ADOPITIVE TRANSFER OF ALLOTYPE-SPECIFIC SUPPRESSOR CELLS INHIBITS THYMUS-INDEPENDENT IMMUNOGLOBULIN PRODUCTION IN SYNGENEIC ATHYMIC MICE

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Allotype-suppressed mice are (SJL x BALB)F1 hybrids that have developed a suppressor cell population directed specifically against the paternal (SJL) allotype determinant carried on the H chain of γG2a immunoglobulin (Ig-1b) (1). Suppressor cells persist in the lymphoid tissues of these mice and are able, in cell mixture experiments, to suppress Ig-1b production of normal syngeneic cells, both in vivo (2) and in vitro (3, 4). Although suppression usually persists throughout the life of the animal, there are often periods in which some Ig-1b-secreting cells escape from suppression both in situ and after adoptive transfer (1, 2). This is probably related to the fact that Ig-1b-bearing cells are present in suppressed mice despite the lack of Ig-1b in their sera (5), and may reflect fluctuations in the level of the suppressor T cells. Allotype suppression therefore represents a long-term regulatory mechanism in which a very delicate balance is maintained between suppressed and suppressor cell populations.

Investigations into the mechanism of allotype suppression have thus far determined that the effector cells are T cells (3, 4) differing from helper T cells with respect to their Ly determinants (6), and that they release, in vitro, a soluble suppressor factor (3, 6). It has recently been reported that the target for the suppressor cell is not the Ig-1b secreting cell itself, but rather an allotype-specific helper T cell, required for the production of Ig-1b (6).

However, congenitally athymic (nude) mice, presumed to lack functional T cells, have easily detectable serum levels of γG2a (7–10). Similarly, in the experiments described below, (SJL x BALB)F1 nude mice have considerable levels of both Ig-1b and Ig-1a, the γG2a of BALB allotype. It must therefore be assumed that γG2a can be produced in the absence of helper T cells, possibly in response to thymus-independent stimuli. The present studies were undertaken to determine whether the B cell responsible for this thymus-independent Ig-1b would be resistant or sensitive to suppressor cells generated in the normal manner in perinatally suppressed (SJL x BALB)F1 mice. The results to be presented indicate that the B cells are sensitive to suppression and that in these mice, the target is therefore not a thymus-derived cell, but possibly the Ig-1b-producing cell itself.

Materials and Methods

Mice

BALB/cAn mice were obtained from Simonsen Laboratories, Gilroy, Calif., and SJL/J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. The mice used in these experiments were derived from these parent strains and are referred to as follows:
SUPPRESSED MICE. These mice are the (SJL × BALB)F1 progeny of SJL males and BALB females immunized against Ig-1b. They have been described in detail elsewhere (1).

NORMAL MICE. Normal mice are the progeny of SJL males and nonimmune BALB females.

NUDE MICE. These mice are the (SJL × BALB)F1 (nu/nu) progeny of SJL males and BALB females that are heterozygous for the nude gene (nu/+) and have been described in detail elsewhere (1).

Preparation of Cell Suspensions. Cells were obtained from spleen and lymph nodes (axillary, brachial, submaxillary, and mesenteric) by gently rubbing the tissue between two slides into minimum essential medium (Grand Island Biological Co., Grand Island, N. Y.) and passing them through nylon mesh to remove debris. The cells were centrifuged at 1,000 rpm for 10 min and resuspended in medium to the appropriate concentration for counting and injection.

Immunodiffusion. Sera were tested for Ig-1b by double diffusion in agar on microscope slides as previously described (1). Positive reactions are rated as w+ to 4+, according to the strength of the reaction. These values represent a range of 0.025 to >1 mg/ml of serum.

Results

The prebleed level of Ig-1b in the serum of the nude mice ranged from values of 0.5 to 1 mg/ml, whereas that of normal (SJL × BALB)F1 mice of the same age was in the range of 1.5 to 3 mg/ml. Four nude recipients were injected with 10^7 spleen or lymph node cells obtained from a suppressed donor. Within 3 wk after injection of the suppressor cells, Ig-1b was completely absent from the serum of two of the nude mice and was considerably reduced in the other two (Table I).

| TABLE I |

Suppression of Ig-1b Production in (SJL × BALB)F1 Nude and Normal Mice Injected with Spleen or Lymph Node Cells from a Syngeneic Allotype-Suppressed Donor

| Recipient mice | Donor cells* | Ig-1b level (wk after transfer) |
|----------------|-------------|--------------------------------|
|                | Pre-bleed   | 3  5  7  11  14  16  20  42 |
| (SJL × BALB)F1, Nude | | 3+ 4+ 5+ 6+ 7+ 8+ 9+ 10+ |
| 1 Spleen       | 3+  | -  | -  | -  | -  | -  | -  |
| 2 Spleen       | 3+  | -  | -  | -  | -  | -  | -  | Dead |
| 3 Lymph node   | 3+  | -  | -  | -  | -  | -  | -  |
| 4 Lymph node   | 3+  | +  | -  | -  | -  | -  | -  |

| (SJL × BALB)F1, Normal | | 3+ 4+ 5+ 6+ 7+ 8+ 9+ 10+ |
| 1 Spleen       | 4+  | -  | -  | -  | -  | -  | -  |
| 2 Spleen       | 4+  | 2+ | -  | -  | -  | -  | -  |

* Donor was a 9-mo-old suppressed female. Cells were injected i.v. at a concentration of 10^7 cells/mouse.
† Determined by double diffusion in agar. Mice were 3-4+ for Ig-1a throughout.
§ Irradiated with 600 R 1 day before cell transfer.
No significant difference is apparent between recipients of spleen or lymph node cells. By the 5th wk after transfer, all four of the mice were essentially negative for Ig-1b, and continued to be negative through the 11th wk. At this time, a low level of Ig-1b was detected in the serum of two of the mice, but it had disappeared again by the 14th wk. This transient appearance of the suppressed allotype is commonly seen in perinatally suppressed mice and irradiated recipients of suppressor cells. In contrast to the disappearance of Ig-1b, the level of Ig-1a remained high throughout the period of observation. As a control for the efficiency of the suppressor cell population, two normal (SJL × BALB)F₁ recipients, irradiated 1 day earlier with 600 R, were also injected with 10⁷ cells from the same cell suspension. Table I shows that the rate of Ig-1b disappearance was essentially the same in all of the mice. That the disappearance of Ig-1b is the result of specific suppression was established in a related experiment (data not shown) in which nude mice, injected with thymus or spleen cells from nonsuppressed syngeneic donors, continued to demonstrate high levels of serum Ig-1b over a period of up to 8 mo of testing.

The experiment shown in Table I was terminated 10–15 mo after the initial cell transfer. At the time of sacrifice, the mice were healthy and were still negative for Ig-1b. There was no sign of thymic tissue, either by gross morphology or histology, at the time of sacrifice.

To confirm these observations and test for suppressor cell persistence, a second transfer into nude and irradiated normal mice was performed, using as donors suppressed nude mice numbers 1 and 4 (from Table I). As seen in Table II, suppressor cells or their progeny persisted in the nude mouse spleens over the entire 10–15 mo period, and were present in sufficient number to suppress the Ig-1b response in a second group of athymic and normal mice. In addition,

**Table II**

*Survival of Active Suppression through Second Transfer into Athymic and Normal Syngeneic Recipients*

| Recipient mice | Number donor cells injected* | Ig-1b levels (wk after transfer) | Pre- bleed | 2 | 4 | 7 | 11 | 14 |
|----------------|-----------------------------|---------------------------------|-----------|---|---|---|---|---|
| (SJL × BALB)F₁, Nude | Suppressed nude no. 4 | Pre- bleed | 1 (2 x 10⁷) | 3+ | + | - | - | - |
| | (2 x 10⁷) | 3+ | + | - | - | - | - |
| (SJL × BALB)F₁, Normal§ | Suppressed nude no. 1 | Pre- bleed | 1 | 4+ | 3+ | + | - | - | - | - |
| | (2 x 10⁷) | 3+ | + | - | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |
| | (2 x 10⁷) | 4+ | 3+ | + | - | - | - |

* Spleen cells from nude no. 1 (sacrificed 10 mo after the initial transfer) and no. 4 (sacrificed 15 mo after transfer) were injected into recipients at the concentration shown.

† Determined by double diffusion in agar. Mice were 3–4+ for Ig-1a throughout.

§ Irradiated with 600 R 1 day before transfer.
lymph node cells from these mice also suppressed Ig-1b production in irradiated normal mice in typical fashion (data not shown). It is of interest to note that the delay in onset of suppression seen in Table II with the lowest dose (5 × 10^6) of splenic suppressor cells was also observed in another experiment (data not included) in which the radiation dose of normal recipients was reduced to 350 R.

Discussion

The results presented here demonstrate that in the allotype suppression system the production of Ig-1b by congenitally athymic mice can be regulated by suppressor T cells in the same manner as has previously been shown in normal mice. Since there is no evidence for the existence of functional T cells in these mice, the simplest interpretation of these data would be that the suppressor cells act directly on the Ig-1b-producing B cells, or on an intermediate cell which is not thymus processed. The existence of suppressor cells with specificity for immunoglobulin class (11), allotype (1), idiotype (12), and total immunoglobulin (13) is well established. Clear evidence for the B cell as target exists in one of these systems (13) since, after injection of suppressor cells into chickens, plasma cells disappear completely within 1 or 2 wk. Moreover, since specific idiotype suppressor T cells form rosettes with erythrocytes coated with idiotypic Fab fragments (12), it appears at least that the suppressor T cell recognizes the immunoglobulin.

In the case of antigen-specific suppression, it is not clear which cell is the target of the I-J positive suppressor T cell which is generated in response to a protein extracted from other T cells (14, 15). However, Basten et al. (16) suggest that the B cell is the target of specific suppressor T cells in their system, and a similar conclusion has been reached in the recent studies of Warren and Davie (17), who describe the effect of carrier-specific suppressor T cells on antibody-secreting cells in the secondary response.

Since helper T cells were found to be the target of suppression in a system in which the immune response to a specific antigen was studied (6), the possibility cannot be excluded that there may be more than one mechanism operating to maintain allotype suppression. Since allotype-specific and class-specific helper T cells have been suggested to exist (6, 18), they may serve as a target for suppression, either separately or together with B cells at their point of interaction. Alternatively, it may be that T cells, after activation by antigen, express the same receptor for suppressor cell activity as do B cells. Nonetheless, the data presented here suggest either that nude mice have allotype-specific helper cells and that these helper cells are, therefore, not thymus-processed, or that B cells themselves are the direct target for allotype suppression.

Summary

(SJL × BALB)F1, suppressed mice have, in their lymphoid tissues, a population of suppressor T cells directed specifically against a paternal γG_a allotype (Ig-1b). Spleen or lymph node cells from these mice were injected into syngeneic nude mice and the effect on thymus-independent synthesis of Ig-1b in the athymic recipients was determined.
After the injection of suppressor cells, Ig-1b disappeared from the serum of the recipients in a time course similar to that seen in normal mice. These results indicate that suppression occurs in the absence of thymus-derived helper cells, and they suggest that Ig-1b-producing B cells are the target of allotype-suppressor cells.

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References

1. Jacobsen, E. B., and L. A. Herzenberg. 1972. Active suppression of immunoglobulin synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL x BALB/c)F1 mice. J. Exp. Med. 135:1151.
2. Jacobson, E. B., L. A. Herzenberg, R. Riblet, and L. A. Herzenberg. 1972. Active suppression of immunoglobulin allotype synthesis. II. Transfer of suppressing factor with spleen cells. J. Exp. Med. 135:1163.
3. Jacobson, E. B. 1973. In vitro studies of allotype suppression in mice. Eur. J. Immunol. 3:619.
4. Herzenberg, L. A., E. L. Chan, M. M. Ravitch, R. J. Riblet, and L. A. Herzenberg. 1973. Active suppression of immunoglobulin allotype synthesis. IV. Identification of T cells as responsible for suppression by cells from spleen, thymus, lymph node and bone marrow. J. Exp. Med. 137:1311.
5. Okumura, K., C. M. Metzler, T. T. Tsu, L. A. Herzenberg, and L. A. Herzenberg. 1976. Two stages of B-cell memory development with different T-cell requirements. J. Exp. Med. 144:345.
6. Herzenberg, L. A., K. Okumura, H. Cantor, V. Sato, F. W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T-cell regulation of antibody responses: demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. J. Exp. Med. 144:330.
7. Luzzati, A., and E. B. Jacobson. 1972. Serum immunoglobulin levels in nude mice. Eur. J. Immunol. 2:473.
8. Pritchard, H., J. Riddaway, and H. S. Micklem. 1973. Immune responses in congenitally thymus-less mice. II. Quantitative studies of serum immunoglobulins, the antibody response to sheep erythrocytes, and the effect of thymus allografting. Clin. Exp. Immunol. 13:125.
9. Crewther, P., and N. L. Warner. 1972. Serum immunoglobulins and antibodies in congenitally athymic (nude) mice. Aust. J. Exp. Biol. Med. Sci. 50:625.
10. Bloemmen, J., and H. Eysen. 1973. Immunoglobulins and antibodies in congenitally athymic (nude) mice. Eur. J. Immunol. 3:117.
11. Kishimoto, T., Y. Hirai, M. Suemura, and Y. Yamamura. 1976. Regulation of antibody response in different immunoglobulin classes. I. Selective suppression of anti-DNP IgE antibody response by preadministration of DNP-coupled mycobacterium. J. Immunol. 117:396.
12. Owen, F. L., S. T. Ju, and A. Nisonoff. 1977. Binding to idiotypic determinants of large proportions of thymus-derived lymphocytes in idiotypically suppressed mice. Proc. Natl. Acad. Sci. U. S. A. 74:2084.
13. Lerman, S. P., M. D. Grebenau, M. A. Palladino, and G. J. Thorbecke. 1977. Further characterization of the sensitizing bursa cells and of the target for suppression in the transfer of agammaglobulinemia. Adv. Exp. Med. Biol. 88:181.
14. Tada, T. 1977. Regulation of the antibody response by T cell products determined by different I subregions. In Immune System: Genetics and Regulation. ICN-UCLA Symposium on Molecular and Cellular Biology, E. E. Sercarz, L. A. Herzenberg, and C. Fred Fox, editors. Academic Press, Inc., New York. 6:345.

15. Waltenbaugh, C., J. Thèze, J. Kapp, and B. Benacerraf. 1977. Immunosuppressive factor(s) specific for L-glutamic acid$^{16}$-L-tyrosine$^{16}$ (GT) III. Generation of suppressor T cells by a suppressive extract derived from GT-primed lymphoid cells. J. Exp. Med. 146:970.

16. Basten, A., J. F. A. P. Miller, J. Sprent, and C. Cheers. 1974. Cell-to-cell interactions in the immune response. X. T-cell-dependent suppression in tolerant mice. J. Exp. Med. 140:199.

17. Warren, R. W., and J. M. Davie. 1977. Late acting T cell depression of high avidity IgG antibody secretion in the secondary response. J. Immunol. 119:1806.

18. Kishimoto, T., and K. Ishizaka. 1973. Regulation of the antibody response in vitro. VI. Carrier-specific helper cells for IgG and IgE antibody response. J. Immunol. 111:720.