SUPPRESSION OF HAPTEN-SPECIFIC DELAYED-TYPE HYPERSENSITIVITY RESPONSES IN MICE BY IDIOTYPE-SPECIFIC SUPPRESSOR T CELLS AFTER ADMINISTRATION OF ANTI-IDIOTYPIC ANTIBODIES*

By HIROSHI YAMAMOTO,‡ MAKOTO NONAKA, and DAVID H. KATZ

From The Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

During the past few years, the biological effects of anti-idiotypic antibodies have received increasing attention a result of the (*a*) postulated role that idiotype-anti-idiotype responses may play in both regulation and diversification of the immune system, as fostered by Jerne (1) and (*b*) possible clues such anti-idiotypic reactivities might provide to the ultimate clarification and elucidation of the mystery concerning the molecular nature of T-cell receptors (reviewed in 2, 3).

The BALB/c phosphorylcholine (PC)[1]-binding TEPC-15 myeloma protein displays idiotypic determinants (T-15) which represent the dominant idiotype present on anti-PC antibodies produced by BALB/c mice. Previous investigations by others have demonstrated effective modulation of PC-specific immune responses after appropriate exposure of BALB/c mice to anti-T-15 idiotypic antibodies. These include suppression of PC-specific antibody production (4), suppression of PC-specific helper T-lymphocyte activities (5) and induction of T-15 idiotype-specific suppressor T-lymphocyte activities (6).

To conduct an analysis of biological effects of anti-idiotypic antibody (anti-Id) on another form of cell-mediated immunity, we have established a system for the induction of PC-specific delayed-type hypersensitivity (DTH) responses in BALB/c mice. This was accomplished by appropriate sensitization with PC-coupled peritoneal exudate cells (PEC), and was confirmed to be mediated by PC-specific T lymphocytes. The experiments summarized herein demonstrate that administration of appropriate doses of anti-T-15 idiotypic antibodies is effective in specifically inhibiting the induction phase of PC-specific DTH responses in BALB/c mice. Moreover, the results demonstrate quite clearly that administration of anti-Id to BALB/c mice generates idiotype-specific suppressor T lymphocytes which specifically suppress PC-specific DTH responses at both the induction and effector phases of such responses.

* Publication No. 108 from the Department of Cellular and Developmental Immunology and publication No. 1778 from the Immunology Departments, Scripps Clinic and Research Foundation, La Jolla, Calif. Supported by U. S. Public Health Service grant AI-13781 and Biomedical Research Support grant RR0-5514.

‡ Supported by a Fellowship from The Cancer Research Institute, Inc.

[1] *Abbreviations used in this paper:* C, complement; DPPC, Diazonium phenyl-phosphorylcholine; DTH, delayed-type hypersensitivity; Id, idiotype or idiotypic; MEM, minimum essential medium; NMS, normal mouse serum; PEC, peritoneal exudate cells; PC, phosphorylcholine; SC, spleen cells; TNBS, trinitrobenzene sulfonate; TNP, trinitrophenyl.

Materials and Methods

Animals. Female BALB/c mice, aged 8–10 wk, and retired breeders and female A/J mice, aged 8–10 wk, from the Scripps breeding colony were used throughout this study.

Chemicals. Diazonium phenyl-phosphorylcholine (DPPC) was synthesized from *p*-nitrophenyl-phosphorylcholine (Pierce Chemical Co., Rockford, Ill.) by the method of Chesebro and Metzger (7). Trinitrobenzene sulfonate (TNBS) was purchased from Eastman Kodak Co., Rochester, N.Y.

Hapten Derivatization of Cell Preparations

Conjugation of PEC or spleen cells (SC) with PC and trinitrophenyl (TNP) were performed as follows:

PC. PEC were collected from peritoneal cavities of BALB/c retired breeder mice and young adult A/J mice 5 d after injection of 1.5 ml of mineral oil (Fisher Scientific Co., Pittsburgh, Pa.) intraperitoneally. Contaminating erythrocytes were lysed by brief exposure of the PEC to Tris-buffered ammonium chloride solution followed by washing twice with minimum essential medium (MEM) and once with phosphate-buffered (0.005 M) saline (pH 7.2). Washed PEC were resuspended in 1 ml of isotonic borate-buffer pH 8.2 (50–60 × 10^6 per ml) in 12-ml plastic tubes. 6 μmol of DPPC were added three times, at 3-min intervals into the cell suspensions with gentle swirling at room temperature. The pH was monitored with pH indicator paper after every addition of DPPC. 3 min after the last addition of DPPC, the reaction was stopped by the addition of 10 ml of MEM. 1 ml of fetal calf serum was then added slowly down the side walls into the bottom of individual plastic tubes, and the tubes were centrifuged for 10 min at 1,200 rpm. PC-derivatized PEC were then washed twice with MEM.

Single cell suspensions of spleen cells from young adult BALB/c and A/J mice were prepared and erythrocytes removed by treatment with Tris-ammonium chloride solution. PC conjugation of SC was performed exactly the same as PEC.

TNP. For derivatization of PEC with TNP, washed PEC were resuspended in phosphate-buffered (0.01 M) saline (pH 7.2) and reacted with TNBS at final concentration of 5 mM for 15 min at room temperature. The reaction was stopped and cells were washed in the same manner as the procedure for PC coupling. TNP-SC were prepared in the same manner.

Viabilities of haptenated cell preparations were examined by trypan blue dye exclusion test; such analysis revealed that the viabilities were > 80% in every case.

Immunization and Challenge. For the induction of DTH responses to PC and TNP, recipient mice were sensitized by injecting subcutaneously (s.c.) 30–40 × 10^6 syngeneic haptenated PEC divided into two sites of the hind trunk areas (0.1-ml vol/site).

The sensitized mice were challenged 5 d after sensitization with injections into the right footpads of 20–30 × 10^6 syngeneic haptenated SC in 25-μl vol. Footpad thicknesses were measured (in double-blind fashion) with a micrometer 36–48 h after challenge. The magnitude of DTH responses are expressed as the difference in thickness of the right (experimental) and left (control) footpads. Control groups consisted of nonimmunized mice which were injected with the same amount of haptenated SC into the right footpads.

Adoptive Transfer of DTH Responses. 5 d after sensitization of animals with haptenated PEC into hind trunk and cervical areas, regional popliteal, peri-aortic, and cervical lymph nodes were removed and single cell suspensions were prepared. Immune lymph node cells were treated with anti-θ serum (AKR anti-C3H) or normal mouse serum (NMS) plus guinea pig complement (C) as described previously (8). 65 × 10^6 lymph node cells, from normal or immune donors, either treated or untreated with anti-θ serum or NMS, were injected intravenously into naive syngeneic recipient mice and challenged immediately with haptenated SC into right footpads.

Anti-idiotypic Serum. Anti-T-15-idiotypic (anti-Id) serum was raised in A/J mice by the method of Potter and Lieberman (9). Antiserum was obtained from bleedings made on days 27, 35, 42, 49, and 56 after primary immunization, which were pooled and adsorbed by passage through MOPC-167 myeloma protein-conjugated Sepharose 4B to remove anti-allotypic antibodies. The same adsorbed serum pool was used throughout this study. In experiments involving administration of anti-T-15, or A/J NMS as control, the serum was administered as follows: Recipient BALB/c mice were injected with 50 μl of anti-Id serum or NMS per day for four consecutive days (administered i.p. and s.c.). When biological effects of anti-Id on the induction phase of DTH were studied, anti-Id (or NMS) was injected on days)-1, 0, 1, and 2.

When effects of anti-Id on the effector phase of DTH were analyzed, the injections were given on days 4, 5 (twice at 12-h intervals), and 6. All sensitizations with PC-PEC were conducted on day 0 and all challenges with PC-SC were conducted on day 5.

Statistical Analysis of Data. The differences of footpad thickness from right (experimental) and left (control) were expressed as Δ footpad thickness ($\times 10^{-1}$ mm). Arithmetic means and standard errors of means were calculated, and Student's t test was applied to ascertain the statistical significance of the differences.

Results

Induction of Hapten-specific DTH Responses in BALB/c Mice. Groups of BALB/c mice were sensitized on day 0 with either syngeneic PC-PEC or TNP-PEC. 5 d later, these mice and unsensitized control mice were challenged in the right footpads with either PC-SC or TNP-SC and the development of DTH responses, as reflected by footpad thickness, was determined on day 7. As shown in Fig. 1, BALB/c mice, sensitized with PC-PEC, manifested specific DTH responses upon challenge with PC-SC (cf. groups II and I) but failed to respond to challenge with TNP-SC (group III). Conversely, BALB/c mice, sensitized with TNP-PEC, responded to challenge with TNP-SC (cf. groups V and IV) but not to PC-SC (group VI). These data illustrate the hapten specificity of DTH responses induced in this fashion.

Passive Transfer of PC-specific DTH Responses with PC-immune T Lymphocytes. Because it has been well established that DTH responses are mediated by T lymphocytes, a passive transfer experiment was conducted to confirm the requirement for T lymphocytes in the hapten-specific DTH responses generated by immunization with PC-PEC in BALB/c mice. Naive BALB/c recipients were either not injected or injected intravenously with 65×10^6 regional lymph node cells from either normal or PC-PEC-primed donor mice (5 d after immunization), and then challenged shortly thereafter with PC-SC in the right footpads (day 0). Footpad thickness was assayed on day 2. As shown in Fig. 2, uninjected control mice (group I) and recipients of

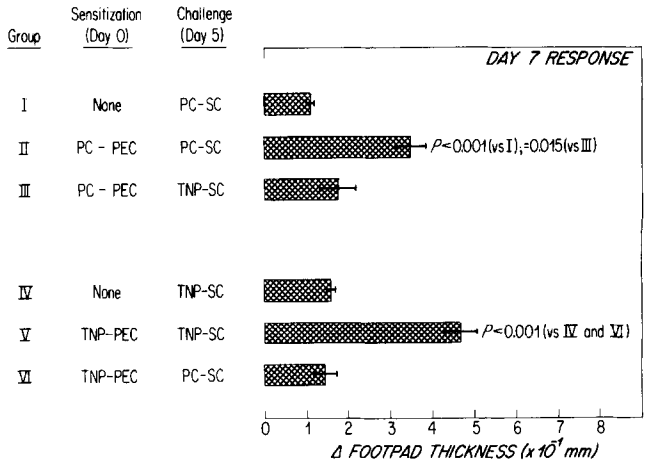

FIG. 1. Specificity of delayed-type hypersensitivity responses in BALB/c mice sensitized with hapten-derivatized syngeneic PEC. BALB/c mice were immunized with either 40×10^6 PC-derivatized syngeneic PEC or 35×10^6 TNP-derivatized PEC subcutaneously into hind trunk area on day 0 and challenged with either 28×10^6 PC-spleen cells or TNP-spleen cells into right footpad on day 5. Footpad thicknesses were measured on day 7.

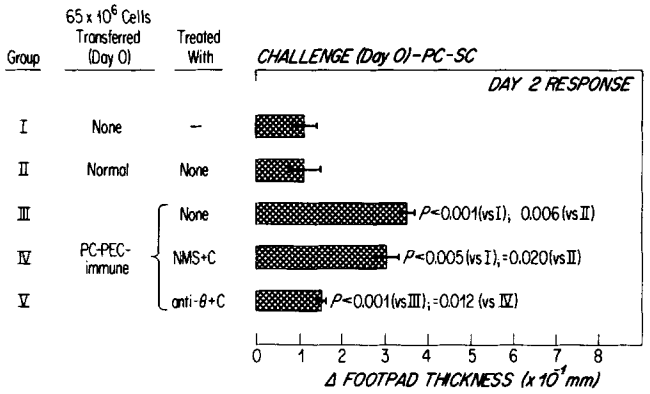

FIG. 2. Demonstration of requirement of T lymphocytes for successful adoptive cell transfer of PC-specific DTH responses in BALB/c mice. 65 × 10^6 regional lymph node cells treated with normal mouse serum or anti-θ serum plus complement, obtained from mice immunized with 40 × 10^6 PC-PEC, were inoculated intravenously into naive syngeneic recipient animals. Immediately after the cell transfer, recipient mice were challenged with 30 × 10^6 PC-SC and footpad thicknesses were measured 2 d later.

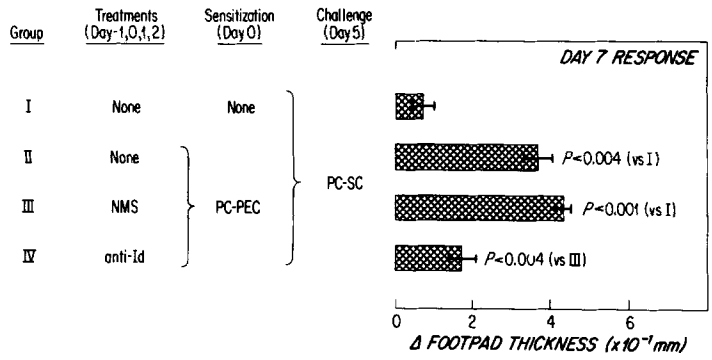

FIG. 3. Effective suppression of sensitization for PC-specific DTH responses by anti-T-15 idiotypic antiserum. Recipient BALB/c mice received four consecutive injections on day −1, 0, 1, and 2 of 50 μl of normal A/J serum (NMS) or A/J anti-T-15 idiotypic serum (anti-Id) in a total vol of 1 ml (1:20 dilution), 0.5 ml i.p. and 0.5 ml s.c. Mice were sensitized with or without 30 × 10^6 PC-PEC on day 0 and challenged with 25 × 10^6 PC-SC.

normal lymph node cells (group II) failed to develop detectable PC-specific DTH responses. Conversely, recipients of PC-PEC-immune lymph node cells displayed significant DTH responses (group III). Pretreatment of PC-PEC-immune lymph node cells in vitro with NMS + C had no appreciable effect on the ability of such cells to positively transfer DTH reactivity (group IV), whereas pretreatment with anti-θ serum + C almost completely abolished the passive transfer of PC-specific DTH responses (group V).

Effects of Anti-idiotypic Antibodies on the Induction and Effector Phases of PC-specific DTH Responses in BALB/c Mice. Having established that (*a*) PC-specific DTH responses could be readily induced in BALB/c mice and (*b*) that such responses were, indeed, mediated by T lymphocytes, experiments were then carried out to investigate the effects of anti-T-15 idiotypic antiserum on such responses. The experiment summarized in Fig. 3 illustrates the substantial inhibitory effects of anti-Id antibodies on the

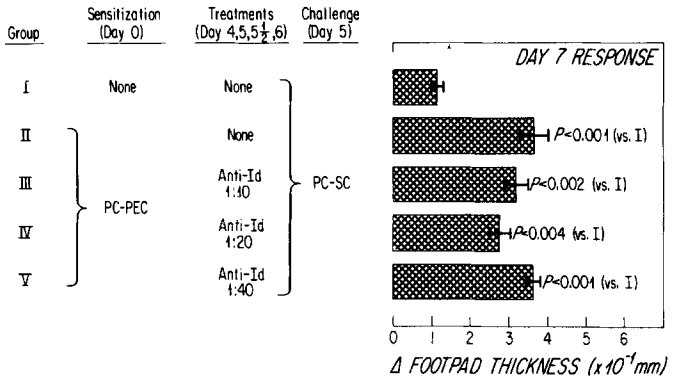

FIG. 4. Failure of anti-idiotypic suppression of PC-specific DTH responses at effector phase. PC-PEC-immunized (38×10^6) BALB/c mice received four consecutive 100 μl injections (1:10 dilution), 50 μl (1:20 dilution) or 25 μl (1:20 dilution) of anti-Id serum on day 4, 5, 5½, and 6. 30×10^6 PC-SC were injected into footpads of recipient mice.

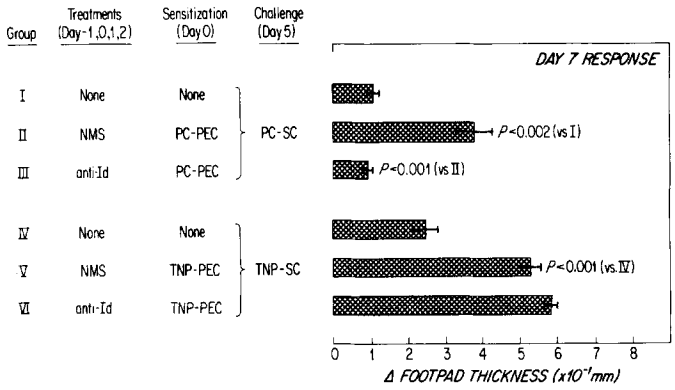

FIG. 5. Specificity of anti-T-15 idiotypic antiserum-mediated suppression of hapten-specific DTH responses. NMS or anti-Id (same procedures and doses as Fig. 3) treated BALB/c mice were sensitized with either 30×10^6 PC-PEC or 23×10^6 TNP-PEC on day 0 and challenged with either 30×10^6 PC-PEC or TNP-PEC on day 5.

induction phase of PC-specific DTH responses. Thus, BALB/c mice sensitized with PC-PEC, and not otherwise treated, developed excellent PC-specific DTH responses (cf. groups II and I), and the administration of A/J NMS immediately before and just after sensitization had no inhibitory effects on the development of such responses (group III). In contrast, administration of anti-Id significantly diminished the development of PC-specific DTH responses (group IV).

In contrast to the ability of anti-Id administration to diminish induction of PC-specific DTH responses (Fig. 3), anti-Id treatment just before and after challenge of previously sensitized mice with PC-SC was ineffective in diminishing the expression of DTH reactivity. As shown in Fig. 4, this ineffectiveness of anti-Id treatment was true over three different concentrations of antiserum employed.

Specificity of Anti-idiotypic Antiserum-mediated Suppression of Hapten-specific DTH Responses. Specificity of the anti-Id-mediated suppression of murine DTH responses, as illustrated in the preceding experiment, was analyzed both in terms of antigen and strain specificity.

ANTIGEN SPECIFICITY. The experiment summarized in Fig. 5 demonstrates the

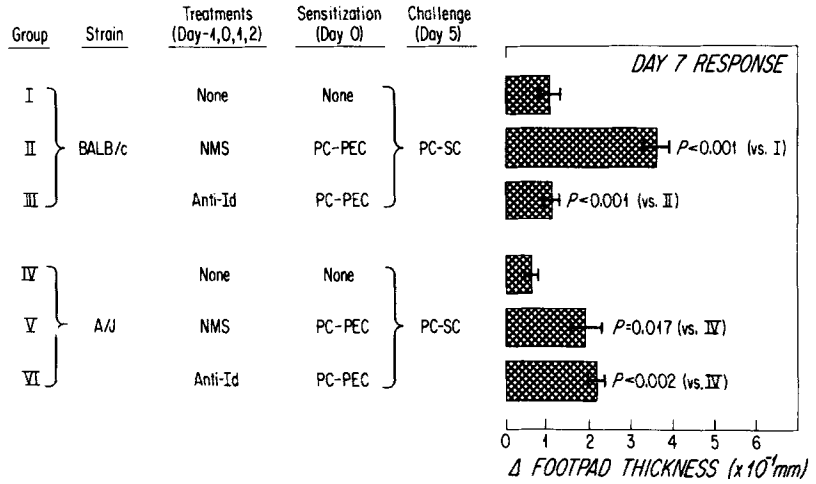

FIG. 6. Strain specificity of anti-idiotypic suppression of PC-specific DTH responses. Recipient BALB/c and A/J mice were treated with NMS or anti-Id (same procedures and doses as Fig. 3) and sensitized with syngeneic PC-PEC (32×10^6 for BALB/c and 25×10^6 for A/J). 5 d later, all recipients were challenged with syngeneic PC-SC (30×10^6 for both strains).

specificity of anti-T-15 idiotypic antiserum treatment for DTH responses that are specific for the PC hapten. This is illustrated by the fact that although anti-Id treatment effectively inhibited induction of PC-specific DTH responses (groups I–III), this same antiserum had no inhibitory effects on the induction of DTH responses specific for the TNP hapten (groups IV–VI).

STRAIN SPECIFICITY. As mentioned above, T-15 is a dominant idiotypic marker present on anti-PC antibodies of BALB/c mice, and the expression of T-15 is closely linked to the Ig-1a allotypic marker (10). A/J mice do not display the T-15 idiotypic marker and, moreover, are capable of developing good anti-T-15 idiotypic antibodies after immunization with the BALB/c TEPC-15 myeloma protein. To document the anti-Id suppression observed in the preceding experiments as true idiotype-anti-idiotype interactions on the development of DTH responses, we felt it essential to ascertain the strain specificity of such suppression. In other words, a true idiotype-anti-idiotype phenomenon should be demonstrable in a mouse strain possessing T-15 as a dominant idiotype, such as BALB/c, but not in a mouse strain lacking the T-15 idiotype, such as A/J. This expectation was confirmed in the experiment summarized in Fig. 6. Thus, anti-Id treatment was clearly effective in inhibiting induction of PC-specific DTH responses in BALB/c mice (groups I–III), but had no inhibitory effect on the development of PC-specific DTH responses in A/J mice (groups IV–VI).

Suppression of PC-specific DTH Responses by Passive Transfer of T Lymphocytes from Anti-Id-treated BALB/c Donor Mice

SUPPRESSION OF THE INDUCTION PHASE OF THE RESPONSE. As summarized in Fig. 7, four groups of BALB/c mice were sensitized with PC-derivatized PEC on day 0. On the same day, three of these groups were injected intravenously with syngeneic donor cells derived from either: (*a*) normal mice, (*b*) BALB/c mice pretreated with anti-Id (a total of 0.2 ml divided into three equal doses, administered i.p. and s.c. given daily on day -3, -2, and -1); donor spleen cells were removed on day 0 and were not treated

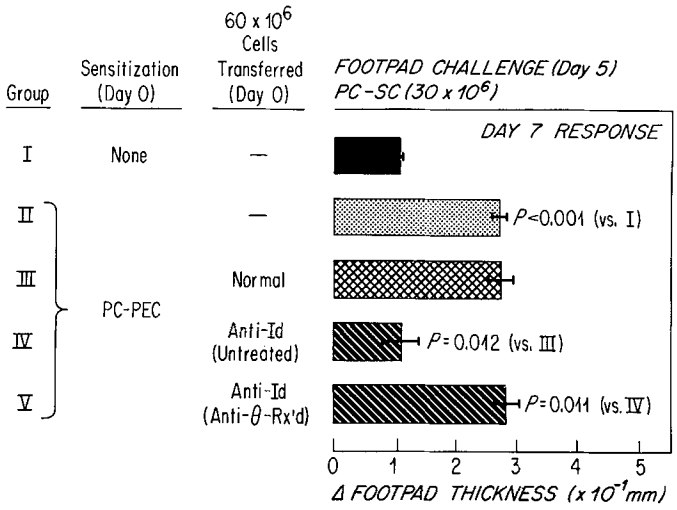

FIG. 7. Suppression of PC-specific DTH responses by passive transfer of T lymphocytes from anti-Id-treated BALB/c donor mice. BALB/c mice were sensitized with 35 × 10^6 PC-derivatized syngeneic PEC subcutaneously into hind trunk area on day 0. On the same day, recipient mice were injected with 60 × 10^6 spleen cells treated with or without anti-θ serum plus complement obtained from either normal or anti-T-15 idiotypic serum-treated (0.2 ml total, on days -3, -2, and -1, i.p. and s.c.) BALB/c mice; the donor spleen cells were either not treated or treated with anti-θ serum + C in vitro before transfer. All animals were challenged on day 5 with 30 × 10^6 PC-coupled syngeneic spleen cells into right footpad. The differences of footpad thickness from right (experimental) to left (control) were measured on day 7.

before transfer, and (*c*) spleen cells from donor mice pretreated with anti-Id (as the others), but which were treated in vitro with anti-θ plus complement (C) before the cell transfer. The fourth group of PC-PEC-sensitized mice received no donor cell transfer. On day 5, these four groups of sensitized mice and an additional group of unsensitized control mice were challenged in the footpads with PC-derivatized SC for elicitation of PC-specific DTH responses.

As shown on the right of Fig. 7, the magnitude of PC-specific DTH reactivities observed 2 d after footpad challenge demonstrate development of very good responses in PC-sensitized mice either not otherwise manipulated (group II) or injected with normal BALB/c donor cells (group III) as compared to the unsensitized control mice (group I). On the other hand, mice sensitized to PC but also receiving the spleen cells from donor mice pretreated with anti-Id (but which were not otherwise treated) displayed complete blunting of PC-specific DTH reactivity (group IV). The capacity of anti-Id-pretreated donor cells to prevent the induction of PC-specific DTH responses was completely abrogated by pretreatment of the donor cells with anti-θ + C before transfer (group V).

SUPPRESSOR T CELLS INDUCED BY ANTI-ID EFFECTIVELY SUPPRESS BOTH THE INDUCTION AND THE EFFECTOR PHASES OF PC-SPECIFIC DTH RESPONSES. In the preceding experiments it was clear that although anti-Id pretreatment was effective in preventing the induction of PC-specific DTH responses (Fig. 3), such treatment had no detectable effect when administered to previously sensitized mice in terms of inhibiting the elicitation phase of such DTH responses (Fig. 4). In the experiment summarized in Fig. 8, we investigated whether the suppressor T lymphocytes induced by anti-Id

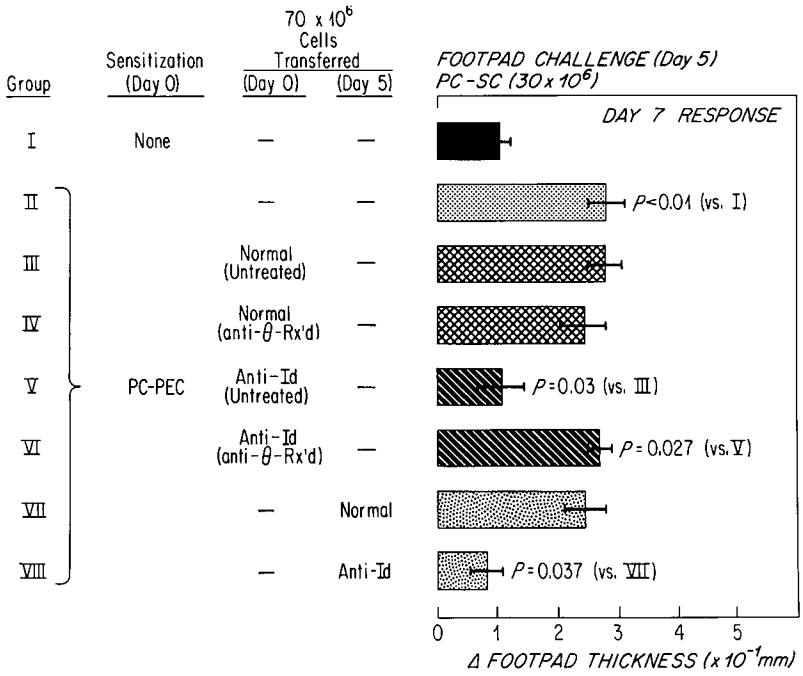

FIG. 8. Suppressor T cells induced by anti-Id effectively suppress both the induction and the effector phases of PC-specific DTH responses. 70 × 10^6 spleen cells obtained from mice which were either not pretreated or pretreated with anti-idiotypic serum 0.2 ml total on day -3, -2 and -1 or days 2, 3, and 4) were inoculated intravenously on day 0 or 5, respectively. Mice of groups V to VIII were sensitized with 40 × 10^6 of PC-derivatized PEC on day 0 and all animals were challenged with 30 × 10^6 PC-SC on day 5. Footpad thicknesses were measured on day 7.

treatment were similarly restricted in their suppressive activity to responses of unsensitized recipients of such cells or whether, alternatively, such cells could likewise ablate the effector phase of DTH responses elicited in previously sensitized mice. The basic protocol employed was similar to that presented in Fig. 7 with the additional aspect that certain groups were sensitized on day 0 and then given donor cells (either normal or anti-Id-pretreated as described above) on the same day as footpad challenge with PC-SC.

Once again (Fig. 8) it is obvious that PC-specific DTH responses could be elicited in BALB/c mice by this sensitization regimen and that such responses were not affected by prior transfer to normal donor cells, either untreated or pretreated with anti-θ + C (groups II–IV versus group I). As in the preceding experiment, passive transfer of spleen cells from anti-Id-pretreated donor mice, not otherwise treated before transfer, on the same day as initial sensitization (day 0) completely prevented induction of PC-specific DTH reactivity (group V), and this suppressive activity was abolished by pretreatment of such donor cells with anti-θ + C before transfer (group VI). When tested for suppressive activity on the effector phase of the response, cells from donor mice pretreated with anti-Id were highly active in this regard (group VIII), an effect clearly related to the anti-Id-pretreatment because donor cells transferred at the same time from normal donor mice had no detectable effects on the elicitation of PC-specific DTH (group VII).

Discussion

In the experiments presented above, we have illustrated the capability of inducing DTH responses specific for the PC hapten in both BALB/c and A/J mice and to the TNP hapten in BALB/c mice by sensitization with either PC- or TNP-derivatized syngeneic PEC. The PC-specific responses in BALB/c mice could be passively transferred with sensitized lymph node cells, and the capacity of such cells to passively transfer DTH reactivity was abolished by pretreatment of such cells with anti-θ antibodies + C.

The possibility of inducing DTH in mice to simple chemical haptens provides an excellent model for studies on the specificity of T-lymphocyte activities. The system we have employed was made possible by the recent studies of Greene et al. (11) who were the first to demonstrate that syngeneic macrophages coupled with TNP were highly immunogenic in terms of inducing hapten-specific DTH responses in mice. We were particularly interested in using the PC system in this way for purposes of investigating idiotypic determinants on T lymphocytes as well as obtaining insights into idiotype-anti-idiotypic immunoregulatory mechanisms, because the T-15-PC system has been extensively studied in terms of idiotypic determinants in BALB/c mice. As compared to certain other haptens which can easily sensitize mice by means such as skin painting, the PC system posed considerably greater difficulties because usual derivatization with PC requires diazonium coupling under alkaline conditions. This obstacle was overcome when, in preliminary experiments, we found that the derivatization of spleen cells or PEC with diazonium phenyl-phosphorylcholine could be successfully achieved at weak alkaline conditions (pH 8.2) with retention of good viability of the derivatized lymphoid cells. This allowed us to establish the basic experimental system described here for the induction of PC-specific DTH, although in the course of our studies, Bach et al. (12) reported the induction of azobenzenearsonate-specific DTH responses using similar procedures.

In recent years, a variety of different experimental approaches have been used to investigate the biological effects of anti-idiotypic antibodies on various aspects of immune reactivity. From the seminal and early work from investigators such as Nisonoff (13, 14), Cosenza, Kohler and colleagues (4, 15), and Cazenave (16), most of which was directed at the effects of anti-idiotypic antibodies on B-lymphocyte activities, have arisen a growing list of investigations of such anti-idiotypic antibody effects on specific T-lymphocyte functions (2, 5, 6, 17–20). Having established the system for induction of PC-specific DTH responses, and confirmed the hapten-specific nature of the response, we then turned to the question concerning the effects of anti-T-15 idiotypic antibodies on such responses. As shown herein, using an A/J anti-T-15 antiserum, the specificity characteristics of which are described in detail elsewhere,[2] we were successful in virtually abolishing the induction of PC-specific DTH responses in BALB/c mice. Suppression of the induction phase of DTH responses by treatment with an anti-Id satisfied all criteria of antigen (Fig. 5) and strain (Fig. 6) specificity.

Regarding the mechanism of successful suppression by anti-Id antiserum in these studies, Eichmann (18) was the first to demonstrate the capacity of anti-idiotypic antibodies to induce antigen-specific, idiotypically-related suppressor T cells capable

[2] Yamamoto, H. and D. H. Katz. 1979. Biological effects of anti-idiotypic antibodies on lymphocyte function. I. Analysis of the effects on B lymphocytes of combining site and framework-directed anti-T-15 idiotypic antibodies. *Cell. Immunol.* In press.

of inhibiting antibody production in mice. Similarly, Nisonoff and colleagues (19) and Bottomly et al. (6) have reported evidence implicating idiotype-specific suppressor T cells in inhibitory effects on antibody responses. Very recently, Greene and colleagues (21, 22), have demonstrated the capacity to elicit azobenzenearsonate (ABA)-specific suppressor T cells and soluble factors derived from such cells which apparently share identical dominant idiotypic determinants with those known to exist on ABA-specific antibody molecules in A/J mice; their work on the soluble suppressor factor provides strong evidence for the association of a *V*-gene product with an *I*-region gene product capable of binding antigen specifically and exerting a biological effect.

The results presented herein in Figs. 7 and 8 document the capacity of anti-Id to induce idiotype-specific suppressor T cells that are effective in negatively regulating PC-specific DTH responses. These suppressor T cells are capable of suppressing both the induction and effector phases of DTH reactions. The results in Fig. 4 revealed that the ability of anti-Id treatment to inhibit PC-specific DTH was limited to the induction phase of the response. The finding in Fig. 8 that suppressor T cells generated by anti-Id treatment are capable of inhibiting also the effector phase of the response does not contradict the results in Fig. 4, but rather indicates that several days are required for generation of Id-specific suppressor T cells after treatment with anti-Id serum.

It is worth noting that the capacity of idiotype-specific suppressor T cells to inhibit primed as well as unprimed effector T cells in the DTH system contrasts with the known predilection of anti-Id antibody inhibitory activities for unprimed, as opposed to primed, idiotype-bearing *B* lymphocytes as pointed out by Pierce and Klinman (23) and Owen and Nisonoff (24). In the case of the B cell, such findings imply that idiotypic receptor specificities might undergo rapid selection in terms of minor idiotypes after proper immunostimulation by the relevant haptenic determinant (25) either alone or perhaps in conjunction with cell interaction (CI) molecules encoded by major histocompatibility complex genes. The present contrasting findings may indicate that T cells mediating DTH responses fail to undergo similarly rapid selection in idiotypic receptor specificities.

Thus, idiotype-specific reactivities, both from a regulatory and inductive point of view, have been demonstrated at virtually all aspects of B- and T-lymphocyte biology. Heretofore, almost the sole missing link to complete the picture was the direct demonstration of such idiotypic control of T cell-mediated DTH responses. The experiments described in this paper provide the first demonstration of such effects in mice and therefore close this isolated gap in our knowledge.

Summary

Delayed-type hypersensitivity (DTH) responses specific for the phosphorylcholine (PC) hapten were induced in BALB/c mice by immunization with syngeneic peritoneal exudate cells (PEC) coupled with diazotized phenyl-phosphoryl-choline. PC-specific DTH responses were elicited in such immunized mice after footpad challenge with PC-derivatized syngeneic spleen cells. Moreover, PC-immune lymph node cells could passively transfer PC-specific DTH responses to naive BALB/c mice and it was possible to demonstrate that the cells responsible for such passively transferred responses were T lymphocytes. Because the T-15 idiotypic determinant displayed on

the TEPC-15 PC-binding myeloma protein is known to be a dominant idiotype associated with anti-PC antibody responses in BALB/c mice, an analysis was made of the effects of anti-T-15 idiotypic antibodies on the induction and expression of murine PC-specific DTH responses. Repeated injections of anti-T-15 idiotypic antiserum, raised in A/J mice by immunization with TEPC-15 myeloma protein, into recipient BALB/c mice both immediately before and after sensitization with PC-PEC virtually abolished the development of PC-specific DTH responses. Although administration of anti-T-15 antiserum effectively inhibited the induction phase of PC-specific DTH responses, these anti-idiotypic antibodies had no suppressive activity at the effector phase of these responses. The inhibition observed with anti-T-15 antibodies was highly specific for the PC hapten, and for PC-specific DTH responses of BALB/c but not A/J mice. Studies were conducted to address the possibility that anti-Id treatment induced suppressor T lymphocytes capable of specifically inhibiting the activity of PC-specific T cells participating in DTH responses. The results demonstrate that idiotype-specific suppressor T cells are, indeed, induced by treatment with anti-Id; moreover, such suppressor T cells, once induced, are highly effective in abrogating both the induction and the effector phases of PC-specific T cell-mediated DTH responses in BALB/c mice.

We express our best appreciation to our colleagues in our laboratory who gave us continuous support and help during this work, especially to Dr. Fu-Tong Liu who kindly synthesized diazonium phenyl-phosphorylcholine and gave invaluable discussions. We thank Anthea Hugus and Cherie Dichter for assistance in preparation of the manuscript and Dr. Norman Klinman who critically commented in a most helpful way on the work and the manuscript.

Received for publication 23 April 1979. Additional data received on 13 July 1979.

References

1. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris)* **125C:**373.
2. Binz, H., and H. Wigzell. 1976. Antigen-binding, idiotypic receptors from T lymphocytes: an analysis of their biochemistry, genetics, and use as immunogens to produce specific immune tolerance. *Cold Spring Harbor Symp. Quant. Biol.* **41:**275.
3. Krawinkel, U., M. Cramer, C. Berek, G. Hammerling, S. J. Black, K. Rajewsky, and K. Eichmann. 1976. On the structure of the T-cell receptor for antigen. *Cold Spring Harbor Symp. Quant. Biol.* **41:**285.
4. Cosenza, H., and H. Köhler. 1972. Specific inhibition of plaque formation to phosphorylcholine by antibody against antibody. *Science (Wash. D. C.).* **176:**1027.
5. Cosenza, H., A. A. Augustin, and M. H. Julius. 1976. Idiotypes and anti-idiotypes as probes in analysis of immunoregulation. *Cold Spring Harbor Symp. Quant. Biol.* **41:**709.
6. Bottomly, K., B. J. Mathieson, and D. E. Mosier. 1978. Anti-idiotype induced regulation of helper cell function for the response to phosphorylcholine in adult BALB/c mice. *J. Exp. Med.* **148:**1216.
7. Chesebro, B., and H. Metzger. 1972. Affinity labeling of a phosphorylcholine binding mouse myeloma protein. *Biochemistry.* **5:**766.
8. Katz, D. H., and D. P. Osborne, Jr. 1972. The allogeneic effect in inbred mice. II. Establishment of antibody production by the graft-versus-host reaction. *J. Exp. Med.* **136:** 455.
9. Potter, M., and R. Lieberman. 1970. Common individual antigenic determinants in five of eight BALB/c IgA myeloma proteins that bind phosphorylcholine. *J. Exp. Med.* **132:**737.

10. Lieberman, R., M. Potter, E. B. Mushinski, W. Humphrey, Jr., and S. Rudikoff. 1974. Genetics of a new IgVH (T-15 idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. *J. Exp. Med.* **139:**983.
11. Greene, M. I., M. Sugimoto, and B. Benacerraf. 1978. Mechanism of regulation of cell-mediated immune responses. I. Effect of the route of immunization with TNP-coupled syngeneic cells on the induction and suppression of contact sensitivity to picryl chloride. *J. Immunol.* **120:**1604.
12. Bach, B. A., L. Sherman, B. Benacerraf, and M. I. Greene. 1978. Mechanisms of regulation of cell-mediated immunity. II. Induction and suppression of delayed-type hypersensitivity to azobenzenearsonate-coupled syngeneic cells. *J. Immunol.* **121:**1460.
13. Hart, D. A., A. L. Wang, L. L. Pawlak, and A. Nisonoff. 1972. Suppression of idiotype specificities in adult mice by administration of antiidiotypic antibody. *J. Exp. Med.* **135:** 1293.
14. Pawlak, L. L., D. A. Hart, and A. Nisonoff. 1973. Suppression of immunological memory for a crossreactive idiotype in adult mice. *Eur. J. Immunol.* **4:**10.
15. Strayer, D. S., H. Cosenza, W. Lee, D. A. Rowley, and H. Kohler. 1974. Neonatal tolerance induced by antibody against antigen specific receptors. *Science (Wash. D. C.).* **186:**640.
16. Cazenave, P. A. 1977. Idiotypic anti-idiotypic regulation of antibody synthesis in rabbits. *Proc. Natl. Acad. Sci. U. S. A.* **74:**5122.
17. McKearn, T. J., F. P. Stuart, and F. W. Fitch. 1974. Anti-idiotypic antibody in rat transplantation immunity. I. Production of anti-idiotypic antibody in animals repeatedly immunized with alloantigens. *J. Immunol.* **113:**1876.
18. Eichmann, K. 1976. Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. *Eur. J. Immunol.* **5:**511.
19. Ju, S.-T., F. L. Owen, and A. Nisonoff. 1976. Structure and immunosuppression of a cross-reactive idiotype associated with anti-*p*-azophenylarsonate antibodies of strain-A mice. *Cold Spring Harbor Symp. Quant. Biol.* **41:**699.
20. Bona, C., and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. I. T suppressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. *J. Exp. Med.* **149:**592.
21. Greene, M. I., B. A. Bach, and B. Benacerraf. 1979. Mechanisms of regulation of a cell-mediated immunity. III. The characterization of azobenzene arsonate-specific suppressor T-cell-derived suppressor factors. *J. Exp. Med.* **149:**1069.
22. Bach, B. A., M. I. Greene, B. Benacerraf, and A. Nisonoff. 1979. Mechanisms of regulation of cell-mediated immunity. IV. Azobenzene arsonate-specific suppressor factor(s) bear cross-reactive idiotypic determinants the expression of which is linked to the heavy chain allotype linkage group of genes. *J. Exp. Med.* **149:**1084.
23. Pierce, S. K., and N. R. Klinman. 1977. Antibody-specific immunoregulation. *J. Exp. Med.* **146:**509.
24. Owen, F. L., and A. Nisonoff. 1978. Effect of idiotype-specific suppressor T cells on primary and secondary responses. *J. Exp. Med.* **148:**182.
25. Mäkelä, O., and K. Karjalainen. 1977. Inherited immunoglobulin idiotypes of the mouse. *Immunological Rev.* **34:**119.