DICHOTOMY BETWEEN THE INDUCTION OF
SUPPRESSOR CELLS AND IMMUNOLOGIC TOLERANCE
BY ADULT THYMECTOMY*

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The generation of immune suppression and the induction of unresponsiveness are two forms of immunologic tolerance. In the past, these two immunologic phenomena have not been clearly distinguished from each other. For example, adult thymectomy has been shown either to prevent or to prolong immunological tolerance (1-3). This apparent contradiction arises from confusion concerning the cellular mechanisms that may underly immune suppression and tolerance. Recent evidence indicates that the elicitation of suppressor cells and the induction of tolerance can be independent of each other, which suggests that the generation of suppressor T cells is not critical for tolerance induction (4). In this report, we show that adult thymectomy prevents the induction of suppression but, concomitantly, fails to influence the induction of immunologic tolerance to the same antigenic determinant. This finding demonstrates that the cellular mechanism underlying the induction of suppressor T cells is distinct from that underlying tolerance.

Materials and Methods

Animals. 3-wk-old male BALB/c mice and C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, Maine)

Thymectomy. 3- to 4-wk-old animals were thymectomized according to the method of Levey and Medawar (5). Sham thymectomy of age- and sex-matched mice involved anesthetizing the animal, opening the thoracic cavity, and applying autoclips to the incision similarly to the thymectomized animals.

Preparation of Tolerogens and Antigens. Murine IgG_{2a} was obtained through starch block electrophoresis from sera of BALB/c mice bearing the RPC₅ tumor, as previously described (6). The nucleoside guanosine was purchased from Sigma Chemical Co. (St. Louis, Mo.). Keyhole limpet hemocyanin (KLH) (Pacific Biomarine Supply Co., Venice, Calif.) was prepared as described elsewhere (7). Guanosine was bound to protein by the method of Erlanger and Beiser (8). Guanosine₃₅-IgG_{2a} (G-γ2a) and guanosine₇₁-KLH (G-KLH) were used as tolerogen and immunogen, respectively. (Subscripts indicate the total molar ratio of hapten substitution on the carrier protein.) Guanosine was coupled to isogenic spleen cells by a modification of the method previously described (9). In brief, spleen cells from 10 C57BL/6 or BALB/c mice were prepared in 15 ml of Minimal Eagle's Medium (MEM) with a tissue grinder. The cells were washed three times in MEM and once in 50 ml of a 50% solution of MEM and 0.15 NaHCO₃ solution; then the cell concentration was determined in a hemocytometer. For every 10⁹ spleen

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cells, 20 mg of guanosine was suspended in 3 ml of 0.15 M NaHCO_3 , then oxidized with 1.5 ml of 0.1 M sodium periodate in saline for 20 min at room temperature; the reaction was stopped with 15 μl of ethylene glycol. The spleen cell suspensions were added dropwise to the oxidized guanosine solution and the mixture was stirred gently at room temperature. After 15 min the binding was stopped with 100 mg *t*-butylamine borane in 5 ml 0.15 M NaHCO_3 . After 3 min at room temperature, the reaction tube was filled to 50 ml with MEM and centrifuged for 15 min at 1,000 rpm. The guanosine-conjugated cells were washed with MEM three more times, after which appropriate adjustments in cell concentration were made. Guanosine-coupled spleen cells were then ready for intravenous injection via tail vein.

Detection of Guanosine Plaque-forming Cells (PFC). The detection of antibody-forming cells to guanosine was done as previously described (9) with guanosine directly linked to sheep erythrocytes as indicator cells. Guanosine-specific direct PFC were assayed in spleen cell suspensions 5 d after challenge with G-KLH.

Experimental Design. Thymectomized and sham-thymectomized mice were rested for 5–6 wk before entering the experiments. Age- and sex-matched normal littermates were used as controls. The basic protocol was: Groups of five to six normal sham-treated or adult thymectomized BALB/c or C57BL/6 8- to 10-wk-old mice were untreated or injected either with guanosine coupled to IgG to induce tolerance or with guanosine coupled to isogeneic spleen cells to induce suppression. Low doses of tolerogen (10–20 μg of G- $\gamma 2a$) were used with a relatively high dose ($40\text{--}80 \times 10^6$) of guanosine-modified spleen cells. Animals of all groups were challenged 5 d later with 0.4 mg of G-KLH given intraperitoneally in complete Freund's adjuvant. Guanosine-specific direct PFC was assayed 5 d later. Statistical analysis of the difference between groups was done according to the Student's *t* test. The geometric mean PFC for each group was also calculated.

Results

A simple experimental model was used in which the same nucleoside (i.e., guanosine) was coupled either to isogeneic spleen cells to elicit suppressor cells or to isologous gammaglobulin to induce tolerance (9).¹ Guanosine was selected among the four nucleosides of DNA because of its immunodominance (10). In both instances, guanosine-specific antibody-forming cells were assayed after immunization with G-KLH. Initial experiments were done in BALB/c mice because they are easily tolerized to guanosine-isologous IgG (11). The results (Fig. 1) show that both control and sham-treated mice can readily be suppressed by guanosine-modified spleen cells, as well as rendered tolerant by G- $\gamma 2a$. The immune response of sham-operated mice appears wider than in controls. In contrast, adult thymectomized mice were not suppressed by guanosine coupled to isogeneic spleen cells; nonetheless, they were readily tolerized by guanosine coupled to isologous gammaglobulin.

Similar results were obtained in several experiments with C57BL/6 mice, which were chosen because one can readily generate suppressor T cells by guanosine-modified spleen cells in this strain.¹ A low dose of tolerogen (10 μg G- $\gamma 2a$) was chosen to determine whether thymectomy would prevent low-dose tolerance induction. Conversely, a high dose of guanosine coupled to isogeneic spleen cells (80×10^6) was selected in an attempt to overcome the influence of thymectomy on the generation of suppressor cells. The data from a typical experiment are shown in Table I. Whereas normal and sham-treated mice can be either suppressed or tolerized, adult thymectomized mice can only be tolerized.

¹ Borel, Y., and M. C. Young. Nucleic acid suppressor T cells. *Proc. Natl. Acad. Sci. U. S. A.* In press.

FIG. 1. Effect of adult thymectomy (ATX) on immune suppression and immunologic tolerance to guanosine in BALB/c mice. Groups of five to six normal, sham-thymectomized or thymectomized 8-wk-old male BALB/c mice were treated as follows: □ No treatment before immunization with G-KLH (control group). ▨ Injected intravenously with 20 μ g of G- γ 2a 5 d before immunization with G-KLH (tolerized group). ■ Injected intravenously with 40×10^6 guanosine-modified spleen cells 5 d before immunization with G-KLH (suppressed group). Each bar represents the geometric mean ($\pm$ SE) of the direct guanosine-specific PFC of each group as assayed 5 d after immunization.

TABLE I

Effect of Adult Thymectomy on Immune Suppression and Immunologic Tolerance to Guanosine in C57BL/6 Mice

Number of mice	Treatment	PFC/spleen ($\pm$ SE)	P
Controls			
5	None	27,705 (4,764)	
5	10 μ g G- γ 2a	6,840 (2,784)	<0.001
5	80×10^6 G-spleen cells	5,538 (2,818)	<0.001
Sham thymectomy			
5	None	19,276 (2,382)	
5	10 μ g G- γ 2a	7,046 (838)	<0.001
5	80×10^6 G-spleen cells	2,219 (1,197)	<0.001
Adult thymectomy			
5	None	14,285 (4,598)	
5	10 μ g G- γ 2a	4,577 (869)	<0.01
5	80×10^6 G-spleen cells	17,128 (3,371)	NS

Discussion

The concept that the thymus is the source of suppressor cells originated when it was shown that intrathymic inoculation of antigen results in the adoptive transfer of antigen-specific suppressor cells (12). Subsequently, it was found that adult thymec-

tomy leads to enhanced activity of cytotoxic T cells directed against syngeneic tumor cells *in vitro* (13); *in vivo* enhanced helper activity (14) and prolonged allograft survival (15) have been noted.

Adult thymectomy has also been used as a technique for analyzing the cellular mechanism of the induction of suppression. Basten et al. (1) showed that mice could not be rendered fully tolerant by deaggregated human gammaglobulin after adult thymectomy. Their interpretation was that the suppressor cell was a relatively short-lived T cell of thymic origin. Nachtigal et al. (2), utilizing human serum albumin, also reported that the capacity to induce tolerance was lost after adult thymectomy. In both instances, tolerance was dependent upon the generation of suppressor T cells. In contrast, Claman and Talmage (3) have shown that adult thymectomy did not prevent tolerance induction to bovine gammaglobulin, but did facilitate its maintenance. This apparent discrepancy on the effect of thymectomy on tolerance can be explained by the recent observation of Parks et al. (4). They have shown that the form of the antigen preparation is critical for the generation of suppressor cells, which may or may not accompany tolerance induction. Therefore, if tolerance induction is independent of suppressor cells, thymectomy would have no effect on tolerance. This is consistent with our results. In addition, the prolongation by adult thymectomy of the state of tolerance might be understood in light of recent work by Waters et al. (16). They have shown that suppressor T cells to the tolerogen are generated when tolerance is broken. If the breaking of tolerance requires suppressor T cells, themselves dependent upon the existence of the intact thymus, then adult thymectomy might not only permit tolerance induction but also facilitate its maintenance.

Our data clearly show that whereas adult thymectomy prevents the generation of suppression, it does not affect the induction of tolerance to the same antigenic determinant. The distinction between the cellular mechanisms of these two immune phenomena is particularly striking in this system, because we are looking at the suppression of a B cell response to a single hapten achieved by two different means. In one instance, guanosine coupled to isologous gammaglobulin was used to induce tolerance; in the other, guanosine was coupled to isogeneic spleen cells to induce suppression (9).¹ In both cases, guanosine-specific PFC were assayed after challenge with G-KLH.

The following conclusions can be drawn from our data: short-lived T₁ lymphocytes are depleted as a result of adult thymectomy (17). These are the Ly123 cells, precursors of the Ly23 T cell subset of the mature T cells. Thus, mature T cells would not be generated and consequently could not suppress a B cell response, either directly or indirectly through their action on Ly1 helper T cells. This conclusion is consistent with the work of others who have shown that, as a result of adult thymectomy, there is a depletion of suppressor T cells acting on delayed hypersensitivity Ly1 T cells (18, 19).

Adult thymectomy clearly diminishes the generation of suppressor T cells that can affect either T or B cell responses. On the other hand, this surgical procedure fails to influence tolerance induction, which is independent of suppressor T cells. Because both T and B cells are rendered tolerant by hapten-isologous IgG (20), and we have tested tolerance with a T-dependent antigen, this indicates that one can induce tolerance in Ly1 helper T cells, the long-lived lymphocytes, directly. Perhaps this is the first indication that one can induce tolerance in the Ly1 subset of T cells (helper

T cells) without suppressor T cells (Ly23). The mechanism is, most likely, receptor blockade (21), which might involve helper T cells as well as B cells.

In view of these findings, and to avoid further confusion in the literature, we propose that "immune suppression" be used to refer to unresponsiveness mediated by suppressor T cells, whereas "immunologic tolerance" be reserved for other cellular mechanisms.

Lastly, the clinical implications of this observation have to be emphasized. Thymectomy might be done in immunodeficiency diseases in which the mechanism is a result of an excess of suppressor T cells, or in leukemia to help amplify cytotoxic T cell responses against autochthonous leukemia antigens (22). This surgical procedure would not, however, impair the induction or the maintenance of tolerance autologous antigens, and consequently would not provoke autoimmunity.

Summary

Adult thymectomy prevents the development of suppressor T cells without impairing the induction of immunologic tolerance to the same antigenic determinant. This finding demonstrates that the cellular mechanisms underlying immune suppression and immune tolerance are different.

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