SPECIFIC ADSORPTION OF H-2-RESTRICTED CYTOTOXIC T CELLS TO MACROPHAGE MONOLAYERS

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Murine cytotoxic thymus-derived (T) cells (Tc cells) specific for modified self cells exhibit H-2 restriction, i.e., they recognize antigenic patterns dependent on self H-2K or H-2D antigens plus a foreign antigen (1, 2). Models to explain this phenomenon fall into two general categories. The first, dual recognition (3–7) states that a Tc cell expresses on its surface two different types of antigen binding sites, one specific for a self H-2K or H-2D determinant, and the other specific for a foreign determinant (X). This model demands that a paired set of binding sites (anti-H-2 plus anti-X) is essential in triggering Tc-cell function, whereas antigen binding to two anti-H-2 sites, or two anti-X sites is not operationally important. The second model (altered self) states that a Tc cell expresses only one type of antigen binding site which is specific for a new antigenic pattern that is dependent upon an interaction or complex between a self H-2K or H-2D molecule and a foreign antigen (1). In this model, the Tc-cell binding site must have affinity only for the complex and not for each of the separate components or it becomes, by definition, a class of dual recognition.

Thus far, there is no conclusive evidence which excludes either model. However, the exquisite specificity of self H-2 recognition, as shown by H-2K mutants (6, 8–10), the variety of viral and other antigens which cause H-2 restricted responses, and considerations of Tc-cell ontogeny (4–7, 11) impose severe strain on altered self models. On the other hand, cold target competition experiments (1) are readily explained by altered self, but require qualification of dual recognition. The salient observations are as follows: Tc-cell-mediated lysis of labeled virus-infected target cells is specifically inhibited only by addition of virus-infected unlabeled competitors which have the same H-2 and viral antigens as the labeled targets; H-2 compatible uninfected competitors, or infected allogeneic competitors are not specifically inhibitory. The problem for dual recognition is that multipoint, high-avidity binding of Tc cells to either H-2 or X antigens, not necessarily both, should cause competition. The model can be salvaged, however, if competition operates not via binding per se, but at the level of the lytic mechanism through inability of Tc cells to lyse more than one target cell simultaneously (12). This form of dual recognition predicts that uninfected self or infected allogeneic cells can bind to Tc cells specific for infected self targets, but are ineffective competitors because they do not activate the lytic mechanism. The present experiments address this problem.

Materials and Methods

General. Details of inbred mice, and use of ectromelia (13), Sendai (14), and influenza A/JAP (15) viruses have been given elsewhere. Trinitrophenyl (TNP) modification was similar to the method used by others (16).
Generation of Effector T, Cells In Vitro. The method used was as described for ectromelia virus (17). Briefly, spleen cells from mice immunized 3–5 wk previously with virus (ectromelia, Sendai, or influenza) were cultured with syngeneic splenic stimulator cells infected with the same virus used for immunization. Stimulator cells were infected with two plaque-forming units virulent ectromelia virus/cell, 1–2 embryo infectivity dose (EID₅₀) U/cell of Sendai virus, or 2 EID₅₀U/cell of influenza virus. A stimulator to responder ratio of 1:10 was used for all viral systems and cultures were held for 5 days at 37°C for Sendai and influenza, and 39°C for ectromelia.

The generation in vitro of secondary TNP-immune T, cells was essentially as for viral systems except that responder spleen cells were from mice primed 2–12 wk previously by subcutaneous injection of 4 × 10⁶ TNP-modified spleen cells in 40 μl PBS into the hind footpad, and responder:stimulator ratio was 4:1.

For primary one-way mixed lymphocyte reactions (MLR) splenic responder cells and γ-irradiated, allogeneic splenic stimulator cells were cultured at 37°C for 5 days at a responder:stimulator ratio of 4:1 (18).

After the harvesting of responder cells from cultures, dead cells were always removed by centrifuging through Ficoll-Isopaque (19).

Adsorption of Immune Lymphocytes to Macrophage Monolayers. The method described by Brondz et al. (20) was modified as follows: peritoneal macrophages were harvested 3 days after intraperitoneal injection of an irritant (4 ml thioglycollate medium, Difco Laboratories, Detroit, Mich.). Almost confluent monolayers were prepared by seeding 7 × 10⁶ peritoneal macrophages in 10 ml F-15 containing 10% FCS in a tissue culture flask (25 cm² growth area, Falcon Plastics, BioQuest, BBL & Falcon Products, Cockeysville, Md.) and incubating at 37°C overnight. Monolayers were rigorously washed with warm medium to remove all nonadherent cells; the cells remaining in the flask were then uniformly large, strongly adherent macrophages. After washing, all medium was drained from the flasks and 2 ml of complete medium containing 10⁷ lymphoid cells (including effector T, cells) from the cultures described above were added to each flask. Adsorption was carried out by incubating at 30°C for 2.5 h in a horizontal position. Collection of the nonadherent cells was achieved by draining the flasks vertically over centrifuge tubes without prior agitation. This procedure inevitably resulted in some loss of nonadherent cells in the small volume of medium remaining in the drained flask. There could also be a degree of nonspecific adherence and trapping as reported in several laboratories (20–24). However, this method avoided the problem of detachment of cells from the adsorbing monolayer which would then contaminate the effector cell suspension and act as cold competitors (1). Because of their large size and morphology, thioglycollate-induced macrophages which became detached could be easily identified during counting of the nonadherent cells harvested after the adsorption procedure. They were never more than 1.5% of the total nonadherent cell population, which was then tested for effector activity on ⁵¹Cr-labeled target cells. Because the maximum effector:target ratio used was 10:1, this level of contamination was never high enough to result in the macrophages acting as cold competitors.

Another potential pitfall in this method is the possibility that T, cell lytic potential becomes exhausted (because of repeated killing) during their 2.5 h in culture at 30°C with an absorbing monolayer that they are capable of recognizing and lysing (22). This possibility was ruled out by control experiments which showed that after 2.5 h at 30°C in contact with a ⁵¹Cr-labeled adsorbing monolayer, T, cells had lysed very few of the labeled cells, but they were able to strongly lyse the labeled macrophages over a further 6 h with the temperature raised to 37°C, the time and temperature used in routine assays.

Cytotoxicity Assay. The method used for L929, P815, and macrophage targets have been described in detail elsewhere (17, 18). Data given are the means of triplicates and have had spontaneous release subtracted for assay times of 6 h at 37°C. Standard errors of the means were always <3% and are omitted for clarity. Significance was determined by Student's t-test.

Results
Specific Depletion of T, Cells Sensitized to TNP-Modified or Virus-Infected Cells. CBA/H effector cells generated against TNP-modified or influenza virus (JAP)-infected syngeneic spleen cells were placed on a panel of monolayers of CBA/H or BALB/c
Table I

Specific Adsorption of TNP-Immune, Virus-Immune, and Alloreactive Tc Cells on Macrophage Monolayers*

| Adsorption monolayers | TNP-1,929 (H-2t) targets plus CBA/H-TNP Tc cells | JAP-1,929 (H-2t) targets plus CBA/H anti-CBA/H JAP Tc cells | P-815 (H-2h) targets plus CBA/H anti-BALB/c Tc cells |
|-----------------------|--------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
|                       | 3:1 | 10:1 | 3:1 | 10:1 | 3:1 | 10:1 | 3:1 | 10:1 |
| CBA/H (H-2t)          | 46.1 | 70.6 | 26.1 | 49.5 | 29.0 | 71.1 |
| CBA/H-TNP             | 12.0§ | 30.1§ | 34.8 | 59.6 | 39.3 | 79.9 |
| CBA/H-JAP             | 49.8 | 80.0 | 12.0§ | 29.2§ | 44.0 | 79.6 |
| BALB/c (H-2b)         | 53.0 | 83.9 | 35.0 | 59.2 | 8.9§ | 29.6§ |
| BALB/c-TNP            | 42.2 | 81.3 | 28.5 | 50.5 | 9.4§ | 27.6§ |
| BALB/c-JAP            | 44.7 | 83.4 | 26.7 | 45.7 | 2.5§ | 15.5§ |

* Data given are means of triplicates of percent-specific ³Cr release at effector-target ratios of 3:1 and 10:1.
§ Significantly less than appropriate controls at the same effector-target ratio (P < 0.001).

Table II

Virus-Specific Adsorption of CBA/H Tc Cells Stimulated by Sendai or Influenza (JAP) Viruses*

| Adsorption monolayer | Sendai-1,929 (H-2b) targets plus Sendai-immune Tc cells | JAP-1,929 (H-2t) targets plus JAP-immune Tc cells |
|----------------------|----------------------------------------------------------|----------------------------------------------------------|
|                       | 3:1 | 10:1 | 3:1 | 10:1 | 3:1 | 10:1 |
| CBA/H (H-2t)          | 15.9 | 38.2 | 26.5 | 59.9 |
| CBA/H-JAP             | 17.3 | 36.6 | 15.7§ | 36.8§ |
| CBA/H-Sendai          | 7.8§ | 14.7§ | 24.1 | 54.9 |
| BALB/c (H-2b)         | 18.3 | 38.4 | 32.5 | 60.4 |
| BALB/c-Sendai         | 14.7 | 31.1 | 35.4 | 60.2 |

* Data given are means of triplicates of percent-specific ³Cr release at effector-target ratios of 3:1 and 10:1.
§ Significantly less than all other groups on the same target at the same effector-target ratio (P < 0.001).

Discussion

Adsorption of alloreactive Tc cells has been reported previously by several groups (20–24), but only one brief report, uncontrolled for virus-specificity, concerns adsorption of macrophages, either TNP-modified, infected with JAP or untreated (Table I). Specific adsorption occurred only when monolayers were homologous to the stimulator cells used in culture, i.e., CBA/H TNP-immune Tc cells were specifically adsorbed only on TNP-modified CBA/H monolayers. Further, CBA/H JAP-immune Tc cells adsorbed specifically on JAP-infected CBA/H monolayers although no reduction of cytotoxicity was observed after contact with TNP-modified or JAP-infected BALB/c monolayers. To ensure that the BALB/c macrophages were capable of specific adsorption, alloreactive Tc cells generated in a MLR were tested on the same panel of monolayers. Adsorption of anti-BALB/c Tc cells occurred on all BALB/c monolayers irrespective of infection by influenza virus or TNP modification. These observations were extended to include BALB/c Tc cells. JAP-immune Tc cells from BALB/c mice can be specifically adsorbed on the appropriate JAP-infected monolayers (data not shown).

To test the virus-specificity of adsorption of virus-immune Tc cells, Sendai and influenza (JAP) viruses were used. Table II illustrates that adsorption is highly virus-specific. Both types of virus-immune Tc cells showed significantly reduced cytotoxicity only after contact with syngeneic monolayers infected with the same virus used for immunization.
tion of H-2 restricted Tc cells (25). Our prime objective was to determine if Tc cells which recognize antigenic patterns dependent on both self H-2 and a given foreign antigen (X) could bind specifically to macrophages displaying (a) self H-2 alone; (b) X alone, or (c) self H-2 plus X together. The results clearly indicated that significant specific binding occurred only in case (c). This adsorption was specific for both H-2 and X antigens in the examples tested. There was clear discrimination between H-2k and H-2d (acting as self markers), and between TNP, influenza (JAP) and Sendai viral antigens (acting as X). Because no specific adsorption of H-2-restricted Tc cells (specific for self H-2 plus X) could be detected on either H-2-bearing or on X-bearing macrophages, these results are consistent with altered self. At face value they are not compatible with dual recognition models in which multiple copies of anti-H-2 and anti-X binding sites, each with measurable affinity for antigen, are displayed on Tc cell surfaces and are thus available to participate in multipoint (high avidity) interaction with antigens on adsorbing cell surfaces. However, the biophysical and genetic problems inherent in altered self models (see references 4-7 for review of salient points) may justify formulation and testing of modifications of dual recognition models which invoke new assumptions concerning the expression and deployment of antigen-receptors in Tc-cell surface membranes, and/or the mechanisms of antigen-dependent triggering of lytic function as a consequence of receptor-antigen interaction.

Summary

These experiments tested whether Tc cells specific for foreign antigen (X) plus self H-2 adsorbed to macrophage monolayers displaying (a) X with allogeneic H-2; (b) self H-2 alone; (c) X plus self H-2. Specific adsorption occurred only in case (c), a result compatible with altered self and requiring further operational assumptions in dual recognition models.

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