

IMMUNE MECHANISMS IN LEUKEMIA
Role of the Ia Antigens*

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We have focused our recent efforts on characterizing the surface properties of T-effector cells in protection against syngeneic tumors. We have utilized a mouse model, the C58 mouse/line I_b syngeneic leukemia system, to evaluate and characterize the lymphoid subpopulations operative in leukemia immunity (1-4). Utilizing an adoptive transfer technique, we have been able to measure the protective capacity of lymphocytes derived from the spleen, bone marrow, and thymus of both normal and immune donors quantitatively (1). We have shown that the protective cell population appears to be Thy 1.2⁺, Ig⁻, and Ly 4.2⁻ T cells (4).

The *I* (immune response) region of the mouse major histocompatibility complex (MHC) codes for a series of lymphocyte membrane alloantigens (Ia), as well as for many immunologically related functions, including immune response genes, graft versus host reactivity, mixed lymphocyte reactivity, and resistance to gross virus leukemogenesis (5). It is now generally agreed that these Ia antigens are expressed on at least a subset of T cells (6-11). We thus wished to determine any possible role that these Ia expressing T cells may play in tumor immunity.

We report here that the selective removal of the small subpopulation of T cells possessing the Ia determinants coded by the *I-A^k*, *I-B^k*, and *I-J^k* regions of the MHC of the mouse abrogates the protective capacity of nylon-wool-purified T cells against challenge by leukemic cells.

Materials and Methods

Mice. All mice were raised in the breeding colonies at the University of Minnesota or at the University of Southern California. C58(*H-2^k*) mice were maintained in Dr. Lukasewycz's colony at the University of Minnesota. The colony was started with breeder groups furnished by Dr. William H. Murphy at the University of Michigan. The origin of these mice has been described (12). Mice were 8-10 wk old when used experimentally.

I_b Cells. The methods used to prepare stocks of I_b cells were as described previously (1). Spleens were removed from mice moribund with transplanted leukemia and teased into minimum essential medium (MEM) supplemented with 5% fetal calf serum (FCS) (Grand Island Biological Company, Grand Island, N. Y.). All procedures were carried out aseptically. Total and viable cell counts (trypan blue dye exclusion) were performed.

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Immunization of Mice. Procedures for immunizing mice to I_b cells have been described (1). In brief, fresh I_b cells prepared as described above and at a final concentration of 1×10^7 cells/ml were incubated in a 0.02% formalin solution for 18 h at 4°C. Mice received a single i.p. injection (1.0 ml) of the formalinized cells and were observed for 15 days to assure that none died of the immunizing preparation. Mice thus immunized could survive a challenge dose of 10^7 viable I_b cells and served as donors of immune spleen cells (12).

Adoptive Cell Transfer. Mice were immunosuppressed by cytoxan treatment (250 mg/kg body weight); 24 h later they were injected with a counted number of I_b -primed T cells; 24 h after this injection they were challenged with a partially formalin-killed preparation of I_b cells. These preparations contained approximately 10^8 viable cells and are lethal to immunosuppressed, unprotected mice (1, 2).

Spleen Cell Preparation. Immune spleen cells (ISC) were obtained from mice immunized 15 days previously. Donor mice were killed by cervical dislocation, and a single cell suspension was prepared as described for I_b cells.

Nylon Wool Columns. Thymus-derived lymphocytes were prepared from whole ISC preparations using nylon-wool (LP-1 Leukopak Leukocyte Filter, Fenwall Laboratories Inc., Morton Grove, Ill.) as described by Julius et al. (13). Such cells were routinely 90–95% Thy-1 positive as determined by anti-Thy 1.2 serum and complement treatment. Less than 5% of these cell populations are Ig^+ by immunofluorescence.

Antisera. Antisera specific for the Ia antigens were produced as previously described (14). Cell experiments were performed using a single-pool antiserum (A.TH \times B10.HTT) F_1 anti-A.TL which contains antibodies against the *I-A^k*, *I-B^k*, and *I-J^k* regions, but not the *I-E* and *I-C* regions. This antiserum might contain anti-Ia.1, 2, 3, 19, plus the specificity coded by *Ia-4*. This serum has cytotoxic activity for Ia.3 and against promoter T cells (15).

Antiserum Treatment of Cells. For blocking experiments nylon wool-purified T cells were incubated with 2.0 ml of a 1:10 dilution of the anti-Ia serum for 20 min at room temperature and for an additional 20 min at 4°C. Appropriate viability counts (trypan blue exclusion) were made at the end of each procedure. Cells were then washed by centrifugation in MEM-FCS and resuspended at the appropriate concentration.

To determine if the protective cells carried Ia determinants, 1×10^7 cells were incubated with 2.0 ml of a 1:20 dilution of the anti-Ia serum for 20 min at room temperature and 20 min at 4°C, centrifuged, and resuspended in 2 ml of guinea pig complement (Microbiological Associates, Bethesda, Md.) diluted 1:2 in MEM, and incubated for 30 min at 37°C. The cells were then washed twice by centrifugation in media, counted in a hemocytometer, and adjusted to the appropriate concentration.

Results and Discussion

Protective Capacity of T Lymphocytes Bearing the Ia Determinants. To ascertain the role of Ia^+ T lymphocytes in protecting immunosuppressed mice against leukemic challenge, we treated nylon wool-purified T-cell populations with antisera against a restricted portion of the *I* region of the mouse *H-2* complex. We tested graded log doses of T cells treated with such antisera or with normal mouse serum (NMS), with and without addition of exogenous complement, for their ability to protect against I_b cell challenge (Table I). 10^6 untreated, nylon-wool-purified T cells were able to protect immunosuppressed recipients. This is in agreement with our previous findings showing the protective cell bears Thy 1.2⁺, Ig^- , and Ly 4.2⁻ cell surface antigens (4). Treatment with specific antisera alone, NMS alone, complement alone, or with NMS and complement does not alter the protective capacity of the transferred T cells. Treatment of the T lymphocytes with anti-Ia antisera and complement removes only 10–15% of the total T-cell population. The lymphocytes remaining after such treatment are not protective, even at the 1×10^6 transferred cell level. This suggests that the small subpopulation of T cells bearing the Ia determinants coded by the *I-A*, *I-B*, and *I-J* regions of the *H-2* complex appear to have a

TABLE I
Elimination of the Protective Class of Immune T Cells by Treatment with Anti-Ia Serum and Complement

Immune T Cells		Survivors of leukemic challenge				
Treated with:	No. viable cells transferred	Experiment no.			Totals	Percent
		I	II	III		
—	1 × 10 ⁶	5/5	5/5	4/4	14/14	100
—	1 × 10 ⁵	3/5	2/5	1/4	6/14	43
—	1 × 10 ⁴	0/5	0/5	0/4	0/14	0
Anti-Ia	1 × 10 ⁶	4/5	5/5	4/4	13/14	93
Anti-Ia	1 × 10 ⁵	2/4	4/5	2/4	8/13	62
Anti-Ia	1 × 10 ⁴	0/5	0/5	0/4	0/14	0
Anti-Ia + C	1 × 10 ⁶	0/5	0/5	0/4	0/14	0
Anti-Ia + C	1 × 10 ⁵	0/6	0/5	0/4	0/15	0
Anti-Ia + C	1 × 10 ⁴	0/5	0/5	0/5	0/15	0
NMS	1 × 10 ⁶	5/5	5/5	4/4	14/14	100
NMS	1 × 10 ⁵	2/5	3/5	1/5	6/15	40
NMS	1 × 10 ⁴	0/5	ND	ND	0/5	0
NMS + C	1 × 10 ⁶	5/5	5/5	5/5	15/15	100
NMS + C	1 × 10 ⁵	2/5	3/5	2/4	7/14	50
NMS + C	1 × 10 ⁴	0/5	0/5	0/5	0/15	0
C	1 × 10 ⁶	4/4	4/5	4/4	12/13	92
C	1 × 10 ⁵	2/5	ND	1/5	3/10	30
C	1 × 10 ⁴	0/5	ND	ND	0/5	0
—	No cells	0/5	0/5	0/5	0/15	0

significant role in tumor immunity. We have not completely eliminated the possibility that the depletion of Ia⁺ macrophages may be responsible for the lack of protection seen by the treated cell population. This seems unlikely in view of our preliminary findings (unpublished) that T-cell populations depleted of macrophages by glass wool filtration are competent in providing protection. Whether this protective capacity is relegated to cells bearing determinants coded by one, two, or all three of the regions tested is yet to be determined.

The role of Ia determinants as a marker for functional T-cell subsets has recently been explored. It has been shown that the Ia determinants are expressed on suppressor T cells and map in the *I-J* region (16). Further, we have recently shown the Ia antigens expressed on concanavalin A-reactive T cells to be restricted to the *I-J* subregion (15). Stout et al. have studied Ia on Fc receptor-positive T cells by blocking of Ig binding and have determined that a portion of the FcR⁺ T cells are blocked by antibodies directed at *I-A*- or *I-C*-coded determinants (17). The relationship between the Ia-positive T cells capable of protecting mice against leukemic challenge and Ia⁺ T cells with these other functions is yet to be determined.

It is of interest to point out that normal cells, i.e., cells from nonimmune donors, possess the capacity to protect against line I_b leukemia, but only with a 100-fold increase in cells transferred in this assay (1). Studies are now underway to determine if this protective capacity of normal cells can also be abrogated by specific antisera. If it cannot, it would suggest that the Ia determinants are expressed as a result of antigenic stimulation. This seems a real possibility since

Ia expression on T cells appears more easily detected on activated T cells (18, 19).

It is important to consider the results reported here with two other pieces of information. It has been accepted that Ia antigens are not expressed on cytotoxic T cells specific for MHC-coded determinants using negative selection by anti-Ia and complement such as presented here (20), although a contradictory result has appeared (21). The second bit of information is that although it is clear that cytotoxic T cells specific for MHC determinants express the phenotype Ly-1⁻, Ly-2⁺3⁺ (22), a recent report suggests that the effectors for syngeneic tumor cells are Ly-1⁺2⁺3⁺ and therefore represent a distinct subset from the effectors specific for MHC alloantigens (23). The effectors that function in the adoptive transfer system reported here for protection against the syngeneic L₁ leukemia are clearly Ia⁺ in contrast to the cytotoxic T cells directed at the MHC-coded antigens. This together with the previous report provides strong evidence that the effectors in immunity against syngeneic tumors are a different lymphocyte class than those directed at allogeneic cells. Assuming we are not eliminating a putative Ia⁺ amplifier population, we would predict the effector cells in this system would be Ly-1⁺2⁺3⁺ and Ia⁺ and represent a uniquely marked T-lymphocyte subset distinct from both suppressors Ly-1⁻2⁺3⁺, Ia⁺, and helpers Ly-1⁺2⁻3⁻, Ia⁻. Further studies to determine which Ly antigens are expressed and any possible subregion restriction of the Ia antigens expressed on the effectors are underway.

Summary

We have shown that the selective removal of cells possessing Ia determinants coded by the *I-A*, *I-B*, and *I-J* regions of the *H-2* gene complex completely abrogates the protective capacity of nylon-wool-purified T lymphocytes against leukemic challenge. This suggests that the Ia antigen bearing T cells play an important role in tumor immunity.

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