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Absence of Expression of Ia Antigen on Human Cytotoxic T Cells

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Two major human immunoregulatory T-cell subsets have been defined with heteroantisera and monoclonal antibodies directed at stable cell-surface antigens (Reinherz and Schlossman 1980). One subpopulation of T cells, reactive with a monoclonal antibody termed anti-T4, comprises approximately 60 percent of the peripheral T-cell compartment and contains the inducer cell for T-T and T-B interactions (Reinherz et al. 1979 a, Reinherz et al. 1979 b). Moreover, this same population of lymphocytes is responsible for the production of nonspecific helper factors (Reinherz et al. 1980 a). A second subpopulation, accounting for 20 to 30 percent of peripheral T cells, is reactive with anti-TH2 heteroantisera (Evans et al. 1978 a), as well as with anti-T5 and anti-T8 monoclonal antibodies (Reinherz et al. 1980 b, Reinherz et al. 1980 c). The latter population, termed TH2+ (T5+/T8+) defines the human cytotoxic/suppressor subset (Reinherz and Schlossman 1979, Reinherz et al. 1980 b), and is reciprocal to the T4+ inducer population. Thus, the human T4+ subset is analogous to the murine Lyt1 inducer subset, while the TH2+ (T5+/T8+) subset is equivalent to the Lyt23 cytotoxic/suppressor subset (Cantor and Boyse 1977).

Additional studies have indicated that further heterogeneity exists in the human T-cell population. For example, following activation in MLC, some T cells express HLA-D-like (Ia) antigens, whereas others remain Ia-negative (Evans et al. 1978 b, Reinherz et al. 1979 c, Ko et al. 1979). The present study was undertaken to characterize further the human cytotoxic T lymphocyte and determine whether or not it expressed Ia antigens. For this purpose, we utilized a monoclonal antibody directed at an invariant region of the human Ia antigen (I1), as well as two other monoclonal antibodies defining cell-surface antigens on the inducer (T4+) and cytotoxic/suppressor (T8+) subsets. In the results to be reported below, it will be shown that although 60 percent of T cells express Ia antigen following MLC, only the T8+ Ia-T cell is the cytotoxic effector cell in CML.

Lymphocyte populations were isolated as follows. Human peripheral blood mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals,

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Piscataway, New Jersey) density centrifugation. Subsequently, lymphocytes were separated into E-rosetting-positive (T) and E-rosetting-negative subpopulations as previously described (Reinherz and Schlossman 1979). The T-cell population was then sensitized in MLC as described below.

Monoclonal antibodies anti-T4 (OKT4) and anti-T8 (OKT8) were produced and characterized as previously described (Reinherz et al. 1979 a, Reinherz et al. 1980 c). In brief, these two antibodies were shown to be restricted in their reactivity to cells of T lineage. Anti-T4 reacted with approximately 60 percent of peripheral T cells representing the inducer T-cell subset, while anti-T8 defined the human cytotoxic/suppressor population comprising 30 percent of peripheral T cells. The anti-I1 monoclonal antibody (OKI1) was shown to precipitate a 29 000 and 34 000 dalton bimolecular glycoprotein membrane structure which defined the human Ia antigen (Reinherz et al. 1979 c). This antigen is found on all peripheral B cells, a fraction of peripheral Null cells and monocytes, and is lacking on resting T cells. In contrast, activated T cells may express Ia antigen.

Sensitization cultures for cell-mediated lympholysis were established by combining T lymphocytes with mitomycin-treated stimulator cells in microtiter plate wells as previously described (Reinherz et al. 1979 a). Unsensitized T cells were cultured with media alone. All cells were placed in culture at 2×10^6 cells/ml in RPMI 1640 (Grand Island Biological Company, Grand Island, New York) containing 20 percent human AB serum, 1 percent penicillin-streptomycin, 200mM L-glutamine, 25mM HEPES buffer (Microbiological Associates, Bethesda, Maryland), and 0.05 percent sodium bicarbonate and incubated in a 5 percent CO_2 humid atmosphere at 37° C. After 6 days, the allosensitized T cells were harvested, washed, and separated into aliquots of 10×10^6 viable cells. Subsequently, cells were centrifuged at 300 xg and cell pellets resuspended in 0.8 cc of a 1 : 200 dilution of anti-T4, anti-T8, anti-Ia, normal ascites control, or PBS alone and incubated for 60 min at 24° C. Next, 0.2 cc of fresh rabbit complement was added to the antibody-treated populations and cells incubated for an additional hour at 37° C in a shaking water bath. At the end of this last incubation, cells were spun down, and the remaining viable cells enumerated by Tryphan blue exclusion. The antibody-treated populations were then washed two times and equal numbers of viable cells tested for effector function diluted in media 199 containing 10 percent fetal calf serum (FCS) (Microbiological Associates). Thus, untreated and antibody-treated killer populations were added to ^{51}Cr sodium chromate labeled target cells at effector : target ratios of 40 : 1, 20 : 1, and 10 : 1. Specific cytotoxicity was determined after a 4 hour incubation as previously described (Sondel et al. 1975). Specificity of cell-mediated lympholysis was tested by utilizing an additional allogeneic mononuclear target population unrelated to the mononuclear cells used in the initial sensitization phase of MLC.

Cytofluorographic analysis of all cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG (G/M FITC; Meloy Laboratories, Springfield, Virginia) on a Cytofluorograf FC200/4800A (Ortho Instruments, Westwood, Massachusetts) as described (Reinherz et al. 1979 d). MLC-stimulated lymphocytes were cultured overnight at 37° C after selective lysis and then analyzed on the Cytofluorograf.

Prior studies demonstrated that two distinct T-cell subsets could by defined by

anti-T4 and anti-T8 monoclonal antibodies and that following activation in MLC, T cells expressed Ia antigens (reviewed in Reinherz and Schlossman 1980a). As shown in Table 1, following MLC stimulation, 55 percent of recovered T cells were T4+, while 39 percent were T8+, and approximately 60 percent expressed Ia antigens. Lysis with anti-T4 and complement left 42 percent of the cells, diminished the total number by 58 percent, and as expected, eliminated the T4+ population (less than 2 percent T4+ cells) when reanalyzed. In addition, the absolute number of T8+ cells did not change and their percentage increased. In contrast, following, lysis with anti-T8 and complement, 61 percent of the starting population remained, of which 90 percent were T4+. Thus, removal of T8+ cells did not diminish the absolute number of T4+ cells. These results confirm prior studies indicating that the T4+ population in peripheral blood was distinct and reciprocal to the T8+ population (Reinherz at al. 1980c).

Complement-mediated lysis with anti-T4 or anti-T8 did not, however, eliminate all Ia+ T cells in either case (Table 1). Following lysis with anti-T4 or anti-T8, 31 percent and 33 percent of cells were Ia+, respectively. These findings suggested that the Ia+ population was not restricted to either T-cell subset alone. In reciprocal studies, treatment with anti-Ia and complement resulted in lysis of 54 percent of cells. The residual cells had a similar distribution of T4+ and T8+ subsets to the starting T-cell population.

To determine whether pretreatment of MLC-stimulated T cells with antibody and complement would abrogate cell-mediated lympholysis, unfractionated T cells, or T cells obtained following treatment with monoclonal antibody and complement, were examined for their cytotoxic potential. As shown in Table 2, in two separate experiments, the unfractionated T-cell population mediated 29 percent and 48 percent killing, respectively, at a 40:1 effector:target ratio. At lower E/T ratios, diminished but significant killing was seen. Pretreatment of activated T cells with anti-T4 and complement resulted in significant enhancement of cytotoxicity in each of the two experiments. In contrast, anti-T8 and complement virtually abrogated cytotoxic effector function at all E/T ratios tested.

The above results were consistent with previous studies which demonstrated that the T8+ population contained the cytotoxic effector T-cell population (Reinherz et al. 1980b). Perhaps more importantly, it should be noted that following anti-Ia and complement pretreatment of allosensitized T cells, there was no diminution in cytotoxic function at any E/T ratio. Rather, increased activity was

Table 1. Lysis of MLC-stimulated T cell subpopulations with monoclonal antibody and complement

Treatment of Lymphoid populations	Percent cells recovered	Percent reactivity of residual cells with monoclonal antibodies		
		anti-T4	anti-T8	anti-Ia
Control	100	55	39	60
Anti-T4+C'*	42	<2	85	31
Anti-T8+C'	61	90	<2	33
Anti-Ia+C'	46	52	32	<5

* C'=complement.

Table 2. Effect of antibody and complement lysis on T-cell cytotoxic function

Pretreatment	Percent killing by residual cells		
E:T Ratio:	40:1	20:1	10:1
Exp. 1			
Control	29±2	25±3	20±2
Anti-T4+C'*	50±2	29±3	26±3
Anti-T8+C'	6±1	6±2	2±3
Anti-Ia+C'	45±5	39±6	26±3
Exp. 2			
Control	48±7	39±2	17±2
Anti-T4+C'	58±3	45±2	35±2
Anti-T8+C'	12±2	6±2	7±1
Anti-Ia+C'	57±4	43±2	37±3

*C'=complement.

noted following treatment with anti-Ia and complement. Moreover, it should be noted that although 60 percent of MLC-stimulated cells expressed Ia antigen, their removal augmented the degree of cytotoxicity. The cytotoxic cells obtained following anti-Ia and complement treatment were specific for the sensitizing population in MLC and did not kill unrelated targets. Finally, in the absence of sensitization in MLC, lysis of target populations was less than 5 percent in all cases (data not shown).

In earlier studies utilizing one anti-Ia heteroantisera, anti-P23, 30, it was shown that treatment of MLC-stimulated cells with the latter and complement markedly diminished cytotoxicity in CML (Evans et al. 1978 b). The present study suggests that the rabbit anti-P23,30 heteroantisera detected additional determinants other than the specific portion of the invariant region defined by anti-Il, despite the fact that both appeared to define a similar bimolecular glycoprotein complex. Further support for distinctions between the monoclonal anti-Ia and rabbit anti-P23,30 were seen in functional studies where it was shown that anti-P23,30 blocked T-macrophage binding and antigen presentation and proliferation in MLC, whereas anti-I1 did not (unpublished data). Analysis of multiple monoclonal anti-Ia antibodies directed at common framework structures also demonstrates that some monoclonal antibodies block soluble and alloantigen proliferation in vitro, whereas most do not (Charron et al. 1979). Findings reported in the present study and those above, support the notion that multiple HLA-D-like antigens may be expressed on activated T cells and that, more importantly, unique Ia antigens might be expressed selectively on subsets of T lymphocytes. The findings reported above indicate that cytotoxic effector cells bear a T8+ Ia− phenotype as defined by these monoclonal antibodies. This appears to be analogous to findings in murine systems (McDevitt et al. 1976, Beverly et al. 1976).

Given prior studies demonstrating the presence of the Ia antigen on TH2+ (T5+/T8+) T cells with functional suppressor activity (Reinherz et al. 1979 e,

Reinherz and Schlossman 1980) and the present results showing the absence of Ia antigen on cytotoxic effector cells, it remains a possibility that the expression of this Ia antigen may distinguish activated suppressor cells from cytotoxic cells. Still to be determined is whether a population of activated suppressor cells might lack the Ia antigen or could be defined by unique I-region antigens in man.

The nature of cell-surface antigens found on human cytotoxic effector T cells was examined utilizing a series of monoclonal antibodies. Anti-T4 was previously shown to react with the human inducer subset, whereas anti-T8 identified the human cytotoxic/suppressor subset. Treatment of MLC-stimulated T cells with anti-T4 and complement resulted in enrichment of lytic activity. In contrast, anti-T8 and complement virtually abrogated cytotoxicity. Since prior studies demonstrated that both T4+ and T8+ T cells expressed Ia antigens following MLC stimulation, their presence on T8+ cytotoxic effector cells was examined. Despite the fact that 60 percent of MLC-activated T cells expressed Ia antigens, their elimination with monoclonal anti-Ia antibody and complement enhanced, rather than eliminated, cytotoxicity. These studies support the notion that the T8+, Ia− T cell is the cytotoxic effector cell.

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