Brief Definitive Report

IDIOTYPE-SPECIFIC T LYMPHOCYTES
II. Capability of Generation of Idiotype-specific T Lymphocytes is Determined by Corresponding V_H Gene*

BY HIROSHI YAMAMOTO, SOJI BITOH, AND SHIGEYOSHI FUJIMOTO

From the Department of Immunology, Kochi Medical School, Kochi 781-51, Japan

During the past decade, the immunological significance of idiotype-antiidiotype interactions has received increasing attention, since the antiidiotypic antibodies have various interesting immunobiological activities (1). Among the various cellular interactions, the lymphocytes that specifically recognize idiotypic determinant(s) may have important roles for both regulation and diversification of the immune system.

To conduct an analysis of immunoregulatory roles of cellular interaction via idiotypic determinant(s), we have established a system to detect idiotype-specific T cells. The cytotoxic T lymphocyte (CTL) population obtained from mice immunized with MOPC-104E (M104E) myeloma tumor cells was co-cultured with dextran B1355S-immune BALB/c B lymphocytes. It was revealed that M104E cross-reactive idiotypic (CRI) antidextran antibody responses were effectively inhibited in the presence of the CTL population in an H-2-restricted manner. The surface phenotypes of the inhibitory cell were Thy-1^+, Lyt-1^-, Lyt-2^+, and I-J^- (2). In this paper, using the various Igh-1-congenic strains of mice, we show that the capability for the induction of idiotype-specific T lymphocytes is genetically determined by the producibility of CRI in that strain of mouse.

Materials and Methods

Animals. BALB/cCr mice were obtained from Shizuoka Agricultural Cooperative Association, Shizuoka, Japan. The following three Igh-1-congenic strains of mice, maintained in our animal facility by sister-brother matings, were also used: C.AL-20 (Igh-1^d) and CB-20 (Igh-1^b), originally from Dr. A. Nisonoff, Brandeis University, Waltham, MA and Dr. D. H. Sachs, National Institutes of Health (NIH), respectively, were obtained from The Institute for Cancer Research, Osaka University; BAB-14, originally from Dr. D. E. Mosier, NIH, was obtained from Dr. A. Yano, Shinshu University, Matsumoto, Japan. Female mice at the age of 5–7 wk were used throughout the study.

Reagents. Immunization, preparation, and specificity analysis of the anti-M104E idiotypic antibody were exactly the same as previous reports (2, 3). Dextran B1355S from *Leuconostoc mesenteroides* NRRL B1355 and its dinitrophenylated (DNP) form were used as described elsewhere (2, 3).

Induction of CTL. CTL immune to M104E were induced as follows: 10^6 viable M104E myeloma cells were inoculated into BALB/c or various congenic mice intradermally in their backs. 7–8 d later, the tumor mass was surgically removed and, 10–14 d after operation, spleen cells of those mice were restimulated in vitro with mitomycin C-treated M104E cells. CTL activity was assessed on day 5 of culture. To see the effect of CTL populations on idiotypic antibody-producing B lymphocytes, aliquots of CTL cultures

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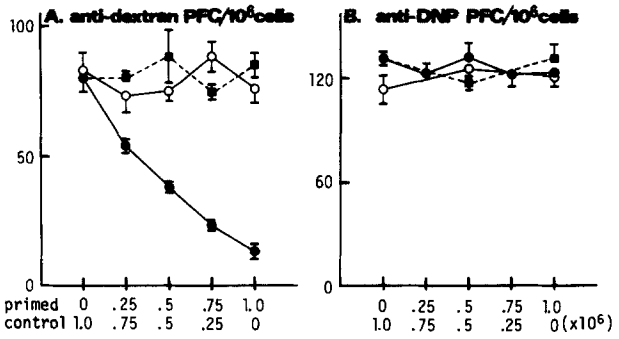

FIGURE 1. Antigen-specific inhibition of antibody production by CTL population immune to M104E myeloma tumor cells. CTL populations for M104E were obtained from BALB/c and C.AL-20 mice on day 4 of culture (the cytolytic activities of aliquots of culture cells on day 5 were 25.6% and 21.5%, respectively, at an effector/target cell (E/T) ratio of 40:1). CTL population for M167 of BALB/c was also prepared using same procedures as for M104E (CTL activity was 17.5% at an E/T of 40:1). 10^6 of T cell-depleted BALB/c spleen cells immune to DNP-dextran (50 μg, 48 h earlier) were co-cultured with graded numbers of various CTL populations (primed) for 3 d. To keep the culture conditions constant, 4-d cultured M104E or M167 immune spleen cells without in vitro restimulation of homologous tumor cells (control) were added as filler cells. Unstimulated spleen cells showed no significant CTL activities. The effect of M104E CTL population from BALB/c (●) and C.AL-20 (○) on antidextran (*A*) and anti-DNP (*B*) antibody responses, and of M167 CTL from BALB/c (■) on both antibody responses are indicated. Each value represents the arithmetic mean and SE of four to five individual cultures.

were harvested on day 4 and were mixed with BALB/c spleen cells treated with anti-Thy-1.2 plus complement from the animals that had been immunized with 50 μg of DNP-dextran intraperitoneally 48 h before. The cultures were continued for 3 d without any antigenic stimulations and direct (IgM) antidextran, and anti-DNP plaque-forming cell (PFC) responses were enumerated as described before (2). To keep the culture conditions constant, aliquots of responder CTL that had not been restimulated in vitro with tumor cells were used as filler cells in this series of experiments. The CTL activity of in vivo primed and in vitro nonrestimulated cells were negative, as were unprimed cells.

Results

In a previous report (2), we showed that the M104E idiotype-specific T lymphocytes, probably CTL, have an idiotype-specific inhibitory activity in an H-2-restricted manner on antidextran antibody production of dextran-immune B lymphocytes. In the course of these experiments, we found that the C.AL-20 mouse, which is an Igh-1-congenic strain, had a capability equivalent to parental BALB/c mice of inducing CTL for M104E by the same immunization regimens as BALB/c. To determine the inhibitory activity of the CTL population obtained from C.AL-20 mice on antidextran antibody production, it was co-cultured with DNP-dextran B1355S-primed B lymphocytes from BALB/c mice for 3 d and the effect on antibody responses was investigated. As shown in Fig. 1, the addition of graded numbers of the CTL from the secondarily restimulated BALB/c spleen cells clearly depressed antidextran antibody responses in a dose-dependent manner (Fig. 1*A*, ●). In contrast, the CTL from C.AL-20 had no effect on the antidextran antibody production of BALB/c B lymphocytes (○). The depression of the response was also not seen at all by co-culture with an M167-specific CTL population (■). Moreover, anti-DNP responses of BALB/c B lymphocytes were entirely unaffected by either the anti-M104E CTL or anti-M167 CTL popula-

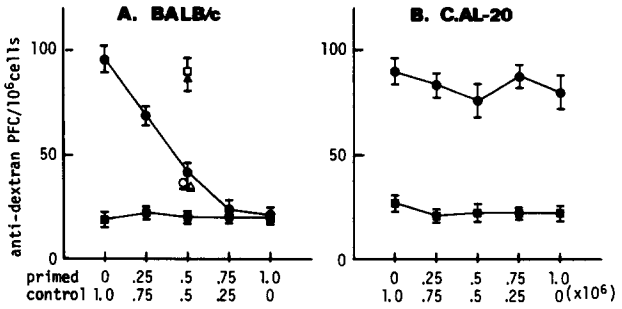

FIGURE 2. Idiotype-specific inhibition of antidextran antibody production by M104E immune lymphocytes. M104E-immune CTL populations of BALB/c and C.AL-20 mice were co-cultured with 10^6 dextran-immune BALB/c B lymphocytes by the same methods as described in Fig. 1 legend. Total antidextran antibody responses in the presence of BALB/c (*A*) and C.AL-20 (*B*) CTL populations are shown (●). To investigate the contents of CRI-negative plaques, PFC of the aliquots of individually cultured cells were developed in the presence of 0.8 ng/ml of specific antiidiotypic antibodies (■). CTL activities of BALB/c and C.AL-20 at an E/T of 40:1 were 45.5% and 43.9%, respectively. Effects of the following 1:1 mixtures of the cells on total antidextran responses were also tested: primed BALB/c plus control C.AL-20 (○), primed BALB/c plus primed C.AL-20 (△), control BALB/c plus control C.AL-20 (□), and control BALB/c plus primed C.AL-20 (▲).

tion.

The idiotypic specificity of the inhibition of antidextran antibody production were then analyzed by plaque-inhibition assay, since the dominant idiotypes (70–80% in our studies) of antidextran B1355S antibodies in BALB/c mice have a CRI to that of M104E myeloma protein (4, 5). The results summarized in Fig. 2 show that although the inhibition of idiotype-positive antibody production by the CTL population of BALB/c was seen (Fig. 2 *A*, ●), CRI-negative plaques that could not be blocked by specifically purified rabbit antiidiotypic antibodies were not affected. On the other hand, antibody responses, either CRI positive or negative, of BALB/c lymphocytes were not affected by the addition of CTL population obtained from C.AL-20 mice (Fig. 2*B*). The noninhibition by C.AL-20 CTL population was not due to the active suppression of the inhibitory activity by some of the suppressor cell population, since inhibitory activity of the primed BALB/c CTL population was not affected by the addition of unrestimulated control or primed cells from CAL-20 mice (Fig. 2*A*, ○, △, respectively). In the CAL-20 strain, even though it shows a comparable CTL activity against M104E to that of BALB/c, the CTL for tumor-associated antigen(s) other than idiotypic determinant on M104E might be dominated. Those results strongly indicate that the CTL population obtained from C.AL-20 mice have no specificity to the CRI, in striking contrast to that from BALB/c.

The experiments were extended to other Igh-1-congenic strains such as BAB-14 and CB-20 mice. As shown in Fig. 3, the inhibition of antidextran antibody response of BALB/c B lymphocytes was caused by the addition of the CTL population obtained from either BALB/c or BAB-14. In contrast, the T lymphocytes derived from CB-20 and C.AL-20 did not inhibit the antibody production. On the other hand, concomitantly immunized anti-DNP responses of BALB/c lymphocytes were not affected by the presence of CTL populations from any strains of mice. C.AL-20, BAB/14, and CB-20 have different Igh-1 allotypes than BALB/c. However, BAB-14 is a recombinant strain that occurs

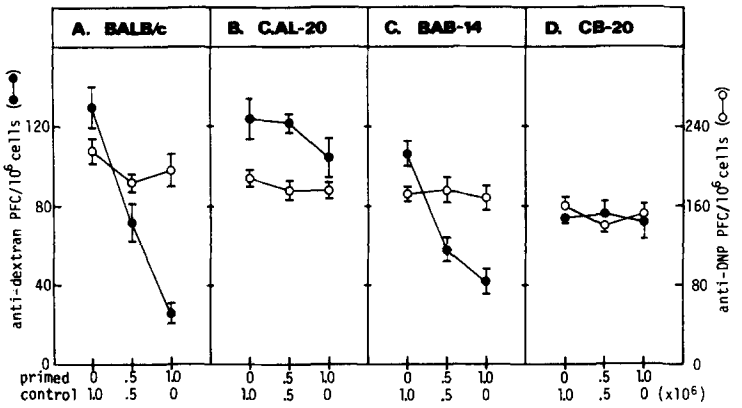

FIGURE 3. Inhibition of antidextran antibody responses by M104E immune CTL populations obtained from various Igh-1-congenic strains of mice. 10^6 cells of DNP-dextran–immune BALB/c B lymphocytes were co-cultured with CTL populations obtained from BALB/c (*A*), C.AL-20 (*B*), BAB-14 (*C*), and CB-20 (*D*). CTL activities at an E/T of 40:1 were 24.8%, 15.2%, 12.6%, and 31.6%, respectively. Antidextran (●) and anti-DNP (○) PFC/10^6 B cells are shown.

in the V_H gene cluster and can respond to dextran B1355S by producing M104E CRI (6), while the other two strains cannot. The results strongly suggest that the capability to induce idiotype- and H-2-restricted (H-2-restrictive phenomenon described in reference 2) T lymphocyte activity strongly correlates to the producibility of the CRI in a particular strain of mice.

Discussion

In a previous paper (2), we described the experimental system used to detect idiotype-specific T lymphocytes. Taking advantage of the expression of M104E myeloma protein on the tumor cell surface that shares CRI to dominant antidextran B1355S antibodies produced by BALB/c mice, it was shown that the M104E-specific CTL population induced by immunization of the tumor cells significantly inhibited antidextran antibody production in an idiotype-specific manner. Moreover, it was found that the T lymphocytes could recognize major histocompatibility complex (MHC) products simultaneously with idiotypic determinant(s) on the B lymphocytes. Using the same system, we compared the inducibility of idiotype-specific T cells in various Igh-1-congenic strains of mice. It was revealed that the induction of M104E idiotype-specific and MHC-restricted T lymphocytes could only be seen in strains BALB/c and BAB-14, which have a potential to produce the CRI. The inability of C.AL-20 and CB-20 to induce inhibitory T cells for antibody production might be due to insufficient clonal expansion for the T cells because of the absence of the corresponding V_H gene products.

Recently, several investigators (7–9) demonstrated the existence of Igh-linked loci-restricted T cell activities in various experimental systems. The report of L'Age-Stehr (10) showed that the antigen (SRBC)-specific T cells that had been primed in environments having a certain V_H haplotype exhibited efficient helper T cell activity for the B cells derived from mice with the corresponding V_H allele. Thus the immunoglobulin-dependent helper T cells, whether from V_H-identical or -nonidentical strains to the B cells, are adaptively sensitized to gain V_H

preference. Our results are very similar to hers although we are dealing with a well-characterized idiotype and although we also first revealed that the idiotype-specific T cells have been sensitized a priori to the relevant V_H gene products.

The experiments reported by Bottomly and Mosier (11) clearly demonstrated that the capability to produce enough T-15 idiotypic antibody is an important element in the induction of T-15 idiotype-specific helper T lymphocytes. In contrast to our previous results (2) and those reported here showing that the T cells are MHC restrictive, Bottomly (12) reported that idiotype-specific T cells are not restricted to MHC products. The reason for this discrepancy is not clear, but one possibility is that it is related to the subsets of T cells used. It is not yet clear how and when the T cells are educated by MHC and V_H gene products to learn what is self. Future experimental progress with various chimeric animals could provide a definitive answer to this question.

It is known that idiotype-specific T lymphocytes can be induced by extensive immunization of the idiotype in syngeneic animals (14–18). However, for the precise analysis of the roles of idiotype-antiidiotype interactions in both regulation and diversification of the immune system, it is important to note that the idiotype-specific T cell is a counterpart to the relevant idiotype and that the clones must be induced by the expansion of the cells possessing the idiotypic determinant(s). In this context, evidence from our series of experiments strongly supports the idea that the V_H gene products are primary antigenic stimulants, i.e., internal images, for the generation and construction of an idiotype network system, as originally postulated by Jerne (19).

Summary

MOPC-104E (M104E) idiotype-specific and major histocompatibility complex-restricted T lymphocyte activities were investigated in BALB/c (Igh-1^a) and its Igh-1-congenic strains of mice, such as C.AL-20 (Igh-1^d), BAB-14 (Igh-1^b), and CB-20 (Igh-1^b). Idiotype-specific T lymphocytes could be induced in BALB/c and BAB-14 by immunization of viable M104E tumor cells followed by surgical removal and in vitro restimulation with the homologous tumor cells. On the other hand, C.AL-20 and CB-20 mice did not show the idiotype-recognizing capacity even though they could mount comparable cytotoxic T lymphocyte activities against M104E to BALB/c and BAB-14. The results strongly suggest that the inducibility of M104E cross-reactive idiotypy closely paralleled the producibility of corresponding idiotype-specific T lymphocytes. Genetically defined V_H gene products might act as internal images that construct idiotype network systems.

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References

1. Janeway, C. Jr., E. E. Sercarz, and H. Wigzell, editors. 1981. Immunoglobulin Idiotypes. Academic Press, Inc., New York.
2. Yamamoto, H., S. Bitoh, M. Torii, and S. Fujimoto. 1983. Idiotype-specific T

lymphocytes. I. Regulation of antibody production by idiotype-specific H-2-restricted T lymphocytes. *J. Immunol.* 130:1038.

3. Inada, T., H. Yamamoto, S. Bitoh, S. Fujimoto, and T. Hamaoka. 1982. Detection and characterization of idiotype-specific enhancing cells generated in mice immunized with idiotype. *J. Immunol.* 129:2069.
4. Blomberg, B., W. R. Geckeler, and M. Weigert. 1972. Genetics of the antibody response to dextran in mice. *Science (Wash. DC).* 177:178.
5. Riblet, R., B. Blomberg, M. Weigert, R. Liberman, B. A. Taylor, and M. Potter. 1975. Genetics of mouse antibodies. I. Linkage of the dextran response locus, V_H-DEX, to allotype. *Eur. J. Immunol.* 5:775.
6. Riblet, R., M. Weigert, and O. Makela. 1975. Genetics of mouse antibodies. II. Recombination between V_H genes and allotype. *Eur. J. Immunol.* 5:778.
7. Janeway, C. Jr. 1980. Idiotypic control: the expression of idiotypes and its regulation. *In* Strategies of Immune Regulation. E. E. Sercarz and A. J. Cunningham, editors. Academic Press, Inc., New York. 179–198.
8. Eardley, D. E., F. W. Shen, H. Cantor, and R. K. Gershon. 1979. Genetic control of immunoregulatory circuits. Genes linked to the Ig locus govern communication between regulatory T-cell sets. *J. Exp. Med.* 150:44.
9. Nutt, N., J. Haber, and H. H. Wortis. 1981. Influence of Igh-linked gene products on the generation of T helper cells in the response to sheep erythrocytes. *J. Exp. Med.* 153:1225.
10. L'Age-Stehr, J. 1981. Priming of T helper cells by antigen-activated B cells. B-cell primed Lyt-1^+ helper cells are restricted to cooperate with B cells expressing the IgV_H phenotype of the priming B cells. *J. Exp. Med.* 153:1236.
11. Bottomly, K., and D. E. Mosier. 1979. Mice whose B cells cannot produce the T-15 idiotype also lack an antigen-specific helper T cell required for T-15 expression. *J. Exp. Med.* 150:1399.
12. Bottomly, K. 1981. Activation of the idiotypic network: enviornmental and regulatory influences. *In* Immunoglobulin Idiotypes. C. A. Janeway, E. E. Sercarz, and H. Wigzell, editor, Academic Press, Inc., New York. 517–532.
13. Janeway, C. Jr., N. Sakato, and H. N. Eisen. 1975. Recognition of immunoglobulin idiotypes by thymus-derived lymphocytes. *Proc. Natl. Acad. Sci. USA.* 72:2357.
14. Woodland, R., and H. Cantor. 1978. Idiotype-specific T-helper cells are required to induce idiotype-positive B memory cells to secrete antibody. *Eur. J. Immunol.* 8:600.
15. Hetzelberger, D., and K. Eichmann. 1978. Recognition of idiotypes in lymphocyte interactions. I. Idiotypic selectivity in cooperation between T and B lymphocytes. *Eur. J. Immunol.* 8:846.
16. Bona, C., R. Hoogie, P. A. Cazenave, C. Leguern, and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. II. Immunity to anti-MOPC-460 idiotype antibodies increases the level of anti-trinitrophenyl antibodies bearing 460 idiotypes. *J. Exp. Med.* 149:815.
17. Rohrer, J. W., B. Odermatt, and R. G. Lynch. 1979. Immunoregulation of murine myeloma: isologous immunization with M315 induces idiotype-specific T cells that suppress IgA secretion by MOPC-315 cells *in vivo*. *J. Immunol.* 122:2011.
18. Abbas, A. K., L. L. Perry, B. A. Bach, and M. I. Greene. 1980. Idiotype-specific T cell immunity. I. Generation of effector and suppressor T lymphocytes reactive with myeloma idiotypic determinants. *J. Immunol.* 124:1160.
19. Jerne, N. K. 1974. Towards the network theory of the immune system. *Ann. Immunol. (Paris).* 125C:373.