T LYMPHOCYTES SPECIFIC FOR IMMUNOGLOBULIN ALLOTYPE

I. Igh-1b-specific T Cells Demonstrated by Suppression In Vivo and Cytotoxicity In Vitro*

BY H. RALPH SNODGRASS, DARCY B. WILSON, AND MELVIN J. BOSMA

From the Division of Research Immunology, Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; The Wistar Institute, Philadelphia, Pennsylvania; and The Institute for Cancer Research, Philadelphia, Pennsylvania

Thymus-derived lymphocytes (T cells) exert profound regulatory effects, both positive and negative, on immunoglobulin (Ig) production (1). Examples of negative T cell regulation include suppression of Ig isotypes (2–4), allotypes (5–7), and idiotypes (8–11). With respect to allotype suppression in mice, two systems are of particular interest. The first is concerned with mice derived from matings of BALB/c females previously immunized with paternal IgG2a allotype (Igh-1b), which fail to produce Igh-1b (12). In the second system, C.B-17 mice, an Ighb-positive strain with the BALB/c background, fail to produce Igh-1b as a result of transferring lymphocytes from BALB/c donors previously immunized with Igh-1b (7, 13). The suppression in both systems is chronic, mediated by T cells, can be adoptively transferred, and is specific for Igh-1b.

Despite similarities in these two systems, two different mechanisms of allotype suppression have been inferred. In the first, T cells are thought to suppress Igh-1b production indirectly by eliminating a specific Igh-1b helper cell (6, 14), whereas in the second system, T cells are presumed to suppress or eliminate Igh-1b-producing B cells directly. Support for the latter mechanism comes from two observations: T cells of mice immune to Igh-1b specifically suppress Igh-1b production in athymic nude mice (15) and inhibit the growth of an Igh-1b-producing plasmacytoma (13).

What is missing in both of the above systems is direct evidence bearing on the specificity of the T cells in question. To investigate this point, we examined whether BALB/c mice immune to Igh-1b contain Igh-1b-specific T cells. Two different functional assays were used: (a) T cells were rosetted with Igh-1b- or Igh-la-coated erythrocytes and tested for their ability to suppress Igh-1b production in vivo; and (b) T cells were tested for their ability to lyse various Ig-producing cell lines. Our results indicate that BALB/c mice can generate Igh-1b-specific T cells detectable in both assays, and they support the hypothesis that the B cell is a direct target of allotype-specific suppression.

* Supported by grants AI-10961, AI-13323, CA-09140, CA-04946, CA-06927, CA-15822, and RR-05539 from the U. S. Public Health Service, grant IN-140 from the American Cancer Society, and by an appropriation from the Commonwealth of Pennsylvania.
Materials and Methods

Mice. Two Igh-congenic strains were used: C.B-17 (Ighb) and BALB/cAnNcr (Igh°). C.B-17 refers to BALB/cIcr mice that carry the Igh gene complex of C57BL/Ka (Ighb). C57BL/6NIcr, C.B-17, and C57BL/Ka are not known to differ with respect to the Ighb complex. All mice were bred and maintained at the Institute for Cancer Research.

Cell Lines. The cell lines used in this study are listed in Table I. CB101, B79, and U10 were derived from plasmacytomas generously supplied by Dr. Michael Potter (National Cancer Institute, Bethesda, Md.). CB101NP is a variant cell line of CB101 that no longer synthesizes detectable Igh-lb heavy chains. The Igh-lb-producing hybridomas, 540A6 and R3-367, were derived from the fusion of a BALB/c myeloma (P3 × 63Ag8) with C57BL/6 spleen cells and were gifts from Dr. Barbara Knowles (The Wistar Institute, Philadelphia, Pa.) and Dr. Theresa Imanishi-Kari (Massachusetts Institute for Technology, Boston, Mass.), respectively. S10P is an Igh-4b-producing hybridoma obtained by the fusion of SP2 (16) with C.B-17 spleen cells according to the technique of Kennett et al. (17). All cell lines were maintained in vitro and periodically recloned to maintain a homogeneous phenotype.

Purification of Igh-lb Proteins. The Ig proteins in ascites fluids of mice bearing the Igh-lb-producing plasmacytomas, CBPC101 and BEPC79, were precipitated with (NH₄)₂SO₄ (45% vol:vol), redissolved in 0.2 M NaCl, and dialyzed extensively against 10 mM NaCl. The resulting euglobulin precipitate was redissolved in 0.5 M NaCl, 100 mM Tris, pH 8, and subjected to gel filtration over a column of Biogel A0.5 (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with the same buffer. The ascending limb of the 7S peak was collected and concentrated by vacuum dialysis against 150 mM NaCl, 10 mM Tris, pH 8. Purified Igh-1a, Igh-4a, and Igh-4b myeloma proteins were obtained by similar techniques.

R3-367, an Igh-lb protein that binds (3-nitro-4-hydroxyphenyl)acetyl (NP), was specifically purified over NP-affinity columns. These columns were prepared by first substituting Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.) with amino groups by resuspending CNBr-activated Sepharose in ethylenediamine (25% vol:vol, pH 9). NP was subsequently attached by an overnight incubation at 25°C of the amino-substituted Sepharose (50% vol:vol in 3% NaHCO₃, pH 8.5) with an equal volume of dioxane containing NP-cap-succinimide (2.5 mg/ml; Biosearch, San Rafael, Calif.) (18). Ascites fluids from mice bearing R3-367 tumors were allowed to clot, clarified by centrifugation, and then mixed with an equal volume of Alsever’s solution and passed through an NP column. The column was washed with phosphate-buffered saline (PBS) until the eluate OD was <0.005. The anti-NP antibodies were then eluted with 5 × 10⁻⁴ M (3-nitro-4-hydroxy-5-iodophenyl)acetyl-cap, and dialysed extensively against PBS. The purity of these preparations was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Immunizations. Immunization of BALB/c mice against purified Igh-lb proteins of different tumors (CBPC101, BEPC79, and R3-367) consisted of multiple intraperitoneal injections of 100 µg of Igh-lb. The first injection was in complete Freund’s adjuvant, the second was in incomplete adjuvant, and subsequent injections were in buffered saline. Hyperimmunization of BALB/c mice with C57BL/6 serum was done in the manner described earlier (19).

Igh-lb Suppression. The ability of immune T cells to induce Igh-lb suppression in vivo was assessed by a protocol described elsewhere (7). Briefly, immune BALB/c T cells (2 × 10⁶) and C.B-17 spleen cells (2 × 10⁶) were cotransferred (intravenously) into sublethally irradiated (550 rad) BALB/c recipients. Recipient sera were tested in Ouchterlony assays for production of Igh-lb.

Rosetting of Ig-binding T Cells. Purified myeloma proteins or salt fractionated goat-anti-mouse Ig (GAM Ig) were coupled to sheep erythrocytes (SRBC) by means of CrCl₃ according to the procedure of Goding (20). Briefly, 1 ml of CrCl₃ (0.01% wt:vol in saline) was slowly added to a 3-mI vortexing solution of SRBC (33% vol:vol) and Ig (0.3 mg/ml). The mixture was allowed to stand for 10 min at 25°C and washed twice with PBS.

Rosette-forming T cells were prepared and purified as described by Parish and McKenzie (21). Nylon wool-purified T cells were spun down with a 100-fold excess of SRBC, allowed to

---

1 Abbreviations used in this paper: GAM Ig, goat anti-mouse Ig; HBSS, Hank’s balanced salt solution; NP, (3-nitro-4-hydroxyphenyl)acetyl hapten; PBS, phosphate-buffered saline; SRBC, sheep erythrocytes; Tc, cytotoxic T cells; Ts, suppressor T cells.
stand 60 min at 25°C, and gently resuspended in 1 ml Hanks' balanced salt solution (HBSS). The cell suspension was layered over a solution of Ficoll (5.6%)/Isopaque (9.6%) and spun at 2,000 g for 15 min at 25°C. The cell pellet was washed twice with HBSS, and the SRBC were lysed with NH₄Cl (22).

**Generation of Cytotoxic T (Tc) Cells.** Single cell suspensions were prepared from spleens of immunized mice and the erythrocytes were lysed by treatment with NH₄Cl. 5 x 10⁶ spleen cells were cultured with 1 x 10⁵-10 x 10⁵ irradiated (2,000 rad) CB101 cells in a total volume of 2 ml in Linbro tissue culture dishes (Flow Laboratories, Hamden, Conn.). The culture medium consisted of RPMI-1640 (Microbiological Associates, Walkersville, Md.), 10% fetal calf serum, 5 x 10⁻⁵ M 2-mercaptoethanol, 50 μg/ml gentamycin (Schering Corp., Kenilworth, N. J.), and 2 mM glutamine. Cultures were incubated at 37°C in a humidified incubator for 5-6 d.

**Cytotoxicity Assay.** After 5-6 d in culture, the effector cells were harvested and tested in a standard ⁵¹Cr release assay (23). In the majority of experiments, the spontaneous release values were <20% of the maximum release, which was determined by detergent lysis. Experiments in which the spontaneous release was >35% were discarded. All results are reported as percent specific lysis, which is calculated as 100 x (EXP - spontaneous release)/(maximum release - spontaneous release), where EXP equals the counts per minute of chromium released in the presence of effector cells. All values represent the mean of quadruplicate measurements. In all cases, the standard errors were <5% and therefore they are not given.

**Antiserum and Complement Treatments.** Anti-Thy-1.2 was prepared in AKR/J mice by multiple immunizations with C3H/J thymocytes. For T cell depletion, 0.2 ml of this antiserum was added to 2.5 x 10⁷ cells. After 30 min on ice, the cells were washed twice and resuspended in 2 ml guinea pig serum (1:7 dilution), as a source of complement, and incubated for 45 min at 37°C. Under these conditions, >95% of thymocyte and <5% of bone marrow cells are killed.

**Results**

**Evidence for Suppressor T (Ts) Cells Specific for Igh-1⁰ (Igh-1⁰-specific Ts Cells).** Cell rosetting techniques were used to evaluate whether BALB/c mice immune to Igh-1⁰ contained Igh-1⁰-specific Ts cells. Two rosetting procedures were used. In the first, nylon wool-purified T cells, derived from BALB/c mice immunized with C57BL/6 serum, were rosetted with SRBC coupled with Igh-1⁰ or Igh-1⁰ proteins. In the second procedure, the T cells were first incubated with soluble Igh-1⁰ or Igh-1⁰, washed, and rosetted with SRBC coupled with GAM Ig. In both procedures, the rosetting cells were purified by Ficoll-Isopaque gradients; the SRBC were subsequently lysed with NH₄Cl and the T cells were admixed with C.B-17 spleen cells and injected into irradiated (550 rad) BALB/c recipients. The results (Table II) demonstrate that all the mice receiving T cells rosetted with Igh-1⁰ were suppressed for Igh-1⁰ production at 4 wk (groups A and E), whereas only one out of five recipients was suppressed in the control groups (groups B–D, F, and G). These findings indicate that some allotype

**Table I**

| Cell line | Plasmacytoma | Origin | Ig product |
|-----------|--------------|--------|------------|
| CB101     | CBPC101      | C.B-20 | 1⁰, k      |
| CB101NP   | —            | CB101 variant | None      |
| B79       | BEPC79       | CXBE   | 1⁰, k      |
| U10       | UPC10        | BALB/c | 1⁰, k      |
| S40A6     | —            | C57BL/6-BALB/c hybridoma | 1⁰, 4⁰, k |
| R3-367    | —            | C57BL/6-BALB/c hybridoma | 1⁰, k, λ  |
| S10P      | —            | C.B-17-BALB/c hybridoma | 4⁰, k     |
H. R. SNODGRASS, D. B. WILSON, AND M. J. BOSMA

TABLE II

Ability of Igh-Ib-rosetted T Cells to Suppress Igh-Ib Production

| Group | Rosetting conditions* | Number of animals positive for Igh-Ib at various times after cell transfer (wk)$ | 2 | 3 | 4 |
|-------|-----------------------|---------------------------------------------------------------------------------|---|---|---|
| A     | Igh-Ib-SRBC           |                                                                                 | 0 | 0 | 0 |
| B     | Igh-Ib-SRBC           |                                                                                 | 5 | 5 | 4 |
| C     | SRBC                  |                                                                                 | 5 | 5 | 5 |
| D     | No T cells            |                                                                                 | 5 | 5 | 4 |
| E     | Igh-Ib + GAMIg-SRBC   |                                                                                 | 1 | 1 | 0 |
| F     | Igh-Ib + GAMIg-SRBC   |                                                                                 | 5 | 5 | 4 |
| G     | GAMIg-SRBC            |                                                                                 | 5 | 5 | 5 |

* Nylon wool-purified splenic T cells from BALB/c mice that had been immunized with C57BL/6 serum were incubated with SRBC to which Ig had been directly coupled via the CrCl technique, or alternatively, the T cells were incubated with soluble Ig, washed, then incubated with SRBC coupled with a polyspecific (GAMIg). The rosetting cells were purified by Ficoll-isopaque gradients.

† The rosette-forming cells from 10 x 10^6 T cells were admixed with 2 x 10^6 C.B-17 spleen cells and injected intravenously into irradiated (550 rad) BALB/c recipients (five animals per group). The presence of Igh-Ib was monitored for up to 10 wk. All animals expressed Igh-Ib at 10 wk.

Fig. 1. Lysis of CB101 target cells by spleen cells from Igh-Ib-immunized BALB/c mice. Spleen cells from Ig^b^-immune or nonimmune BALB/c mice were cultured with or without the myeloma cells indicated. After 5–6 d, the resulting cells were tested in ^51Cr-release cytotoxicity assays using CB101 cells as targets.

Ts cells are specific for the Igh-Ib allotype. All recipients expressed Igh-Ib at 10 wk (data not shown).

Allotype-specific Tc Cells. Spleen cells from Ig^b^-immune BALB/c mice were restimulated in vitro with an irradiated Igh-Ib-producing myeloma, CB101, and then tested for cytolytic activity against radiolabeled CB101 cells. Fig. 1 illustrates the mean cytolytic activity from eight such experiments. These experiments demonstrate that both in vivo priming and in vitro restimulation are required to generate detectable cytolytic activity. Importantly, CB101 NP, a non-Ig-producing variant of CB101, does not effectively restimulate Tc cell activity.

The cells responsible for the cytolytic effect are T cells; this is demonstrated by the
observation that prior treatment of these killer cell populations with anti-Thy-1.2 and complement abrogates their lytic ability (Table III).

Specificity of the Tc Cells. The functional specificity of cytolytic T cells, which were primed in vivo with C57BL/6 immune serum and restimulated in vitro with CB101 myeloma cells, was assessed by comparing their lytic potency on a variety of different cell lines. The results show that only Igh-1b-producing cell lines are lysed (Fig. 2). Targets that were not lysed included the non-Ig-producing variant of CB101 (CB101NP), an Igh-1a-producing myeloma (U10), an Igh-4b-producing BALB/c × C.B-17 hybridoma (S10P), as well as C.B-17 mitogen-induced (lipopolysaccharide and concanavalin A) blast cells.

These data suggest that Tc cells generated in this fashion are specific for determinants of the Igh-1b allotype. The experiments presented in Table IV support this suggestion by demonstrating that purified Igh-1b can serve as the priming immunogen. Chromatographically purified Igh-1b myeloma proteins (CB101 and B79) and an affinity-purified Igh-1b hybridoma protein (R3-367) all efficiently prime the BALB/c Tc cell response (Table IV).

Fig. 3 demonstrates that BALB/c anti-Igh-1b Tc display an apparent major histocompatibility complex restriction; they lyse Igh-1b-producing targets of the

| Experiment | Complement | Anti-Thy-1.2 + complement |
|------------|------------|--------------------------|
| 1          | 45         | 1.2                      |
| 2          | 56         | 2.5                      |

**Table III**

*Sensitivity of Cytotoxic Activity to Anti-Thy-1.2 and Complement Treatment*

Effector cells were generated as described in Materials and Methods from spleen cells of mice that had been immunized in vivo with C57BL/6 serum, and restimulated in vitro with CB101 myeloma cells. Before treatment, the effector: target ratio was 40:1 and no adjustment was made in cell numbers after the various treatments.

**Fig. 2.** Specificity of BALB/c-anti-Ig b Tc cells. The lytic potency of BALB/c Tc cells that were primed in vivo with C57BL/6 serum and restimulated in vitro with CB101 myeloma cells was tested on radiolabeled target cells as indicated.
H. R. Snodgrass, D. B. Wilson, and M. J. Bosma

TABLE IV

Ability of Purified Igh-1b to Prime Tc Cells That Lyse CB101 Target Cells

| BALB/c Tc cells generated by*  | Percent specific lysis of\$  |
|-------------------------------|-----------------------------|
| In vivo immunization          | In vitro restimulation CB101| CB101 NP |
| CB101 protein                 | CB101                       | 62       | 5    |
| None                          | None                        | 1        | 0    |
| B79 protein                   | CB101                       | 55       | 7    |
| None                          | None                        | 3        | 3    |
| R3-367 protein                | CB101                       | 58       | 5    |
| None                          | None                        | 1        | 1    |
| None                          | CB101                       | 0        | 2    |

* Spleen cells from BALB/c mice that had been immunized as indicated were cultured either with or without CB101 myeloma cells and tested in \$Cr-release assays as described in Materials and Methods.
\$ Values of specific lysis reported were obtained at effector:target ratio of 40:1.

Fig. 3. Major histocompatibility complex restriction of BALB/c-anti-Igh-1b Tc cells. The lytic ability of BALB/c (H-2\(^d\), Igh-1\(^b\)) T cells primed with Igh-1\(^b\) allotype, and restimulated in culture with the Igh-1\(^b\)-producing myeloma, CB101 (H-2\(^d\), Igh-1\(^b\)) were tested on target cells, expressing Igh-1\(^b\) and the same (H-2\(^d\)) or a different (H-2\(^b\)) major histocompatibility complex haplotype.

immunizing H-2\(^d\) haplotype but not Igh-1\(^b\) targets of the B79 line expressing a different major histocompatibility complex haplotype, H-2\(^b\).

Effects of Anti-Igh-1b Tc Populations on Igh-1b Expression In Vivo. The final series of experiments explores the possibility that primed and restimulated populations of Igh-1\(^b\)-specific Tc are able to suppress expression of Igh-1\(^b\) proteins in vivo. Table V shows that BALB/c recipients of target C.B-17 spleen cells and anti-Igh-1\(^b\) effector Tc populations fail to express Igh-1\(^b\) allotype while continuing to express the Igh-4\(^b\) allotype.

Discussion

The preceding results provide direct evidence for the existence of allotype-specific T cells. T cell populations primed in vivo with Igh-1\(^b\) proteins and restimulated in vitro with Igh-1\(^b\)-expressing myeloma cells are able to suppress the production of
Table V

Ability of Tc Cell Populations to Induce Suppression of Igh-1b Production In Vivo

| Experiment | T cell population* | Percent specific lysis of CB101 targets§ | C.B-17 donor allotypes present 3-4 wk after cotransfer§ |
|------------|--------------------|------------------------------------------|--------------------------------------------------|
| 1          | Primary            | 60                                       | Igh-1b:0/8, Igh-4b:8/8                           |
| 2          | Primary            | 53                                       | Igh-1b:0/3, Igh-4b:5/5                           |
| 3          | Normal             | 2                                        | Igh-1b:5/5, Igh-4b:5/5                           |

* Primary Tc cells were generated by restimulating Igh-1b-immunized BALB/c spleen cells with CB101 myeloma cells in vitro for 5 d.

§ Effector:target ratios were 50:1 for the primary Tc cells.

§§ 5 x 10^6 primary Tc cells or normal lymph node cells were cotransferred with 5 x 10^6 C.B-17 spleen cells into irradiated (550 rad) BALB/c recipients. The presence of C.B-17 allotypes was monitored weekly. The values represent the number of animals expressing the indicated allotype over the total number of animals in the group.

Igh-1b proteins in vivo, and they are also specifically lytic for target cells expressing Igh-1b proteins.

Earlier studies in mice have shown that chronic suppression of Igh-1b production is mediated by T cells (5, 7). These studies concluded that the target of Ts was a helper T cell population, and from this it seems unlikely that the specificity of Ts would be directed to an Ig allotype.

To investigate this point, the specificity of a Ts cell population capable of regulating allotype expression was examined by using rosetting procedures. Table II illustrates that Ts cells can be demonstrated with two different procedures to bind Igh-1b specifically. T cells that form rosettes with Igh-1b-coupled SRBC are active in suppressing expression of Igh-1b in vivo, whereas the cells that rosette with Igh-1a are not. In addition, Ts cells that bind soluble Igh-1b with sufficient affinity to be rosetted by anti-Ig-coated SRBC are also capable of suppressing Igh-1b in vivo. There are currently many examples in the literature of antigen-binding Ts cells (24-28); therefore the finding that allotype Ts cells bind Igh-1b is not without precedent.

It is of interest that the nonrosetted immune BALB/c spleen cell population was also capable of Igh-1b suppression in vivo (unpublished results). This could result either from a technical shortcoming, i.e., that rosetting was incomplete, or alternatively, immune spleen cell populations may contain allotype suppressor T cells with specificity directed to determinants other than the Igh-1b allotype itself.

The most straightforward interpretation of the results of the Igh-1b rosetting experiments is that Ts exist having surface receptors specific for Igh-1b and that interaction of these cells with target Igh-1b B cells results in their suppression or elimination. This interpretation is supported by the demonstration in earlier studies that Igh-1b myelomas fail to grow in allotype-suppressed hosts (13).

Specificity of Ts Cells from Igh-1b-immune BALB/c Mice. The studies described here also show that priming BALB/c mice with C57BL immune serum in vivo and restimulation with Igh-1b myeloma in vitro induces cytotoxic T cells that lyse Igh-1b-producing myelomas (Figs. 1 and 2). Several findings support the conclusion that these Tc cells are specific for allotypic determinants present on the Igh-1b molecule: (a) in vivo priming is required for generating cytotoxic cells and this can be
accomplished with purified Igh-1b myeloma proteins (Table IV); (b) restimulation in vitro is also required and only Igh-1b-producing myelomas are effective stimulators; a nonproducing variant is not effective (Fig. 1); (c) once stimulated, these cytotoxic cells are lytic only for Igh-1b-producing target cells. Target cells not lysed include Igh-1a- and Igh-4b-producing cell lines as well as an Ig-non-producing variant of CB101, CB101NP (Fig. 2), which does not secrete or produce any detectable Igh-1b (unpublished results); and (d) idiotypes are apparently not involved because two independent Igh-1b-producing cells can serve as targets (Fig. 2) and three different myeloma proteins can serve as priming immunogens for this Tc response (Table IV).

Tumor-specific antigens in these experiments can be ruled out by the observation that affinity-purified Ig can serve as the priming immunogen (Table IV), and by the finding that CB101NP is not lysed (Fig. 2). The involvement of allotype-linked minor histocompatibility differences between BALB/c and C.B-17 mice (29-31) can also be excluded. These antigens are expressed on mitogen-induced blasts, and would also be expected to be present on an Igh-4b-producing hybridoma cell line that is partly of C.B-17 origin. Nevertheless, these particular target cells are not lysed by allotype-primed killer cells (Fig. 2).

The strongest evidence supporting the conclusion that these Tc are specific for allotypic determinants of the Igh-1b molecule is presented in the accompanying paper (32). Tc cells cloned from bulk populations similar to those described in Fig. 2 display functional specificity identical with the bulk populations. Incubation of these cloned Tc cells with affinity-purified Igh-1b results in a marked and specific reduction of the cytotoxic ability of these cells.

Another important aspect of killer cell specificity is that controlled by gene products of the major histocompatibility complex. Preliminary results indicate that allotype-specific cytotoxic populations are restricted to the BALB/c (H-2^d) haplotype. These studies are limited, however, because target cell lines expressing Igh-1b and major histocompatibility complex haplotypes other than H-2^d are not available.

Involvement of Tc Cells in Allotype Suppression. Because both T cell effector functions, suppression and cytotoxicity, seem to be mediated by T cells of like specificity for determinants of the Igh-1b molecule, the question arises whether both functions are mediated by the same T cell. The present studies indicate that at the level of bulk populations of effector cells, these two functions cannot be separated. Both can be induced by priming with purified Igh-1b proteins and both show specificity for Igh-1b proteins, one by rosetting and the other by cytotoxicity. Moreover, as described in the accompanying paper, a cytotoxic Igh-1b-specific T cell line is able to suppress expression of Igh-1b in vivo (32). We therefore feel that the possibility remains that allotype-specific Tc could have a physiologic role in the regulation of allotype suppression.

Another precedent for the suggestion that Tc may regulate expression of Ig in vivo is the demonstration of Segal et al. (33) that T cells from high zone tolerant mice could kill macrophages pulsed with the tolerizing antigen. However, Abbas et al. (34-36) have shown recently that suppression and cytotoxicity may operate by quite different mechanisms at the level of the target cell. Tc directed against one of the Ig proteins of a double producing hybridoma caused the cessation of synthesis of both Ig proteins and target cell lysis, but when exposed to Ts cells specific for one of the Ig
proteins, synthesis of only the specific protein was suppressed in the double producing hybridoma.

The premise that B cells are the direct target of Ts cells in chronic allotype suppression stands in apparent contrast to mechanisms suggested by Herzenberg et al. (14). In this model, Ts cells are thought to eliminate Igh-1b-specific Th cells and thereby to influence Igh-1b production indirectly. The target determinants of helper T cells that provide the basis for discrimination by Ts cells have not been identified. In our view, these mechanisms are not necessarily mutually exclusive; both could be operative to varying degrees. Consistent with this suggestion is the recent observation, using the neonatal suppression system where helper T cells are thought to be the target of Ts cells, of the existence of Ts cells that inhibit both T-independent Igh-1b production (37) and the growth of an Igh-1b myeloma (38).

It remains unclear in the neonatal suppression system how administration of anti-allotype antibody induces allotype-specific Ts cells. One possibility that could be considered is that the temporary elimination of allotype-producing B cell subpopulations by anti-allotype antibody results in the consequent lack of tolerance in the T cell compartment to Ig allotypic determinants. The later emergence of allotype-positive B cells could then induce activation of allotype-specific T suppressor and/or killer cells. Similarly, in the absence of allotype-positive B cells, one might expect alterations in the expressed repertoire of T cells having specificity for allotype (39).

Summary

We show that determinants of IgG2a of C57BL/6 mice (Igh-1b) stimulate allotype-specific T cells in BALB/c mice. Such cells are detected in two different functional assays; chronic allotype suppression and T cell-mediated cytotoxicity. A population of suppressor T cells capable of inducing chronic Igh-1b suppression was demonstrated by rosetting procedures to possess Igh-1b-specific receptors, a result interpreted as indicating that suppressor T cells may act directly upon allotype-bearing B cells. From similar populations we were also able to demonstrate Igh-1b-specific cytotoxic T cells. Such cells were lytic for target myeloma cells expressing the Igh-1b allotype of IgG2a, and were ineffective against a variant cell line failing to express Igh-1b, and other target cell lines expressing different allotypes or isotypes. The similar specificity of suppressor T cells and cytotoxic T lymphocytes for Igh-1b allotype raises the possibility that the target in allotype suppression is a B cell, and that allotype-specific cytotoxic T cells may play some role in regulation of allotype expression in the suppressed state.

Received for publication 20 April 1981.

References

1. Gershon, R. K. 1974. T-cell control of antibody production. In Contemporary Topics in Immunobiology. M. Cooper and N. Warner, editors. Springer-Verlag, New York. 3:1.
2. Tada, T., K. Okumura, and M. Taniguchi. 1973. Regulation of homocytotropic antibody formation in the rat. VIII. An antigen-specific T cell factor that regulates anti-hapten homocytotropic antibody response. J. Immunol. 111:952.
3. Chiorazzi, N., D. A. Fox, and D. H. Katz. 1977. Hapten-specific IgE antibody responses in mice. VII. Conversion of IgE “non-responders” strains to IgE “responders” by elimination of suppressor T cell activity. J. Immunol. 118:48.
4. Suemura, M., T. Kishimoto, Y. Hirai, and Y. Yamamura. 1977. Regulation of antibody response in different immunoglobulin classes. III. In vitro demonstration of “IgE class-specific” suppressor functions of DNP-mycobacterium primed T cells and the soluble factor released from these cells. J. Immunol. 119:149.

5. 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.

6. Herzenberg, L. A., K. Okumura, and C. M. Metzler. 1975. Regulation of immunoglobulin and antibody production by allotype suppressor T cells in mice. Transplant. Rev. 27:57.

7. Bosma, M. J., and G. C. Bosma. 1976. Chronic suppression of immunoglobulin allotype production in adult congenic mice. Nature (Lond.). 259:313.

8. Eichmann, K. 1975. Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. Eur. J. Immunol. 5:511.

9. Owen, F. H., S.-T. Ju, and A. Nisonoff. 1977. Presence of idiotype-specific T cells that interact with molecules bearing the idiotype. J. Exp. Med. 145:1559.

10. Dohi, Y., and A. Nisonoff. 1979. Suppression of idiotype and generation of suppressor T cells with idiotype-conjugated thymocytes. J. Exp. Med. 150:909.

11. Bona, C., and W. E. Paul. 1979. Suppression of idiotype and generation of suppressor T cells with idiotype-specific helper T cells and their specific removal by suppressor T cells. J. Exp. Med. 149:392.

12. Jacobson, E. B., and L. A. Herzenberg. 1972. Active suppression of immunoglobulin allotype synthesis. I. Chronic suppression after perinatal exposure to maternal antibody to paternal allotype in (SJL X BALB/c) mice. J. Exp. Med. 135:1151.

13. Bosma, M. J., and G. C. Bosma. 1977. Prevention of IgG2a production as a result of allotype-specific interaction between T and B cells. J. Exp. Med. 145:743.

14. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. 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.

15. Bosma, M. J., G. C. Bosma, and J. L. Owen. 1978. Prevention of immunoglobulin production by allotype-dependent T cells. Eur. J. Immunol. 8:562.

16. Shulman, M., C. D. Wilde, and G. Kohler. 1978. A better cell line for making hybridomas secreting specific antibodies. Nature (Lond.). 276:269.

17. Kennett, R. H., K. A. Denis, A. S. Tung, and N. R. Klinman. 1978. Hybrid plasmacytoma production: fusion with adult spleen cells, monoclonal spleen fragments, neonatal spleen cells and human spleen cells. Curr. Top. Microbiol. Immunol. 81:77.

18. Pohlit, H. M., W. Haas, and H. von Boehmer. 1979. Haptenation of viable biological carriers. In Immunological Methods. I. Helkovits and B. Pernis, editors. Academic Press, Inc., New York. 181.

19. Kindred, B., and E. Weiler. 1971. Recessive inheritance of rapid anti-allotype antibody production. J. Immunol. 107:389.

20. Goding, J. W. 1976. The chromic chloride method of coupling antigens to erythrocytes: definition of some important parameters. J. Immunol. Methods. 10:61.

21. Parish, C. R., and I. F. C. McKenzie. 1978. A sensitive rosetting method for detecting subpopulations of lymphocytes which react with alloantisera. J. Immunol. Methods. 20:173.

22. Boyle, W. 1968. An extension of the 51Cr-release assay for the estimation of mouse cytotoxins. Transplantation (Baltimore). 6:761.

23. Thorn, R. M., J. C. Palmer, and L. A. Manson. 1974. A simplified 51Cr-release assay for killer cells. J. Immunol. Methods. 4:301.

24. Tada, T., M. Taniguchi, and T. Takemori. 1975. Properties of primed suppressor T cells and their products. Transplant. Rev. 26:106.
25. Taniguchi, M., and J. F. A. P. Miller. 1977. Enrichment of specific suppressor T cells and characterization of their surface markers. J. Exp. Med. 146:1450.

26. Germain, R. N., S.-T. Ju, T. J. Kipps, B. Benacerraf, and M. E. Dorf. 1979. Shared idiotypic determinants on antibodies and T-cell-derived suppressor factor specific for the random terpolymer l-glutamic acid l-alanine l-tyrosine. J. Exp. Med. 149:613.

27. Greene, M. I., B. A. Bach, and B. Benacerraf. 1979. Mechanisms of regulation of cell-mediated immunity. III. The characterization of azobenzene-arsenate specific suppressor T-cell-derived-suppressor factors. J. Exp. Med. 149:1069.

28. Taussig, M. J., and A. Holliman. 1979. Structure of an antigen-specific suppressor factor produced by a hybrid T-cell line. Nature (Lond.). 277:308.

29. Riblet, R., and C. Congelton. 1977. A possible allotype-linked histocompatibility gene. Immunogenetics. 5:511.

30. Rolink, T., K. Eichmann, and M. M. Simon. 1978. Detection of two allotype-(Ig-1)-linked minor histocompatibility loci by the use of H-2-restricted cytotoxic lymphocytes in congenic mice. Immunogenetics. 7:321.

31. Bosma, M. J., G. C. Bosma, and J. L. Owen. 1978. Prevention of immunoglobulin production by allotype dependent T cells. Eur. J. Immunol. 8:562.

32. Snodgrass, H. R., M. J. Bosma, and D. B. Wilson. 1981. T lymphocytes specific for immunoglobulin allotype. II. Cloned Igh-l-specific cytotoxic T cells. J. Exp. Med. 154:491.

33. Segal, S., E. Tzshoval, and M. Feldman. 1979. Immunological tolerance: high-dose antigen induced suppressor cells from tolerant animals inactivate antigen-presenting macrophages. Proc. Natl. Acad. Sci. U. S. A. 76:2405.

34. Abbas, A. K. 1979. T lymphocyte mediated suppression of myeloma function in vitro. I. Suppression by allogeneically activated T lymphocytes. J. Immunol. 123:2011.

35. Abbas, A. K., S. E. Ratnofsky, and S. J. Burakoff. 1980. T lymphocyte-mediated suppression by myeloma function in vitro. II. Evidence for regulation of hapten-binding myelomas by syngeneic hapten-specific cytolytic T lymphocytes. J. Exp. Med. 152:306.

36. Abbas, A. K., S. J. Burakoff, M. L. Gefter, and M. I. Greene. 1980. T lymphocyte-mediated suppression of myeloma function in vitro. III. Regulation of antibody production in hybrid myeloma cells by T lymphocytes. J. Exp. Med. 152:968.

37. Jacobson, E. B. 1978. Adoptive transfer of allotype-specific suppressor cells inhibits thymus-independent immunoglobulin production in syngeneic athymic mice. J. Exp. Med. 148:607.

38. Jacobson, E. B. 1980. Inhibition of Ig-l myeloma cells in allotype-suppressed mice. 4th Internat. Cong. Immunol. (abstract.)

39. Janeway, C. A., R. A. Murgita, R. I. Weinbaum, R. Asofsky, and H. Wigzell. 1977. Evidence for an immunoglobulin-dependent antigen-specific helper T cell. Proc. Natl. Acad. Sci. U. S. A. 74:4582.