PREVENTION OF IgG2a PRODUCTION AS A RESULT OF
ALLOTYPE-SPECIFIC INTERACTION BETWEEN T AND B
CELLS*

BY MELVIN J. BOSMA AND GAYLE C. BOSMA

(From The Institute for Cancer Research, Fox Chase Cancer Center,
Philadelphia, Pennsylvania 19111)

The production of IgG by B cells is regulated by another population of
lymphocytes called T cells (1, 2). Different subpopulations of T cells serve to help
(Th cells) or to suppress (Ts cells) IgG production (3-5). A striking example of
the latter is neonatally induced Ts cells that suppress production of the paternal
IgG2a allotype in (BALB/c × SJL)F1 mice (6). The reported mode of action
for these Ts cells is that they remove or inactivate allotype-specific Th cells, i.e.,
B cells default in their production of the paternal IgG2a allotype for lack of
specific Th cells (5). We have evidence for another mode by which T cells may
prevent IgG2a allotype production: in this, the B cell is the direct target of
allotype-specific T cells.

Using adult Ig-congenic strains of BALB/c mice, we recently were able to
demonstrate cytotoxic or suppressor T cells (Tcs) which are specific for the IgG2a
allotype of C57BL mice (G2) (7). In the appropriate host, these G2 Tcs cells stop
the normal production of IgG2a allotype but not that of IgG1 or IgG2b allotype.
We now show that G2 Tcs cells are also able to prevent the growth of a congenic,
G2-producing plasmacytoma (CBPC 101). This is interpreted as direct evidence
for allotype-specific interaction between T and B lymphocytes; the possible
significance of such cell-cell interaction for IgG regulation is discussed.

Materials and Methods

Mouse Strains and Allotypes. The strains of mice that relate to this study include BALB/c
and three Ig-congenic partner strains, C.B-17, C.B-20, and C.AL-20. The manner in which these mice
were bred is reported elsewhere (8, 9) C.B and C AL refer to BALB/c mice that carry the Ig
allootypes of the C57BL/Ka and AL/N mouse strains, respectively; the number after the hyphen
(e.g., C.AL-20) indicates how many introgressive backcrosses preceded the derivation of each
strain
IgG2a allotypes of BALB/c (G2+ × + +), C.AL (G2 × × +), and C.B (G2) mice are denoted with the
Potter-Lieberman nomenclature (8). The IgG2a allotype of C57BL (G2), which is not known to be
different from that of SJL mice, is alternately denoted Ig-1b by Herzenberg et al. (5, 6).

CBPC 101 Plasmacytoma. CBPC 101, a G2-producing B-cell tumor, was induced in a C.B-20
mouse by Dr. Michael Potter. We received this tumor in its fifth transplant generation, and for
these experiments we serially transferred CBPC 101 ascites intraperitoneally in C.B-17 mice.

Cell Transfers. Cell counts of spleen cells and CBPC 101 tumor cells were made in a hemocy-

* Supported by National Institutes of Health grants CA-04946, AI-13323, CA-06927, and RR-
05539, and by an appropriation from the Commonwealth of Pennsylvania
tometer and the number of viable cells scored on the basis of their ability to exclude eosin. In some experiments, cells were exposed to AKR mouse anti-Thy 1.2 serum (from Dr J B Smith) and complement; control cell suspensions received the same treatment except that AKR normal serum was substituted for AKR anti-Thy 1.2 serum. This was done as described previously (7). All cell preparations were made up in Hanks' balanced salt solution containing 2% fetal calf serum (Flow Laboratories, Inc., Rockville, Md.) and were injected intraperitoneally in 0.5-1.0-ml vol per mouse.

The sublethal X-irradiation of BALB/c recipients (550 R) was carried out 1 day before cell transfer at the following conditions 190 kV-15 mA; filtration 1 mm Al and 0.5 mm Cu; dose rate 40-45 R min⁻¹. At various times after cell transfer, recipients were bled and their sera reacted with BALB/c anti-G2 in micro-Ouchterlony plates to test for the presence of the G2 marker.

Results and Discussion

G2-specific immunity of normal mice was first inferred from the relative inability of CBPC 101 to grow in BALB/c and in C.AL-20 mice. As shown in Table I, intraperitoneal injections of CBPC 101 cells (5 x 10⁶) into BALB/c or C.AL-20 mice did not result in a tumor, except in 2 of 50 mice injected (groups A–C). When tumor cells of the same preparation as used in groups A and C were given to C.B-17 mice, all recipients died with the tumor 3–7 wk later (group D). The significance of these results is noted by the fact that the CBPC 101 plasmacytoma was induced in a C.B-20 mouse and that mice of the BALB/c, C.AL-20, and C.B-17 (or C.B-20) strains are known to differ from each other only with respect to Ig allotypes. Thus, C.B-17 mice are apparently tolerant to the growth of CBPC 101 because their IgG2a allotype is the same as that of the tumor, whereas BALB/c and C.AL-20 mice have different IgG2a allotypes and resist the growth of CBPC 101 cells.

None of the mice in groups A–C of Table I produced detectable anti-G2. This suggested to us that the tumor elicited a cellular immune response in which allotype-specific T cells were the mediators. To test this idea, we carried out the experiments shown in Table II. Here, we used sublethally X-irradiated (550 R) BALB/c mice as recipients of various cell mixtures containing CBPC 101 cells. X-irradiation before the injection of CBPC 101 cells enabled the tumor to grow as indicated by the production of G2 (group A). Further, all of these recipients died of the tumor within 5 wk. However, as indicated in group B, CBPC 101 cells would not grow and produce G2 when cotransferred with BALB/c spleen cells taken from donors that received CBPC 101 cells 15 wk earlier (from group A of Table I). When the same spleen cell suspension was treated with AKR anti-Thy 1.2 serum and complement (instead of with normal AKR serum and complement as in group B), the tumor did grow (group B'). Thus, it seems clear that spleen cells from CBPC 101-challenged mice contain T cells that can prevent the growth of the tumor. It is important to note that we did not obtain any evidence for suppressed tumor cells in the spleens of CBPC 101-challenged mice as the transfer of anti-Thy 1.2-treated spleen cells alone did not result in any G2 production or obvious tumor growth (group B''). Presumably, suppressed tumor cells would have become active in the absence of suppressor T cells. Thus, the likely fate of CBPC 101 cells in BALB/c mice is death from lysis or from a T-cell factor.

Table II demonstrates further that spleen cells of CBPC 101-challenged BALB/c mice are also able to prevent the G2 production of normal C.B-17 B cells.
TABLE I

**Allotype-Dependent Immunity of Ig-Congenic BALB/c Mice to CBPC 101 Tumor Cells**

| Group | Recipients* | No mice living/no injected (weeks after tumor injection) |
|-------|-------------|---------------------------------------------------------|
|       |             | 1  3  5  7  9  15                                       |
| A     | BALB/c      | 9/9 9/9 9/9 9/9                                      |
| B     | BALB/c      | 28/28 28/28 27/28 26/28                                |
| C     | C AL-20     | 13/13 13/13 13/13 13/13                                |
| D     | C B-17      | 9/9 5/9 1/9 0/9                                      |

* Each recipient was injected intraperitoneally with 5 x 10^6 CBPC 101 cells

TABLE II

**Evidence that BALB/c Immunity to CBPC 101 is Mediated by G2 Tcs Cells**

| Group | Cotransfer of cells into X-irradiated BALB/c recipients | No recipients showing G2/no injected survivors (weeks after cell transfer) |
|-------|----------------------------------------------------------|------------------------------------------------------------------------|
|       | Spleen cells | G2 target cells | 1  2  3  5  7                                               |
| A     | CBPC 101     | CBPC 101        | 5/6 2/2 1/1 All dead                                       |
| B     | CBPC 101     | CBPC 101        | 3/6 0/6 0/6 0/6 0/6                                      |
| B'    | CBPC 101     | CBPC 101        | 4/6 4/5 4/5 All dead                                      |
| B'    | CBPC 101     | CBPC 101        | 0/6 0/6 0/6 0/6 0/6                                      |
| C     | C B-17 spleen | C B-17 spleen   | 0/8 0/8 0/8 0/8 0/8                                      |
| D     | C B-17 spleen | C B-17 spleen   | 0/6 0/6 0/6 0/6 0/6                                      |

* 10^7 BALB/c spleen cells were admixed with 2 x 10^6 CBPC 101 cells or 2 x 10^6 C B-17 spleen cells, the mixture was injected intraperitoneally into X-irradiated BALB/c mice (550 R). X-irradiation alone did not cause any of 15 control mice to die in the following 7 wk (not shown). Immune spleen cells were taken from mice challenged with CBPC 101 cells 15 wk earlier (from group A of Table I). Spleen cells in groups B' and B'' were treated with AKR anti-Thy 1.2 and complement before being transferred to X-irradiated BALB/c recipients, the spleen cells of group B were treated in the same manner except that normal AKR mouse serum was substituted for AKR anti-Thy 1.2. Although not shown, the recipients of group C still continued to produce the IgG2 allotype (F2) of C B-17 mice 7 wk after cell transfer as evident from reacting their sera with anti-G2 serum (7).

This is shown in group C. But as with G2-suppressed C.B-17 mice in earlier experiments (7), the C.B-17 B cells in group C did not stop producing their IgG1 allotype (F2) (see footnote of Table II). Two conclusions can be drawn from these results. First, that CBPC 101 cells do not elicit BALB/c T cells which recognize a non-Ig determinant on all C.B-17 lymphocytes, such as a minor histocompatibility antigen; otherwise, we would expect other C.B-17 allotype markers besides G2 (e.g., F2) to be suppressed also. Second, that T-cell immunity to CBPC 101 must be directed against allotype determinants, i.e., we are not dealing with idiotypic determinants unique to the IgG2a molecules produced by CBPC 101 since spleen cells from CBPC 101-challenged mice could suppress the G2 production of normal C.B-17 B cells. Apparently, G2 Tcs cells are responsible for BALB/c immunity to CBPC 101.

Evidence that G2-bearing B cells are the target of G2 Tcs cells is presented in Table III. As shown in group A, the treatment of tumor cells with anti-Thy 1.2 serum and complement to remove any associated T cells that might derive from the tumor-bearing C.B-17 host did not prevent the CBPC 101 cells from rapidly growing in X-irradiated BALB/c. Therefore, CBPC 101 B cells must not require the presence of specific Th cells (e.g., G2 Th cells) in order to grow. Thus, to prevent tumor growth, G2 Tcs cells must recognize CBPC 101 B cells directly and presumably normal G2-producing B cells as well. Further, this recognition must be specific for Fc allotype determinants. Evidence for this is seen in group B,
Table III
Prevention of CBPC 101 Tumor Growth by G2 Tcs Cells: Suggested Presence of G2 Tcs Cells in Normal BALB/c Mice

| Group | Cotransfer of cells into X-irradiated BALB/c recipients | No. recipients showing G2/no rejected survivors (weeks after cell transfer) |
|-------|--------------------------------------------------------|------------------------------------------------------------------------|
|       | Spleen cells                                          | CBPC 101                                                               |
|       |                                                        | (2 × 10^6)                                                             |
| A*    |                                                        | CBPC 101                                                               |
|       |                                                        | (2 × 10^6)                                                             |
| B*    | BALB/c anti-G*                                        | CBPC 101                                                               |
|       |                                                        | (2 × 10^6)                                                             |
| C*    | BALB/c anti-G*                                        | CBPC 101                                                               |
|       |                                                        | (2 × 10^6)                                                             |
| D     | BALB/c normal                                         | CBPC 101                                                               |
|       |                                                        | (2 × 10^6)                                                             |
| E     | C.B-17 normal                                         | CBPC 101                                                               |
|       |                                                        | (2 × 10^6)                                                             |

where CBPC 101 cells would not grow in the presence of BALB/c spleen cells containing G2 Tcs that had been primed against purified Fc fragments of C57BL/6 7S Ig (7) and from mice that were producing G2* (anti-C AL-20 IgG2a). The CBPC 101 cells in groups A-C were treated with AKR anti-Thy 1 2 serum and complement before being transferred into X-irradiated BALB/c recipients.

Consistent with the results of Table I, Table III also shows that normal BALB/c (but not C.B-17) spleen cells can prevent the growth of CBPC 101 tumor cells. Cotransferring BALB/c spleen and CBPC 101 cells at a ratio of 20:1 into X-irradiated BALB/c mice resulted in only 2 of 12 recipients showing G2; both of these animals died of large tumors (group D). Studies are in progress to determine whether normal G2-producing B cells (G2 antibody-forming cells) can elicit a similar response of BALB/c spleen cells, i.e., the prevention of normal G2 production.

The question prompted by the above results is how can spleen cells of normal BALB/c mice mount so rapid and effective a response against tumor cells that bear an allotype antigen that these mice have never seen experimentally? To account for this, we suggest that not only is the G2 marker very antigenic, but that a considerable number of G2 Tcs cells or their precursors are present in normal BALB/c mice. In addition to the suggested presence of G2 Tcs cells, we have unpublished evidence that BALB/c mice also contain Tcs cells directed against IgG2a allotype determinants (G4) of C.AL-20 mice. This raises an interesting possibility that different mouse strains may contain different allotype-specific Tcs cells, and that the stimulus for generating such Tcs cells may be provided by allotype antigens normally undetectable (hidden allotypes). Irregularities or specific stress of this regulatory system could result in detectable production of hidden allotypes. In fact, similar to our earlier findings (9), we can now show with radioimmune techniques the transient appearance of allotypes indistinguishable from those of BALB/c IgG1 and IgG2a in C.B-17 mice.1 Addi-

1 Bosma, M., and C. L. De Witt. Manuscript in preparation
tional evidence for the regulatory control of allotype production is the observed nonallelic behavior of Ig allotypes in rabbits (10, 11). Also, the production of unexpected allotypes by normal (and neoplastic, 12) human lymphocytes has been reported (13, 14); however, several laboratories (15, 16) have not been able to confirm these observations (13, 14). Structural evidence consistent with the idea of regulatory control of allotype production is also available. Most constant region (C) allotypes of human heavy (H) chains correspond to multiple antigenic differences (16), some of which (in the case of IgG3) have been localized to different Cβ regions (17). Moreover, constant region allotypes of light chains (Cλ) in rat (18) and in rabbit (19, 20), as well as rabbit allotypes on H chain variable regions (VH) (16), correlate not with a few but with many amino acid differences. Evolutionary considerations of such "complex" allotypes have led some (18-21) to suggest that these allelic differences may reflect the products of different linked genes and that the basis for the genetic polymorphism is in the control of such genes.

The advantage of regulating IgG production by allotype-specific Tcs cells is not clear although one could suppose in the above context that hidden mouse allotypes reflect Cβ allotype genes that are associated with different sets of VH genes; this, then, would suggest a way of controlling the expression of select sets of VH genes.

Summary

BALB/c T cells, which can prevent normal C57BL IgGα allotype (G2) production of Ig-congenic partner mice (C.B mice), are shown capable of preventing the growth and G2 production of a C.B plasmacytoma (CBPC 101). Such cytotoxic or suppressor T cells are clearly allotype-specific (G2 Tcs cells). And since CBPC 101 B cells do not require specific helper T cells in order to grow, we infer that G2-bearing B cells (normal or neoplastic) must be the direct target of G2 Tcs cells. This mode of T cell prevention of allotype production contrasts that reported for suppressor T cells in (BALB/c × SJL)F1 mice.

We thank Dr. M. Potter for C.AL-20 mice and for the CBPC 101 myeloma, Dr. J. B Smith for antithy 1.2 sera, and Mrs Eva Cunningham for technical assistance.

Note Added in Proof. Our experiments do not exclude the possibility that in addition to G2 determinants, G2 Tcs cells could recognize another determinant specific for G2-producing B cells.

Received for publication 3 November 1976.

References
1. Möller, G. 1969. Transplantation reviews, vol. 1. Williams and Wilkins Co., Baltimore.
2. Gershon, R. K. 1974. T cell control of antibody production. Contemp. Top. Immunobiol. 3:1.
3. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. J. Exp. Med. 141:1376.
4. Cantor, H., and E. A. Boyse. 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly+ cells in the generation of killer activity. J. Exp. Med. 141:1390.

5. 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 the specific removal by suppressor T cells. J. Exp. Med. 144:330.

6. Herzenberg, L. A., E. L. Chan, M. M. Ravitch, R. S. Riblet, and L. A. Herzenberg. 1973. Active suppression of immunoglobulin allotype synthesis. III. Identification of T cells as responsible for suppression by cells from spleen, thymus, lymph node, and bone marrow. J. Exp. Med. 137:1311.

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

8. Potter, M., and R. Lieberman. 1967. Genetic studies of immunoglobulins in mice. Cold Spring Harbor Symp. Quant. Biol. 32:187.

9. Bosma, M. J., and G. C. Bosma. 1974. Congenic mouse strains: the expression of a hidden immunoglobulin allotype in a congenic partner strain of BALB/c mice. J. Exp. Med. 139:512.

10. Strosberg, A. D., C. Hamers-Casterman, W. van der Loo, and R. Hamers. 1974. A rabbit with the allotypic phenotype: a1a2a3 b4b5b6. J. Immunol. 113:1313.

11. Mudgett, M., B. A. Fraser, and T. J. Kindt. 1975. Non-allelic behavior of rabbit variable-region allotypes. J. Exp. Med. 141:1448.

12. Pothier, L., H. Borel, and R. A. Adams. 1974. Expression of IgG allotypes in human lymphoid tumor lines serially transplantable in the neonatal Syrian hamster. J. Immunol. 113:1398.

13. Lobb, N. 1968. The synthesis of immunoglobulin γG by cultured human lymphocytes. Aust. J. Exp. Biol. Med. Sci. 46:397.

14. Rivat, L., D. Gilbert, and C. Ropartz. 1973. Immunoglobulin allotypic specificities in mixed leucocyte cultures. Immunology. 24:1041.

15. Litwin, S. D., T. H. Hütteroth, and L. Balaban. 1976. Synthesis of Gm allotypes by human tonsillar lymphocytes in culture: concordance with serum phenotype. J Immunol. 116:1045.

16. Kunkel, H. G., and T. Kindt. 1975. Allotypes and idiootypes. In Immunogenetics and Immunodeficiency. B. Benacerraf, editor. University Park Press, Baltimore. 56.

17. Natvig, J. B., and H. G. Kunkel. 1968. Genetic markers of human immunoglobulins: Gm and Inv systems. Ser Haematol. 1:66.

18. Gutman, G., E. Loh, and L. Hood. 1975. Structure and regulation of immunoglobulins: kappa allotypes in the rat have multiple amino-acid differences in the constant region. Proc. Natl. Acad. Sci. U S. A. 72:5046.

19. Farnsworth, V., R. Goodflesh, S. Rodkey, and L. Hood. 1976 Immunoglobulin allotypes of rabbit kappa chains: polymorphism of a control mechanism regulating closely-linked duplicated genes? Proc. Natl Acad. Sci. U S. A. 73:1293.

20. Strosberg, A. D. 1976. A possible control by regulatory allelic genes of allotypic expression. Biochem. Soc. Trans. 4:41.

21. Bodmer, W. F. 1973. A new genetic model for allelism at histocompatibility and other complex loci: polymorphism for control of gene expression. Transplant Proc. 4:1471.