertoire in an antigen-independent manner, presumably by idiotype-anti-idiotype interactions. Comparable results were also

obtained by transferring purified T cells from old or young donors to auto-reconstituting young mice.

SUMMARY AND CONCLUSIONS

These studies have shown that the alterations in the repertoire of antibody produced by old mice is not due to an intrinsic defect in the bone marrow or in the B-lymphocyte population arising from the bone marrow but rather to a selective downregulation by auto-anti-idiotypic antibody and idiotype-anti-idiotype interactions, shifting the idiotype distribution in the peripheral B-cell population. Thus, the clonal distributions of B cells generated by bone marrow of old and young mice are very comparable. The age-related differences in antibodies expressed by young and old mice are, to a great extent, determined by the activity of a peripheral regulatory immune network. This immune cellular network operates prior to exposure to antigen, presumably on the basis of an idiotype-anti-idiotype network between T and B lymphocytes. After exposure to antigen, a network of idiotype-anti-idiotype antibody interactions also contributes to differences in the immune responses of old and young mice to foreign antigens.

If the expressed repertoire of antibody reflects down-regulation of auto-anti-idiotypic antibody, comparable repertoires of B-cell clones would be expected to be recovered from old and young mice if B cells from old mice were rescued from selective peripheral downregulatory influences active in old mice. Support for this hypothesis has been obtained by generating B-cell hybridomas from young and old mice immunized with TNP bovine gamme globulin (Marcenario et al. 1989). The same number of anti-TNP hybridomas and a comparable number of IgG and high-affinity antibody-producing clones were recovered from the spleens of young and old mice. Thus, the actual B-cell clonal repertoires of young and old mice appear to be similar although the expressed repertoires of antibody-producing lymphocytes from old and young mice are very different. This conclusion has considerable impact on strategies that could be employed to reverse the senescence of humoral immunity. Strategies to counter downregulatory influences which constrain the expression of the B-cell population should be more effective than attempts to reconstitute the repertoire of B lymphocytes in aged individuals.

Finally, the mechanisms underlying these age-associated shifts in the expressed humoral antibody response can be attributed to life-long interactions with self and foreign antigens. The overall shift may be described as a decreased reactivity to foreign antigens and a complementary increase in reactivity with self antigens. Such changes probably follow the involution of the thymus with consequent decrease in MHC-restricted T-cell recognition of foreign antigens (a defect in positive selection within the thymus) and increase in T-cell reactivity to self antigens (a defect in negative selection within the thymus).

ACKNOWLEDGMENTS

The authors are grateful to Antonio Coutinho for many discussions and his sharing of unpublished research results concerning the recursive circuits operative in the immune system. Without the many contributions of Edmond Goidl, Young Tai Kim, and Tadaaki Tsuda, the studies discussed could never have been realized. Finally, the research described in this review would not have been possible without the financial support of the National Institute on Aging (AG 00239 and AG 00541) and of the Roland Harriman Foundation over the past 15 years.

REFERENCES

Anderson, H. R., Dresser, D. W. & Wortis, H. H. (1974) The relationship between the immunoglobulin class of B-cell precursors and the degree of synergism obtained from the presence of T cells. *Clin. Exp. Immunol.* **16**, 393.

Cancro, M. P., Wylie, D. E., Gerhard, W. & Klinman, N. R. (1979) Patterned acquisition of the antibody repertoire: Diversity of the hemagglutinin-specific B-cell repertoire in neonatal BALB/c mice. *Proc. Natl. Acad. Sci. USA* **76**, 6577.

Gershon, R. K. & Paul, W. E. (1971) Effect of thymus-derived lymphocytes on amount and affinity of anti-hapten antibody. *J. Immunol.* **106**, 872.

Goidl, E. A., Choy, J. W., Gibbons, J. J., Weksler, M. E., Thorbecke, G. J. & Siskind, G. W. (1983) Production of auto-anti-idiotypic antibody during the normal immune response. VII. Analysis of the cellular basis for the increased auto-anti-idiotypic antibody production by aged mice. *J. Exp. Med.* **157**, 1635.

Goidl, E. A., Innes, J. B. & Weksler, M. E. (1976) Immunological studies of aging. II. Loss of IgG and high avidity plaque-forming cells and increased suppressor cell activity in aging mice. *J. Exp. Med.* **144**, 1037.

Goidl, E. A., Michelis, M. A., Siskind, G. W. & Weksler, M. E. (1981) Effect of age on the induction of autoantibodies. *Clin. Exp. Immunol.* **44**, 24.

Goidl, E. A., Schrater, A. F., Siskind, G. W. & Thorbecke, G. J. (1979) Production of auto-anti-idiotypic antibody during the normal immune response to TNP-ficoll. II. Hapten-reversible inhibition of anti-TNP plaque-forming cells by immune serum as an assay for auto-anti-idiotypic antibody. *J. Exp. Med.* **150**, 154.

Goidl, E. A., Thorbecke, G. J., Weksler, M. E. & Siskind, G. W. (1980) Production of auto-anti-idiotypic antibody during the normal immune response: Changes in the auto-anti-idiotypic antibody response and the idiotype repertoire assiciated with aging. *Proc. Natl. Acad. Sci. USA* **77**, 6788.

Harrison, D. E., Astle, C. M. & Lerner, C. (1984) Ultimate erythropoietic repopulating abilities of fetal, young adult and old adult cells compared using repeated irradiation. *J. Exp. Med.* **160**, 759.

Jerne, N. K. (1974) Towards a network theory of the immune system. *Ann. Immunol. (Paris)* **125C**, 373.

Kim, Y. T., DeBlasio, T., Thorbecke, G. J., Weksler, M. E. & Siskind, G. W. (1989) Production of auto-anti-idiotype antibody during the normal immune response. XIV. Evidence for the antigen-independent operation of the idiotype network. *Immunology* (in press).

Kim, Y. T., Goidl, E. A., Samarut, C., Weksler, M. E., Thorbecke, G. J. & Siskind, G. W. (1985) Bone Marrow Function. I. Peripheral T cells are responsible for the increased auto-antiidiotype response of older mice. *J. Exp. Med.* **161**, 1237.

Klinman, N. (1981) Antibody-specific immunoregulation and the immunodeficiency of aging. *J. Exp. Med.* **154**, 547.

Kruisbeek, A. M., Davis, M. L., Matis, L. A. & Longo, D. L. (1984) Self-recognition specificity expressed by T cells from nude mice. Absence of detectable Ia-restricted T cells in nude mice that do exhibit self-K/D-restricted T cell responses. *J. Exp. Med.* **160**, 839.

MacDonald, H. R., Lees, R. K., Sordat, B., Zaech, P., Maryanski, J. L. & Bron, C. (1981) Age-associated increase in expression of the T cell surface markers Thy-1, Lyt-1 and Lyt-2 in congenitally athymic (nu/nu) mice: Analysis by flow microfluorometry. *J. Immunol.* **126**, 865.

Marcenaro, L., Russo, C., Kim, Y. T., Siskind, G. W. & Weksler, M. E. (1989) Immunological studies of aging. Normal B-cell repertoire in aged mice: Studies at a clonal level. *Cell. Immunol.* **119**, 202.

Marcos, M. A., De la Hera, A., Pereira, P., Marquez, C., Toribio, M., Coutinho, A. & Martinez, C. (1988) B cell participation in the recursive selection of T cell repertoires. *Eur. J. Immunol.* **18**, 1015.

Makinodan, T. E., Perkins, E. M. & Chen, M. G. (1971) Immunologic activity of the aged. *Adv. Gerontol. Res.* **3**, 171.

Miller, R. A. (1984) Age-associated decline in precursor frequency for different T cell-mediated reactions, with preservation of helper or cytotoxic effect per precursor cell. *J. Immunol.* **132**, 63.

Naor, D., Bonavida, B. & Walford, R. L. (1976) Autoimmunity and aging: the age-related response of mice of a long-lived strain to trinitrophenylated syngeneic mouse red blood cells. *J. Immunol.* **117**, 2204.

Paul, J. R. & Bunnel, W. W. (1932) Anti-SRBC agglutinin with age. *Am. J. Med. Sci.* **183**, 90.

Roberts-Thomson, I. C., Whittinghom, S., Youngchaiyud, U. & Mackay, I. R. (1968) Aging, immune response and mortality. *Lancet* **II**, 24.

Schrater, A. F., Goidl, E. A., Thorbecke, G. J. & Siskind, G. W. (1979) Production of auto-anti-idiotypic antibody during the normal immune response to TNP-ficoll. I. Occurrence in AKR/J and BALB/c mice of hapten-augmentable, anti-TNP plaque-forming cells and their accelerated appearance in recipients of immune spleen cells. *J. Exp. Med.* **150**, 138.

Szewckuk, M. R. & Campbell, R. J. (1980) Loss of immune competence with age may be due to auto-anti-idiotypic antibody regulation. *Nature (Lond.)* **286**, 164.

Tsuda, T., Kim, Y. T., Siskind, G. W. & Weksler, M. E. (1988) Old mice recover the ability to produce IgG and high-avidity antibody following irradiation with partial bone marrow shielding. *Proc. Natl. Acad. Sci. USA* **85**, 1169.

Walford, R. L. (1969) *The Immunologic Theory of Aging*. Williams and Wilkins Co., Baltimore, 248 pp.

Weksler, M. E., Innes, J. B. & Goldstein, G. (1978) Immunological studies of aging. IV. The contribution of thymic involution to the immune deficiencies of aging mice. *J. Exp. Med.* **148**, 966.

Yancopoulos, G. D., Malynn, B. A. & Alt, F. W. (1988) Developmentally regulated and strain-specific expression of murine Vh gene families. *J. Exp. Med.* **168**, 417.

Zharhary, D. & Klinman, N. R. (1984) B cell repertoire diversity to PR8 virus does not decrease with age. *J. Immunol.* **133**, 2285.