#141

Selective Expression of Loci in the I–J Region on T Cell Hybrids

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Gene products of the major histocompatibility complex (MHC) in the mouse are responsible for a variety of effector and cooperator functions of immunocompetent lymphoid cells. Specifically, loci mapping to the I-region have been linked to suppression (1), MLR stimulator cells (2) and GVH reactions (3). Ia antigens, which are thought to be the products of the Ir genes, have been found on B lymphocytes, macrophages, sperm and a variety of functionally different subsets of T cells (4). The selective expression of I-region molecules on T cell subsets has led many to postulate that these molecules are important in T-B collaboration (5).

Until recently it has been impossible to examine the antigens on these T cell subsets as they represent only a small fraction of the total number of lymphoid cells. Attempts at enrichment of specific T cells have been only partially successful.

Recently it has been demonstrated that cell hybridization offers the opportunity to preserve and immortalize individual B cells as well as T cells. Köhler and Milstein (6) were able not only to immortalize the B cell by fusion with a myeloma but were also able to immortalize its differentiated function, the production of immunoglobulin. Recent work from our laboratory and others (7,8) has shown that by fusion with a T-lymphoma, T cells may also be immortalized. It has therefore become possible to isolate clonal populations of T cells and to characterize surface alloantigens of such clones.

The production of clonal lines of T cell hybrids has been discussed elsewhere (7,9). The clones reported in this paper have come from two different hybridizations. The "921" clones were made with spleen cells from DNP-KLH primed and boosted (BALB/c x SJL)F_1 mice hybridized to BW5147 (a thioguanine, ouabain resistant thymoma cell line, kindly provided by Dr. R. Hyman of the Salk Institute). The "822" clones were made in an identical fashion except that spleen cells were from DNP-KLH primed and boosted A/J mice.

Three different lots of antisera directed against the I-J^k subregion have been employed in this study. Two of these lots (designated 035 and 055) were made by repeated injections of lymphoid cells of B10.A(5R) into B10.A(3R). The third lot (069) was made by repeated injections of B10.A(5R) lymphoid cells into (BALB.B x B10.A(3R))F_1 mice.

The cytotoxic activity of antisera against the hybrid clones was assayed by a micro-procedure using 2000 cells in Terasaki plates with selected lots of rabbit complement. Unless indicated, all cell lines were grown to a density of 3-4 x 10^6 cells/ml for testing. Quantitative absorptions of cytotoxicity were done to show specificity. Either normal spleen cells or hybrid clones were used for absorptions. The absorptions were done with varying cell numbers and 50 μl of antisera at a 1:10 dilution.

We noticed that the killing of 921 2b with anti-I-J sera varied markedly from test to test. Upon detailed examination, we found that the absolute amount of cytotoxicity was dependent upon the culture cycle of the test cells. Specifically, clone 921 2b, at a density of 2×10^6 cells/ml, was 20% killed with 035 at a 1:10 dilution whereas 24 hr later when the cells were approaching stationary phase of the culture cycle and were at a density of 3×10^6 cells/ml, 90% of the cells were killed with the same concentration of 035. Similar results were found on the three subsequent occasions it was tested. This phenomenon was also noted with other antisera (e.g., anti-Thy-1 and anti-Ly-1 and -Ly-2). Thus we conclude that cells in stationary phase of the culture cycle are more susceptible to complement-dependent lysis than those in log phase.

It is known that mouse alloantisera often contain murine leukemia virus activity (10). The concern that lymphoid hybrid lines are quite likely to bear viral determinants on their surface led us to examine the anti-I-J antisera for antiviral activity. Direct testing by a plate binding radioimmunoassay (kindly performed by Dr. J. Ledbetter) demonstrated that 035 had very little activity against AKR virus whereas 055 had considerably more activity. Further, by immunoprecipitation of ^{125}I-labeled disrupted AKR virus followed by SDS-PAGE analysis, 055 and 069 had high levels of activity against GP-70. These antibodies were effectively removed by absorption with purified AKR virus. The absorptions and SDS-PAGE analysis was kindly done by Dr. R. Nowinski, Fred Hutchinson Cancer Center.

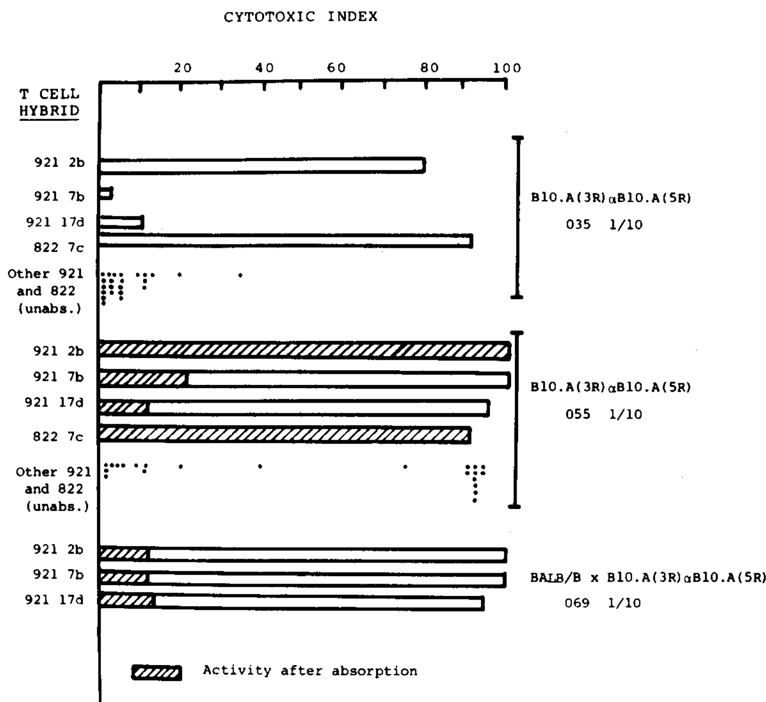

Fig. 1. Expression of loci in the I-J region. Both 055 and 069 were used before and after absorption with 50 µg of AKR virus/30 µl of neat antisera.

An early examination of 24 clones from the 921 series revealed that 921 2b was killed with 035, whereas 921 2b, 927 7b and 921 17d were killed with 055 and 069. Absorptions with appropriate spleen cells indicated that lysis with 035 on 921 2b was specific for I-J region determinants. However, absorptions with 055 and 069 never showed convincing specificity of lysis. When the antiviral antibody was detected in 055 and 069 clones, 921 2b, 7b and 17d were retested using unabsorbed and virus-absorbed antisera. The results are shown in Fig. 1. Serum 055 after virus absorption kills only 921 2b and indeed appears identical to 035 in our assay. However, 069 after absorption no longer kills 921 2b, 7b or 17d, indicating that the only reactivity present in this particular antiserum detected on these T cell hybrids is antiviral in nature.

Recently a similar screening of the 822 hybrids has been done (see Fig. 1). As can be seen, only one 822 clone, 822 7c, was found to be positive with 035. Many clones were found to be positive with 055 before absorption with virus. However, only 822 7c was shown to be killed when virus-absorbed 055 was used. Therefore we conclude that determinants coded for by loci mapping to the I-J region can be detected on T cell hybrids; however, it is essential to be aware of and to remove antiviral antibodies from the antisera used in such studies.

Convincing evidence that the determinants detected on 921 2b are coded for in the I-J region was obtained by absorptions with spleen cells from four congenic pairs of mice. Table 1 shows that a B10.A(3R) (I-J^b) anti-B10.A(5R) (I-J^k) antiserum can be absorbed with B10.A(5R) but not B10.A(3R). A series of other spleen cell populations was also used for absorption purposes. In all cases the absorption with I-J^k haplotype cell populations removed the activity against 921 2b whereas absorption with I-J^b or I-J^s did not remove the activity against this

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TABLE 1

ABSORPTION OF I-J CYTOTOXIC ACTIVITY AGAINST HYBRID CELL LINE 921-2b*

Strain	I-J Haplotype	No. spleen cells (x 10^8) to half absorb anti-I-J**
3R	b	>1
5R	k	0.18
A.SW	s	>1
A/J	k	0.1
B10.S	s	>1
B10.A	k	0.1
BW5147	k	>1

Normal mouse serum + c' cytotoxicity was always $\leq$10%.

*921-2b grown to 3-4 x 10^6/ml (high density culture)

**B10.A(3R) α B10.A(5R) serum #035 was kindly provided by Dr. Hugh McDevitt, Stanford U. Absorptions were done with 50 λ/tube at a 1/10 dilution of antisera with varied cell concentrations.

clone. Thus we conclude that when there are no contaminating antiviral antibodies, it is possible to detect by direct cytotoxicity sepcificities coded for in the I-J region on T cell hybrids. It has been reported by others (11,12) that T cell hybrids made in a similar fashion to ours have specific suppressor activity. It will be interesting to determine if these T cell hybrids express I-J determinants and what correlation there is between immune functional activity and surface phenotype in T cell hybrids.

This work was supported, in part, by grants from the National Institutes of Health: AI-08917, CA-04681, and HD-01287.

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