REGULATION OF CYTOTOXIC T-CELL REACTIVITY TO SYNGENEIC TUMORS BY THE THYMUS*

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Regulation of the generation, appearance, and activity of autoreactive cells has been thought to be controlled by the thymus either by clonal deletion (1), mutation (2), or by the export of cells which suppress reactivity to self (3). It has been recently proposed that major histocompatibility gene products expressed in the thymus may influence the capacity of T cells to react to altered-self determinants (4). Although it remains unclear whether tumors display unique tumor associated antigens on their surface, it has become increasingly important to evaluate if T cells detect tumor cell surface antigens and how this response may be regulated.

Recent data indicate that, in adult life, the thymus may export a specific T-cell subpopulation which controls the immune response either by active suppression (5, 6) or by feedback regulation (7). Removal of the thymus in adult life has been shown not only to enhance both the humoral (8) and cellular (9) immune response but also has been shown to reverse the induction of suppressor T cells in vivo (10). We now report that adult thymectomy results in enhanced T-cell cytolytic activity directed against syngeneic tumor cells in vitro.

Materials and Methods

Adult Thymectomy. C57BL/6 or BALB/c female mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. The mice were thymectomized according to the method of Levey and Medawar (11), and autopsied at the time of sacrifice to ascertain if thymectomy was complete. Sham thymectomy of the age and sex-matched controls involved anesthetizing the animals, opening the thoracic cavity, and applying autoclips to the incision similarly to the thymectomized mice.

Immunoabsorbent Columns. Purified T-cell populations were prepared by using a Sephadex G-200 immunoabsorbent column as previously described (12). In brief, purified rabbit anti-mouse Fab was conjugated to Sephadex G-200 by cyanogen bromide. Approximately 200 x 10^6 spleen cells were resuspended in L-15 medium containing 0.005 M EDTA, 5% fetal calf serum (FCS), and 100 U/ml penicillin-streptomycin and added to the column. The effluent T-cell fraction was collected for 2 h at room temperature. Cells were assessed for T-cell purity by using either fluoresceinated normal rabbit or fluoresceinated rabbit anti-mouse Fab sera. Routinely, the T-cell fraction contained 5% or less immunoglobulin-positive B cells.

In Vitro Sensitization. 2 x 10^6 splenic lymphocytes or splenic T cells (purified utilizing the immunoabsorbent columns) were incubated with either 1 x 10^6 mitomycin-C treated or 2,000 rads irradiated syngeneic tumor cells. In addition, 1 x 10^6 or 10 x 10^6 irradiated syngeneic lymphocytes were incubated with the splenic T cells. The cells were cultured in RPMI-1640 containing 10% FCS, 5 x 10^-5 M 2 mercaptoethanol, 100 U/ml penicillin-streptomycin, 1.2% Hepes buffer, and 1

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mM L-glutamine (complete medium). All cultures were incubated in 35-mm tissue culture dishes (BioQuest, BBL & Falcon Products, Becton, Dickinson & Co., Cockeysville, Md.) at 37°C, in a 5% CO₂ incubator on a rocking platform for 6 days. 0.1 ml of complete medium was added daily before cell harvest.

**Trinitrophenyl (TNP) Modification of Tumor Cells.** Tumor cells were treated with TNBS according to the methods of Shearer. (13).

**Cytolytic Assay.** Tumor cells, syngeneic lymphocytes, or phytohemaglutinin (PHA) stimulated lymphocytes were treated with 150 μCi sodium chromate<sup>51</sup>, washed three times with cold phosphate-buffered saline containing FCS, and incubated 4-8 h with varying numbers of cells obtained from the in vitro cultures. The percent cytotoxicity was calculated as follows:

\[
\text{percent cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{freeze thaw release} - \text{spontaneous release}} \times 100.
\]

In each experiment, normal lymphocytes were incubated with chromium<sup>51</sup>-labeled target cells to assess nonimmune release.

**Results and Discussion**

**The Effect of Adult Thymectomy on the Generation of Cytolytic T Cells Directed Against Syngeneic Tumors.** C57BL/6 (H-2<sup>b</sup>) mice were sham thymectomized (sham tx) or thymectomized (tx) at 8- to 10-wks of age. 2- to 3-mo after surgery, T cells were incubated in vitro with either EL-4 (H-2<sup>b</sup>) tumor cells or normal syngeneic lymphoid cells. As seen in Fig. 1, T cells from thymectomized mice generated a high degree of cytolytic activity directed against EL-4. In contrast, cells from sham tx mice failed to generate any significant reactivity. A series of experiments, summarized in Table I, show that T cells from tx mice had significantly enhanced reactivity directed against EL-4 in contrast to cells from sham tx animals. In addition, as seen in Table I, autoreactivity (as determined by lysis of syngeneic lymphoid targets) could be detected in only one of four experiments. Thus, T cells obtained from adult thymectomized animals not only had a significantly enhanced capacity to recognize and lyse syngeneic tumor cells but, in addition, failed to kill syngeneic lymphoid cells.

**Effect of Adult Thymectomy on the Generation of Cytolytic T Cells Directed Against TNP-Modified Tumor Cells.** BALB/c (H-2<sup>d</sup>) mice were sham tx or tx at 8- to 10-wk of age. 2-3-mo after surgery, the splenic T cells were isolated and incubated in vitro with either P815 or TNP-modified P815 tumor cells. As seen in Fig. 2, cells from sham tx mice had either little or no cytolytic T-cell reactivity. For example, the specific cytolytic reactivity of T cells from sham tx mice at a 50:1 lymphocyte to target cell ratio varied from 0 to 20%. In contrast, cells from tx mice had significantly greater reactivity; at the same lymphocyte to target cell ratio, the specific cytolysis varied from 38 to 50%. Additional experiments were done to ascertain if chemical modification of the tumor by TNP would increase cytolytic T-cell function. A representative experiment, Fig. 2 B, shows that, while TNP-modification of the tumor enhanced the cytolytic activity of T cells from sham tx mice, in comparison, the reactivity of T cells from adult tx mice was significantly increased. In addition, even though cells from the adult tx animals had high degrees of cytolytic activity directed against P815-TNP, there was only slight cytolytic reactivity against P815 tumor cells not treated with TNP. No reactivity against either normal lymphoid or PHA-stimulated lymphocyte blast target cells could be detected (Fig. 2 C). It should be emphasized that the PHA-stimulated lymphocytes were fully susceptible to...
Fig. 1. Induction of cytolytic T cells against syngeneic tumor cells from either sham tx (●) or tx (○) mice. T cells from C57BL/6 (H-2b) mice were cultured with EL4 (H-2b) tumor cells and cytotoxicity assayed as described in Materials and Methods.

Table I

Specific Reactivity of Cytolytic T Cells Directed Against Syngeneic EL-4 Tumor Cells

| Specific release | Specific release |
|------------------|------------------|
|                  |                  |
| EL-4 (target)    | (Splenic lymphoid cell target) |
| Exp.             | %                | %                |
| 1                | Sham tx 08       | 0                |
|                  | tx 22            | 0                |
| 2                | Sham tx 48       | 18               |
|                  | tx 80            | 10               |
| 3                | Sham tx 04       | 0                |
|                  | tx 16            | 0                |
| 4                | Sham tx 03       | 0                |
|                  | tx 20            | 0                |

T lymphocytes were obtained from sham tx or tx C57BL/6 (H-2b) mice, incubated in vitro with EL-4 (H-2b) tumor cells, and assessed for cytolytic reactivity at a ratio of 25 effectors: 1 Crabeled EL-4 or C57BL/6 lymphoid target cell. The results presented represent four independent experiments.

The experiments show that adult thymectomy resulted in the enhanced capacity of splenic T cells to respond to and lyse syngeneic tumor cells in vitro. Moreover, cells from tx mice which lysed syngeneic tumor cells did not kill either normal or PHA-stimulated lymphocytes, showing that T cell recognized and reacted to unique determinant on the tumor cell surface. T cells from tx
animals also generated significantly enhanced cytolytic activity against chemically modified tumor cells. These experiments taken together suggest that the thymus exports a subpopulation of T cells sensitive to adult thymectomy which regulate the reactivity of T cells directed against syngeneic tumor cell surface determinants.

Summary

Adult thymectomy has been shown to result in the enhanced capacity of splenic T cells to respond to and lyse syngeneic tumor cells in vitro. In addition, T cells from thymectomized mice which kill syngeneic tumor cells do not lyse either normal lymphoid or mitogen-stimulated syngeneic lymphoblast target cells. These findings indicate that the thymus exports a subpopulation of T cells sensitive to adult thymectomy which regulates the generation of cytolytic T cells directed against syngeneic tumor cells.

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