

ANTIIDIOTYPES AGAINST  
ANTI-H-2 MONOCLONAL ANTIBODIES

V. In Vivo Antiidiotypic Treatment Induces  
Idiotypic-specific Helper T Cells

BY HUGH AUCHINCLOSS, JR., JEFFREY A. BLUESTONE, AND  
DAVID H. SACHS

*From the Transplantation Biology Section, Immunology Branch, National Cancer Institute,  
National Institutes of Health, Bethesda, Maryland 20205*

The idiotype network hypothesis proposed by Jerne (1) has led to considerable interest in whether physiologic immunoregulation is accomplished by the interaction of receptor molecules and anti-receptors directed at unique idiotypic determinants. Another issue of importance, related to mechanistic questions, is whether exogenously administered anti-receptor antibodies might be used to manipulate immune responses. Because of the particular importance of major histocompatibility complex (MHC)<sup>1</sup> antigens in both transplantation and normal immunity, we have undertaken studies to determine whether antiidiotypic (anti-Id) reagents might be used to modify immune responses to these antigens.

In the past, we have been unable to prepare anti-Id reagents against heterogeneous populations of anti-H-2 antibodies presumably because no single species of antibody was present in sufficient quantity to elicit a detectable anti-Id response. This problem has recently been overcome by using monoclonal anti-H-2 antibodies as the source of receptor material, and large quantities of xenogeneic anti-Id reagents have been generated by this means (2). However, again because of the heterogeneity of the anti-H-2 responses, the question arises whether anti-Id generated against monoclonal antibodies would have any effect on in vivo anti-H-2 humoral responses. We have recently reported (2, 3) the results of several studies demonstrating that treatment of mice in vivo with xenogeneic anti-Id induced the expression of molecules (Id') that share some of the idiotopes with the original Id but may or may not bind H-2 antigens. In addition, mice treated with xenogeneic anti-Id and subsequently grafted with skin bearing the original MHC antigen developed a significantly higher percentage of Id-positive alloantibodies than were detected in the alloantisera of untreated animals (3). These results led to the conclusion that treatment with anti-Id against monoclonal anti-H-2 antibodies can significantly alter B cell immune responses to MHC antigens (3-5).

The present studies were undertaken in an attempt to determine the mechanism by

<sup>1</sup> *Abbreviations used in this paper:* anti-Id, antiidiotypic; CFA, complete Freund's adjuvant; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; FMF, flow microfluorometry; Id', anti-Id-induced molecules sharing idiotopes with the original Id; MHC, major histocompatibility complex; PBS, phosphate-buffered saline.

which treatment with anti-Id against B cell products leads to an alteration of the expressed B cell repertoire. To this end we have asked whether T cells from anti-Id-treated mice play a role in the selection of the idiotopes expressed by B cells. The results of these studies indicate that T cells can be found in the spleens of anti-Id-treated animals which are capable of altering the B cell idiotypic repertoire in adoptively transferred hosts. These data provide evidence that T cell functions are also affected by administration of anti-Id against anti-MHC antibodies and suggest that such T cell functions are involved in the observed alterations of repertoire expression.

### Materials and Methods

*Animals.* BALB/cAnN and BALB/cAnN *nu/nu* mice were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). In several experiments, nude mice were screened with strain-restricted typing sera (6) to assure genetic homogeneity in the strain. BALB.K and C.B20 mice were produced from stock kindly provided by Dr. Michael Potter (National Institutes of Health, Bethesda, MD). BAB.14 mice were provided by Dr. Martin Weigert (Institute for Cancer Research, Philadelphia, PA) and subsequently bred in our own colonies. Other mice were obtained from The Jackson Laboratory (Bar Harbor, ME) or were bred in our own colonies.

*Monoclonal Antibodies.* Clone 11-4.1 (IgG<sub>2a,k</sub>) was raised in a CKB-immunized BALB/c mouse by Oi et al. (7) and obtained through The Salk Institute (San Diego, CA). The antibodies of this clone are specific for the K<sup>k</sup> antigen with cross-reactivities against K<sup>d</sup> and the p and r haplotypes. The myeloma LPC-1 (IgG<sub>2a,k</sub>), which possesses no known binding specificity, was obtained from Dr. Michael Potter and produced as an ascites fluid in BALB/c mice.

*Production and Purification of Anti-Id.* Xenogeneic anti-Id were prepared in both miniature swine and rabbits as described in detail previously (2, 3). Briefly, pigs were immunized repeatedly at approximately monthly intervals with 200  $\mu$ g intramuscularly (rabbits with 100  $\mu$ g) of protein A-purified 11-4.1 antibodies in complete Freund's adjuvant (CFA). Anti-mouse immunoglobulin (Ig) levels were monitored by passive hemagglutination of sheep erythrocytes coated with LPC-1 Ig. Animals were exsanguinated after anti-Ig antibody levels had plateaued. Sera were absorbed with LPC-1 coupled to Sepharose 4B (8) until all detectable anti-normal mouse Ig activity had been removed. Anti-Id antibodies were then absorbed to and eluted from 11-4.1 coupled columns using 4 M guanidine-HCl, pH 7.0, as the eluant. Antibodies were dialysed into phosphate-buffered saline (PBS) and stored at  $-20^{\circ}\text{C}$ .

*In Vivo Treatment of Mice.* Mice were treated with 20–50  $\mu$ g of anti-Id intraperitoneally in saline on days 0 and 3. Id' antibodies were detectable within 2 wk and for at least 6 mo thereafter. Skin grafting was performed using tail skin of BALB.K donors (3).

*Enzyme-linked Immunosorbent (ELISA) Inhibition Assay for Detection of Id'.* Micro-ELISA plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 200  $\mu$ l of protein A-purified 11-4.1 antibodies (0.5–2  $\mu$ g/ml) in PBS at  $4^{\circ}\text{C}$  for at least 18 h before each assay. PBS-Tween (0.05% Tween 20, P-1379; Sigma Chemical Co., St. Louis, MO) was used as the diluent for subsequent steps and all incubations were for 1 h at room temperature. Plates were washed three times between steps.

Inhibitors were serially diluted in 25- $\mu$ l volumes in round-bottomed microtiter wells and then incubated with 25  $\mu$ l of normal pig or rabbit Ig (2 mg/ml), which have been shown to swamp potential anti-xenogeneic constant region antibodies. Simultaneously, xenogeneic anti-Id was incubated with normal mouse serum to minimize nonspecific inhibition. The concentration of anti-Id was chosen from titration curves such that the quantity ( $\sim 1$   $\mu$ g) of antibodies that bound detectably to the Id-coated plates was used. Anti-Id was incubated with inhibitors or controls for 1 h, after which the contents of each well were transferred to the 11-4.1-coated ELISA plates. Binding of xenogeneic anti-Id was detected 1 h later with either horseradish peroxidase-coupled goat anti-rabbit IgG (N. L. Cappel Laboratories, Cochranville, PA) or peroxidase-coupled goat anti-pig IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), depending on which species of anti-Id had been used. Plates were finally developed with

*o*-phenylene diamine substrate (P2,393-8; Aldrich Chemical Co., Milwaukee, WI) and the reaction was stopped with 8 N H<sub>2</sub>SO<sub>4</sub>. Absorbance was read at 492 nm in a Titertek multiscan spectrophotometer (Flow Laboratories, Inc., McLean, VA). Percent inhibition was calculated according to the formula:

$$\text{percent inhibition} = \frac{\text{OD}_{492}(\text{uninhibited}) - \text{OD}_{492}(\text{test sample})}{\text{OD}_{492}(\text{uninhibited})} \times 100.$$

For each plate, the multiscan reader was blanked on the absorbance produced by the background binding of peroxidase-coupled goat antibodies to Id-coated plates when no xenogeneic anti-Id had been added. Control samples tested in each assay to assess the validity of the assay included the purified Id used to coat the plate, an irrelevant monoclonal Ig, sera from mice treated with irrelevant anti-Id or normal immunoglobulin of the same species, and normal mouse serum. Three- or four-point titrations were performed for each test sample, although in most cases we report only the inhibition obtained with a 1:2 dilution of 25  $\mu$ l of test sera.

*Assessment of Anti-H-2 Antibodies.* Anti-H-2 antibodies were detected as described previously by both complement-mediated cytotoxicity (9) and by flow microfluorometry (FMF) using the fluorescence-activated cell sorter (FACS) (2, 3). For analysis by the FACS, aliquots of 10<sup>6</sup> B10.A lymph node cells were washed in Hanks' balanced salt solution containing 0.1% bovine serum albumin and 0.1% Na azide (staining medium). Cells were pelleted (440 g, 5 min) and resuspended in 25  $\mu$ l of test serum from individual mice for 45 min at 4°C, washed twice, and resuspended in 25  $\mu$ l of a mixture of fluorescein isothiocyanate (FITC)-goat F(ab)<sub>2</sub> anti-mouse IgG<sub>2</sub> and FITC-F(ab)<sub>2</sub> anti-mouse IgG<sub>1</sub> at a final dilution of 1:64 of each antibody. The suspension was incubated for an additional 30 min at 4°C, washed twice, and resuspended in 0.4 ml of staining medium for analysis by FMF (FACS II; B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA) as previously described (10).

Expression of idiotopes on anti-H-2K<sup>k</sup> antibodies was assessed by an antigen-binding inhibition assay. 25  $\mu$ l of serum was mixed with 25  $\mu$ l of either anti-Id (anti-11-4.1, 250  $\mu$ g/ml) or an irrelevant anti-Id or normal Ig. After a 1-h preincubation of the serum and the inhibitors, binding to cells bearing H-2K<sup>k</sup> antigens was assessed by FMF as described above. Percent inhibition was calculated according to the formula:

$$\text{percent inhibition} = \left[ 1 - \frac{\text{mean fluorescence of cells with test sera + anti-Id}}{\text{mean fluorescence of cells with test sera + control Ig}} \right] \times 100.$$

Such a calculation gives a minimal representation of Id-positive anti-K<sup>k</sup> antibodies since anti-Ia<sup>k</sup> antibody binding that would not be expected to bear 11-4.1 idiotopes may also be present.

#### *Adoptive Transfer of T Cells*

**PREPARATION OF T CELLS.** T cell purification was performed using a modification of the method of Binz and Wigzell (11). Glass bead columns (Ferro Corporation, Cataphote Division, Jackson, MI) (8–12 ml of packed beads) were washed with PBS, incubated for 2 h with normal mouse serum, washed, then coated with pig anti-mouse Ig by overnight incubation, and washed again. Lymph node and spleen cell suspensions were prepared in Medium 199 (Gibco Laboratories, Gibco Div., Lawrence, MA), erythrocytes were lysed with a hypotonic buffer, and up to 200  $\times$  10<sup>6</sup> cells were added in 2–3 ml to each column. After 15 min, nonadherent cells were collected by washing with medium 199. Cells were washed, counted, and injected intravenously in 1 ml/mouse. Cell yields from columns were 20–30% and Ig<sup>+</sup> cell contamination was ~5% as assessed by indirect fluorescence using fluorescence microscopy.

**NUDE TRANSFERS.** 6–8-wk-old nude animals were injected with T cells as indicated and then treated with anti-Id on the days shown. Thymus transplants into nudes were performed using whole thymuses from 1–2-d-old newborn mice placed in subcutaneous pockets on the flank. These mice were treated with anti-Id at least 2 mo after transplantation, at which time they were able to reject foreign skin grafts.

**BALB/C TRANSFERS.** Naive BALB/c mice were irradiated with 150–200 rad gamma irra-

diation (cesium). T cells were injected within several hours. Mice were skin-grafted on the following day and sera were analyzed for alloantibodies several weeks later. Some mice received subsequent immunization with spleen cells from the same strain as the initial skin graft to boost anti-H-2 antibody activity.

*Chimera Production.* Allogeneic chimeras were produced as described previously (12). Recipient mice were irradiated with 950 rad cesium and injected several hours later with  $10\text{--}20 \times 10^6$  donor bone marrow cells treated with rabbit anti-mouse brain antiserum plus complement.

## Results

*Anti-Id Treatment Alters B Cell Immune Responses.* The system used for the present studies involved anti-Id prepared against the anti-H-2K<sup>k</sup> monoclonal antibody 11-4.1. Table I summarizes results from previously published studies (2, 13, 14) in which BALB/c mice were treated in vivo with pig or rabbit anti-11-4.1. First, mice treated with 20–30  $\mu\text{g}$  of anti-Id in saline on days 0 and 3 produced serum antibodies several weeks later that were capable of inhibiting the binding of anti-Id to idiotypic-coated ELISA plates. This inhibition has been shown to result from the expression of idiotypic-bearing molecules which we have called Id'. Second, 10 of 45 (22%) BALB/c mice treated with anti-Id developed anti-K<sup>k</sup> antigen-binding activity even though they had never been exposed to the K<sup>k</sup> antigen. These antibodies were idiotypic-positive, as indicated by the ability of anti-Id to inhibit their binding to the K<sup>k</sup> antigen. Third, 13 of 27 (48%) animals treated with anti-Id and subsequently grafted with K<sup>k</sup> antigen-bearing skin expressed a significantly higher percentage (>30%) of idiotypic-positive anti-K<sup>k</sup> antibodies among their alloantibodies than was ever found in conventional BALB/c anti-K<sup>k</sup> alloantisera. Finally, Fig. 1 illustrates that the ability to express Id' antibodies after anti-Id treatment is influenced by V<sub>H</sub> region genes.

TABLE I

*Effects of In Vivo Xenogenic Anti-Id Treatment on B Cell Immune Responses in the 11-4.1 System\**

	Expression of Id' antibodies‡ (percent inhibition of anti-Id binding)	Expression of Anti-K <sup>k</sup> antibodies§ (percent of animals with anti-K <sup>k</sup> antibodies)	Expression of Id' anti-H-2 antibodies   (percent of anti-K <sup>k</sup> antibodies inhibited by anti-Id)
Sera from normal mice	0–19	0	—
Sera from mice treated with anti-Id	56–92	22	100
Sera from mice grafted with H-2K <sup>k</sup> skin	0–19	100	0–22
Sera from mice treated with anti-Id and grafted with H-2K <sup>k</sup> skin	62–90	100	>30% (in 48% of mice)

\* BALB/c mice were treated with 30  $\mu\text{g}$  of either pig or rabbit anti-Id in saline on days 0 and 3. Sera from individual animals were assayed after 3 wk.

‡ Data are expressed as the range of percent inhibition of anti-Id binding to Id-coated ELISA plates for individual animals.

§ Data represent the percent of animals with antibodies binding to K<sup>k</sup>-bearing lymph node targets when assayed by the FACS. Sera from 45 individual anti-Id-treated animals were tested for antigen-binding activity.

|| Data represent the range of percent inhibition of anti-K<sup>k</sup> binding by anti-Id. Sera from 27 individual anti-Id-treated and skin-grafted mice were tested.

MICE TREATED WITH ANTI-IDIOTYPE	C <sub>H</sub>	V <sub>H</sub>	NUMBER OF MICE		ID' INDUCTION: % INHIBITION OF ANTI-IDIOTYPE BINDING				
					0	25	50	75	100
BALB/c	a	a	4	(68 ± 3)	[Bar chart showing inhibition levels for BALB/c mice]				
C.B20	b	b	5	(19 ± 2)	[Bar chart showing inhibition levels for C.B20 mice]				
BAB. 14	b	a	5	(62 ± 4)	[Bar chart showing inhibition levels for BAB. 14 mice]				
					(N M S)				

FIG. 1. V<sub>H</sub> region genes influence the Id' response to anti-Id treatment. As described in Materials and Methods, mice were treated with 50 µg of pig anti-Id in saline on days 0 and 3. 3 wk later, sera from individual mice were tested for their ability to inhibit the binding of rabbit anti-Id to Id-coated ELISA plates. The inhibition obtained with normal mouse serum (NMS) is also indicated. Data are presented as the mean inhibition for each group ± SEM.

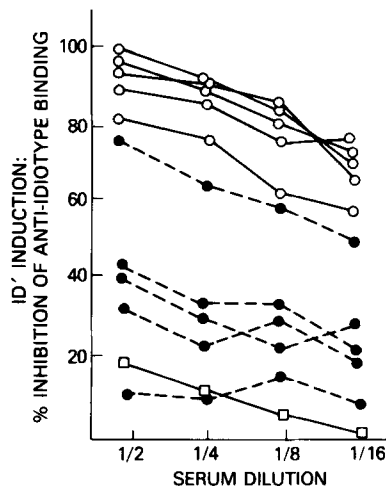


FIG. 2. Nude mice produce fewer Id' antibodies after anti-Id treatment than normal BALB/c animals. Individual BALB/c (○) or BALB/c nu/nu animals (●) were treated with 50 µg of pig anti-Id twice and their sera subsequently assayed for the ability to inhibit pig anti-Id ELISA binding. The inhibition obtained with normal serum (□) is also shown.

Sera from allotype-congenic C.B20 mice (Igh<sup>b</sup>) treated with pig anti-Id inhibited the binding of anti-Id less well than sera from mice with V<sub>H</sub> genes of the Igh-V<sup>a</sup> haplotype. In addition, BAB.14 mice that express the Igh<sup>a</sup> variable region genes but the Igh<sup>b</sup> constant region genes expresses levels of Id' equal to the BALB/c mice. It should be noted that C.B20 mice were able to express some Id' molecules, because in three experiments the percent inhibition obtained by sera of treated C.B20 mice was 29 ± 2 compared with 13 ± 3 obtained with sera from normal mice.

*A Portion of Id' Induction Is T Cell Dependent.* The initial analyses to determine the possible role of T cells in the phenomenon induced by anti-Id treatment were performed using nude mice. Fig. 2 shows the results of a typical experiment in which BALB/c or BALB/c nu/nu mice were treated with pig anti-Id. 3 wk after treatment, sera from individual mice from both groups were assayed for expression of Id' antibodies. The average inhibition was significantly greater in the euthymic BALB/c group than in the athymic BALB/c nude group. Thus, a major portion of

the Id' response to anti-Id treatment appeared to be T cell dependent. The data in Fig. 2 also suggest that some individual nude mice treated with anti-Id responded with nearly normal levels of Id' expression. 1 of the 5 littermates in this group and about 1 in 10 nude animals in a larger number of experiments showed normal responses. Thus, it may be that the expression of some idiotopes can be T cell independent.

To further test the role of T cells in Id' induction, the effect of anti-Id treatment was analyzed in nude mice reconstituted by either adoptive transfer of cells or thymus transplants. Fig. 3 shows the results of several adoptive transfer experiments in which T cells were transferred into BALB/c nude mice, which were then treated with pig anti-Id. The Id' response of mice reconstituted with either BALB/c or C.B20 T cells was significantly greater than that of the untreated nude mice. Further analysis (data not shown) revealed that T cells from anti-Id-treated mice were no better in their ability to reconstitute nudes than unprimed T cells. Experiments in which several T cell doses were used suggested that as few as  $10^5$  T cells/mouse were sufficient to reconstitute the Id' response of nude animals (data not shown). Fig. 4 shows the results of an experiment in which adult nude animals were reconstituted with neonatal C.B20 or BALB/c thymus transplants. Such mice treated 2 mo later with anti-Id produced almost normal levels of Id' antibodies. These experiments thus suggest that a major portion of the Id' response to anti-Id treatment is T cell dependent.

*Igh-V Region Genes Expressed at the B Cell Level Control Id' Induction.* The results above indicated that T cells were involved in the production of idiotope-bearing molecules after anti-Id treatment. The next set of experiments was designed to test whether either  $V_H$ -related genes expressed by the T cells or the environment in which they

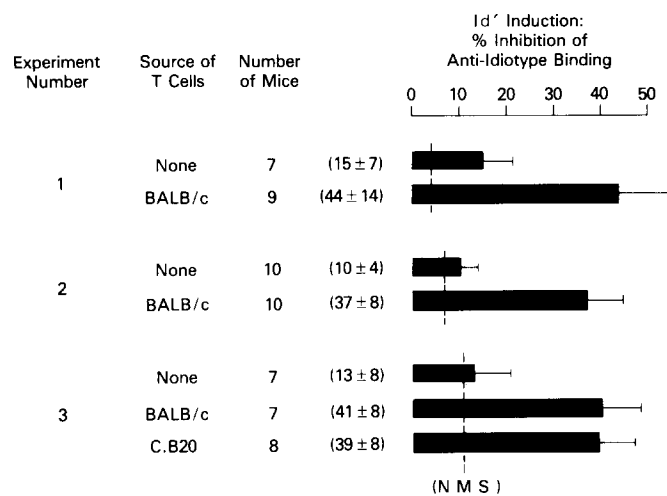


FIG. 3. Adoptive transfer of T cells reconstitutes Id' expression in nude mice. T cells were purified from lymph nodes and spleens of donors as described in the Materials and Methods. Data are presented as in Fig. 1. Experiment 1:  $4 \times 10^5$  unprimed T cells were transferred on day 0, mice were treated with  $30 \mu\text{g}$  of pig anti-Id on days 0 and 3, and inhibition of rabbit anti-Id was tested 2 wk later. Experiment 2:  $5 \times 10^5$  unprimed T cells were transferred on day 0, mice were treated with  $30 \mu\text{g}$  of pig anti-Id on days 0 and 3, and inhibition was tested 10 d later with rabbit anti-Id. Experiment 3:  $5 \times 10^6$  T cells from mice that had been treated previously with pig anti-Id were transferred on day 0, mice were treated with  $30 \mu\text{g}$  rabbit anti-Id on days 1 and 4, and inhibition of pig anti-Id was tested 2 wk later.

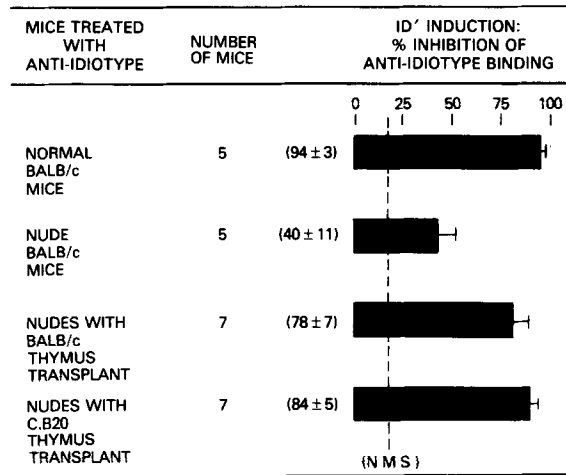


FIG. 4. Thymus transplants reconstitute the ability of nude mice to respond to anti-Id treatment. At least 2 mo after subcutaneous thymus transplantation, mice were treated with 50 µg of pig anti-Id on days 0 and 3 and tested for inhibition of pig anti-Id. Data are presented as in Fig. 1.

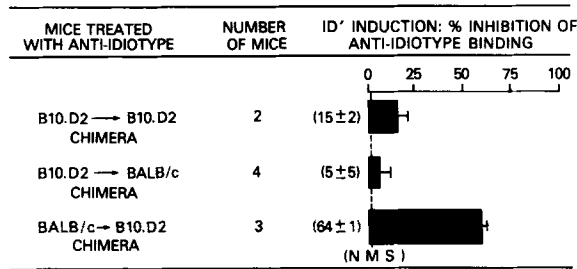


FIG. 5. The environment in which cells mature does not alter the B cell V<sub>H</sub> region genetic control of the Id' response. Chimeras were treated with two doses of 50 µg of pig anti-Id several months after irradiation and bone marrow reconstitution. Sera were tested for inhibition by rabbit anti-Id 3 wk later. Data are presented as in Fig. 1.

matured would affect the ability of B cells to express Id' antibodies. The results of the third experiment shown in Fig. 3 indicate that C.B20 T cells adoptively transferred into nude mice were able to reconstitute the ability of BALB/c B cells to respond to anti-Id treatment even though C.B20 mice produced significantly fewer Id' antibodies than did BALB/c mice in response to direct treatment with anti-Id (3). In addition, as shown in Fig. 4, the ability of nude BALB/c mice to produce Id' antibodies was reconstituted equally well with neonatal C.B20 or BALB/c thymuses. Thus, there did not appear to be any effect of Igh-V region genes at the T cell level or any effect of the Igh thymic maturation environment on T cell ability to reconstitute B cell responses to anti-Id. These experiments were extended further by altering the environment in which not only the T cells but also the B cells matured. A series of allogeneic bone marrow chimeras was produced between mice of different allotypes. As shown in Fig. 5, BALB/c T and B cells that matured in a B10.D2 environment were still capable of producing Id' antibodies, whereas B10.D2 cells produced fewer Id' molecules regardless of the environment in which they matured. Together, these results suggest that V<sub>H</sub> region genes do not control Id' induction at the level of T cells

and are independent of the non-H-2 environment in which either the T or the B cells matured.

*An Id-specific T Cell Is Involved in the Expression of Idiotoxes by B Cells.* At this point, the data suggested that T cells were involved in the expression of the 11-4.1 idiotoxes by B cells. However, a series of experiments in which T cells treated with either Id or anti-Id plus complement were adoptively transferred into nude mice failed to provide evidence for the Id specificity of these T cells (data not shown) and suggested that much of the Id' response by B cells might depend on carrier-specific (15) rather than on Id-specific helper T cells.

The involvement of carrier-specific T cells in the B cell expression of Id' would not eliminate the possibility that additional Id-specific T cells might be induced by anti-Id treatment. Therefore, experiments were designed to examine Id-specific rather than carrier-specific T cells by making use of the previously reported observation that anti-Id-primed BALB/c mice subsequently exposed to  $K^k$  antigens expressed idiotope-positive anti- $K^k$  antibodies, whereas normal BALB/c mice rarely expressed such idiotope-positive antibodies (3). T cells from anti-Id-primed animals were adoptively transferred into lightly irradiated BALB/c mice which were then grafted with  $K^k$  antigen-bearing skin. The anti- $K^k$  antibodies produced by these host animals were tested for the expression of 11-4.1 idiotoxes. Under these conditions, carrier-primed T cells specific for xenogenic determinants of the anti-Id antibodies would not be stimulated by the subsequent H-2 antigen challenge and only Id-specific T cells should be capable of altering the Id of the B cell response.

Adoptive transfer experiments of this type were performed many times in the 11-4.1 system and the data from all experiments are summarized in Fig. 6. As indicated, lightly irradiated BALB/c mice given T cells from mice that had been primed with either normal pig Ig or an irrelevant pig anti-Id (control T cells) only rarely expressed detectable idiotope-positive anti- $K^k$  antibodies after immunization with the  $K^k$  anti-

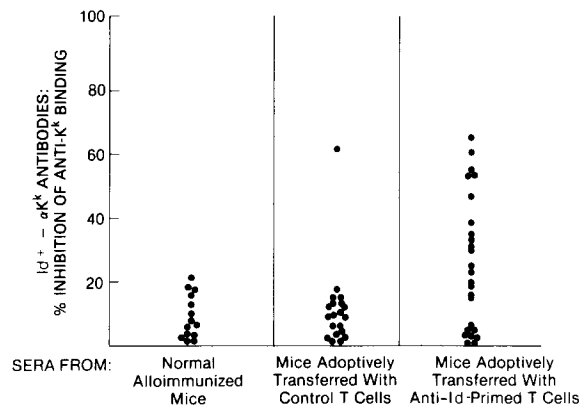


FIG. 6. Adoptive transfer of T cells from anti-Id-treated mice can induce the expression of idiotope-positive anti- $K^k$  antibodies after antigen exposure. BALB/c mice were given 200 rad irradiation and then  $1-5 \times 10^6$  T cells purified from lymph nodes and spleens of donor animals. 1-2 d later mice were skin-grafted with BALB.K tail skin. Subsequent anti- $K^k$  antibodies were tested by FACS analysis for the ability of anti-Id to inhibit their binding to B10.A lymph node targets. Data represent the percent inhibition obtained for antibodies of individual animals. Control T cells came from mice that had previously been treated with either normal pig Ig or an irrelevant pig anti-Id. Primed T cells came from mice that had been treated with pig anti-11-4.1 1-3 mo earlier.



gen. However, BALB/c mice given T cells from animals that had been primed with anti-Id frequently expressed high percentages of idiotope-positive antibodies in their anti-K<sup>k</sup> alloantibody response. Thus, it appeared that T cells from anti-Id-treated mice were able to alter expression of the Id repertoire of naive B cells in their response to the K<sup>k</sup> antigen.

To characterize further the requirements for successful transfer of Id expression, several experiments were performed in which the conditions of adoptive transfer were varied. These analyses indicated that (a) T cells from either anti-Id-primed or anti-Id-primed and K<sup>k</sup> skin-grafted donors could transfer Id expression; (b) T cells from anti-Id-treated mice could transfer Id expression to subsequent hosts whether or not they expressed detectable Id-positive anti-K<sup>k</sup> antibodies; (c) the same pool of T cells injected into several hosts could induce an idiotope-positive B cell response in some animals while failing to do so in others; and (d)  $>10^6$  T cells were apparently necessary to accomplish successful transfer of the expression of Id-positive anti-H-2K<sup>k</sup> antibodies (data not shown). These findings suggested that as many as 10 times more T cells from anti-Id-treated mice were required to demonstrate their Id-specific function than were necessary to reconstitute the ability of anti-Id to induce Id' in BALB/c nude mice.

*T Cells with Igh-V<sup>b</sup> Genes Can Transfer the Expression of Id.* One concern with the experiments described above was that purification of the transferred T cells may not have been sufficient to eliminate contaminating Id-positive anti-K<sup>k</sup> B cells. Such B cells might then have been responsible for the subsequent expression of idiotope-positive anti-K<sup>k</sup> antibodies in the adoptive host. To address this issue, adoptive transfer experiments were performed with T cells from mice of different Igh allotype groups. As shown in Fig. 7A, C.B20 mice (and other mice with Igh-V<sup>b</sup> genes) did not express anti-K<sup>k</sup> antibodies in response to anti-Id treatment. In addition, as shown in Fig. 7B, Igh-V<sup>b</sup> mice did not express idiotope-positive anti-K<sup>k</sup> antibodies when they

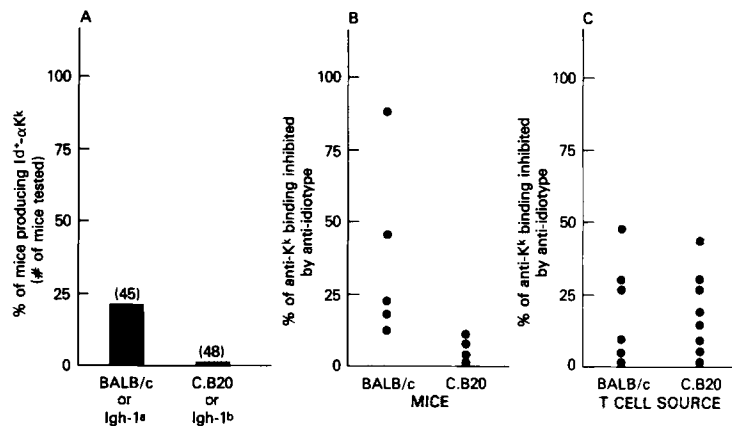


FIG. 7. Although C.B20 B cells cannot express idiotope-positive anti-K<sup>k</sup> antibodies, their T cells can transfer Id expression. Panel A shows the percent of all animals tested by FACS analysis that have expressed anti-K<sup>k</sup> activity after anti-Id treatment alone. Panel B shows the percent inhibition by anti-Id of anti-K<sup>k</sup> antibodies from either BALB/c or C.B20 mice that had been treated with pig anti-Id and subsequently grafted with K<sup>k</sup>-bearing skin. Panel C shows results of an experiment similar to those in Fig. 6 except that T cells from C.B20 mice that had been treated with pig anti-Id were also used for adoptive transfer.

had been first primed with anti-Id and subsequently grafted with  $K^k$  antigen-bearing skin. Nonetheless, the results shown in Fig. 7C indicate that T cells from such C.B20 anti-Id-primed mice were able to transfer the expression of Id into naive BALB/c animals. Because C.B20 cells appear to be unable to express 11-4.1-idiotope-positive anti- $K^k$  antibodies, their contamination of the C.B20 T cell inoculum could not account for the subsequent idiotope-positive antibody response. To further document this point, the allotype of the antibodies in the idiotope-positive alloantisera of adoptive hosts was determined by a hemagglutination inhibition assay capable of detecting 10 ng of contaminating antibody. Only antibodies of the Igh-C<sup>a</sup> allotype were detected in these sera when T cells of Igh-C<sup>b</sup> allotype had been adoptively transferred (data not shown). Thus, it appears that T and not contaminating B cells from anti-Id-primed mice were responsible for the shift in the Id repertoire of B cell antibodies.

### Discussion

The principal goal of the studies reported here was to determine whether Id-specific T cells are involved in the expression of B cell Id. The results indicate that (a) T cells are involved in at least a portion of the Id' response to anti-Id treatment; (b) the ability of anti-Id to induce Id' is influenced by  $V_H$  region genes expressed at the B cell level independent of the environment in which either T or B cells mature; and (c) Id-specific T cells capable of selecting the Id repertoire of the B cells that respond to MHC antigens can be detected in anti-Id-primed spleens by adoptive transfer.

Adoptive transfers into nude mice demonstrated that T cells were involved in the expression of Id' antibodies. Questions arose as to whether the influence of  $V_H$  region genes acted at the level of B or T cells and whether the environment in which T or B cells matured would have any influence on the Id' response. The issue was of interest partly by analogy to MHC-linked Ir gene-regulated responses, which have been shown previously to be influenced by the thymic environment in which the T cells mature (16). In addition, there is some evidence to suggest that T cells that regulate Id expression recognize Id only in association with allotypic determinants and that the ability to recognize  $V_H$ -linked determinants may be determined by the thymic maturation environment (16, 17). The results reported here do not suggest a role of thymic environment in Id' expression. However, the successful reconstitution of the Id' response by C.B20 T cells, with genes of the low responder haplotype, indicates that such control of immune responsiveness does not occur at the T cell level. Thus, it is not surprising that BALB/c T cells maturing in a low responder environment were also capable of reconstituting Id' induction. The additional finding that B cells were not affected by their maturation environment indicates that  $V_H$  region genes encoded in the B cells were responsible for controlling the level of Id' induction.

The finding that B cell  $V_H$  region genes regulate the expression of idiotope-bearing antibodies after xenogeneic anti-Id treatment has implications for the mechanism of V gene expression. Several investigators have recognized that the Id repertoire expressed by mice after antigen challenge may not accurately reflect the availability of structural genes encoding antibodies specific for that antigen. For example, Primi et al. (18) noted that nonspecifically activated, purified B cells can express the M460 idiotope, even though this idiotope may not be detectable in the antibody response after dinitrophenyl immunization. In the present chimera studies, B10.D2 cells (Igh<sup>b</sup>

allotype) were examined in a BALB/c environment, which is normally permissive for idiotype expression. Furthermore, T cells of the Igh<sup>b</sup> allotype were shown to permit the expression of B cell idiotopes when transferred to BALB/c hosts. Nonetheless, B10.D2 B cells exposed to anti-Id in BALB/c hosts expressed few Id' molecules. The simplest explanation for this finding is that the number of idiotope-bearing molecules induced by anti-Id treatment reflects the number of available structural genes that encode receptors expressing these determinants. Thus, the system of Id' induction by xenogeneic anti-Id may provide a more accurate probe for the presence of structural genes within the V<sub>H</sub> region than the analysis of the normal immune response to antigen (19). The ability of transferred T cells from anti-Id-primed mice to select the Id of B cells that respond to the K<sup>k</sup> antigen suggests that Id-specific T cells can occur in this system. Two major alternative explanations for these results might alter this conclusion. First, it was possible that anti-Id-primed B cells might contaminate the T cell inoculum and produce the Id-positive antibodies subsequently detected. This issue was addressed by using C.B20 T cells for the adoptive transfer experiments. B cells from such primed C.B20 mice were never found to express idiotope-positive anti-K<sup>k</sup> antibodies and thus their presence in the T cell population could not account for the subsequent expression of Id by the adoptive transfer host. The alternative explanation was that xenogeneic anti-Id itself was transferred with the T cell population and subsequently induced idiotope-positive anti-K<sup>k</sup> antibodies. At least two considerations make this explanation unlikely. First, it does not appear that sufficient amounts of anti-Id antibodies would be available for transfer to induce idiotope-positive antibodies because the measurable half-life of these xenogeneic anti-Id antibodies is in the range of several days, whereas some of the mice used as T cell donors in these experiments were primed as long as 3 mo before transfer. Second, if anti-Id were transferred with the T cell inoculum, one would expect that these antibodies would induce the expression of non-H-2K<sup>k</sup> binding antibodies in addition to antigen-binding molecules. However, no such Id' molecules could be detected in the adoptively transferred animals by the standard ELISA assays (data not shown). Thus, it appeared unlikely that either contaminating B cells or xenogeneic anti-Id could account for the transfer of Id expression by the T cell population.

The finding that T cells can affect the B cell Id repertoire reflects the function of Id-specific T cells. This finding does not, however, identify the binding specificity of the T cells involved and the experiments reported here do not directly address this issue. Two types of T cells might potentially be able to transfer the expression of idiotope-positive anti-H-2 antibodies. First, T cells with idiotope-bearing receptors might be induced by anti-Id treatment. Such T cells could subsequently act on host B cells either through an intermediate anti-Id receptor either in the adoptive host or inoculum or by providing antigen-specific T help which might shift the antibody response toward a particular epitope of the K<sup>k</sup> antigen that is recognized by idiotope-positive antibodies. Second, T cells with receptors specific for B cell Id might be primed in donor mice, not by the xenogeneic anti-Id itself but rather by the idiotope-positive B cells induced in the donor by the xenogeneic reagents. Indeed, the recently reported studies of Nutt et al. (20) and L'Age-Stehr (21) have suggested that the T cells that regulate Id expression may actually be primed by the Id initially expressed by B cells. Although our system for analysis of Id-specific T cells after anti-Id treatment in vivo is different from these studies since it is based on antigen exposure,

the actual mechanism of T cell priming may still be the same. Such T cells could potentially recognize isotypic rather than idiotypic determinants if the expression of the 11-4.1 Id were a function of the particular antibody subclass. However, this possibility appears unlikely because the 11-4.1 idiotope has been found on both IgG<sub>1</sub> and IgG<sub>2</sub> antibodies as detected in our FACS analyses. Thus, either Id or anti-Id T cells might be involved in the transfer of Id expression to B cells.

The ability of C.B20 T cells to transfer the expression of Id to BALB/c B cells raises the question of how either Id or anti-Id T cell receptors could arise in a strain whose V<sub>H</sub> region genes do not permit the expression of Id-positive anti-K<sup>k</sup> B cell products. However, mice with V<sub>H</sub> region genes of the b haplotype do express some idiotope-bearing molecules after anti-Id treatment (Fig. 1) even if they do not possess the necessary genes to encode idiotope-positive anti-K<sup>k</sup> antibodies (Fig. 7). T cells may therefore exist in C.B20 mice that either express or recognize these individual idiotopes. When transferred to subsequent BALB/c adoptive hosts whose B cells can express idiotope-positive antigen-binding molecules, such T cells could function to select idiotope-positive anti-K<sup>k</sup> antibodies. Thus, it appears that neither the inability of C.B20 mice to express idiotope-positive anti-K<sup>k</sup> antibodies nor their weak expression of Id' molecules is inconsistent with the suggestion that either Id or anti-Id T cells are involved in the regulation of B cell Id expression.

Based on the considerations discussed above, a possible model for the effects of xenogeneic anti-Id treatment might include the following steps: (a) Xenogeneic anti-Id treatment in vivo triggers those B cells expressing idiotopes in common with the original monoclonal anti-H-2 antibody. (b) The expansion of idiotope-positive B cell clones is dependent on T cell helper factors probably generated by recognition of xenogeneic F<sub>c</sub> molecules as carrier determinants. These helper T cells would function in a similar manner as the carrier specific helper T cells reported by others (22-23). The number of such B cell clones and their ability to bind the original H-2 antigen is dependent on the nature and quantity of the structural genes in the V<sub>H</sub> region encoding those Id determinants. (c) In addition, once induced, these idiotope-positive B cell clones may subsequently induce idiotope-recognizing helper T cells. Such anti-Id helper T cells can function in the presence of an additional antigen challenge to alter the Id repertoire of the resulting B cell antibody response. Most of these hypothetical steps are experimentally testable and further studies are in progress directed at these issues.

The studies reported here represent a first step in the effort to determine whether xenogeneic anti-Id reagents prepared against B cell products can be used to modify T cell immune responses to alloantigens. The results indicate that T cell functions can be altered by such manipulations. However, the functions identified have so far been limited to those regulating B cell responses and it remains unclear whether anti-Id treatment can have any effect on T effector cell-mediated responses to MHC antigens. Additional experiments have therefore been undertaken and remain in progress to determine whether T cell mediated transplantation immunity can also be altered by xenogeneic anti-Id made against monoclonal anti-MHC antibodies.

### Summary

Mice have been treated in vivo with xenogeneic anti-idiotypes prepared against a murine monoclonal anti-H-2K<sup>k</sup> antibody, 11-4.1. B cell immune responses have been

found to be altered by such treatment as evidenced by a modification in the idiotypic repertoire of the humoral response to H-2 antigens. Transfer of purified T cells into nude mice before anti-idiotypic treatment showed that T cells are involved in the induction of idiotope-bearing antibodies by xenogeneic antiidiotypic. Studies using bone marrow chimeras indicate that the environment in which either T or B cells mature does not appear to alter  $V_H$  region genetic control of induction of antiidiotypic-induced molecules. By adoptive transfer studies, T cells from antiidiotypic-treated mice were found capable of modifying the idiotypic repertoire of B cells subsequently exposed to antigen even when the T cells were obtained from antiidiotypic-primed mice of inappropriate allotype. Although it still must be determined whether idiotypic or antiidiotypic T cells are involved in such B cell idiotype regulation, these results indicate that some T cell functions are altered by xenogeneic antiidiotypes prepared against B cell products and suggest that T cell immunity to major histocompatibility complex antigens may also be affected by such reagents.

The authors wish to thank Marian Knode for expert technical assistance in the purification of antiidiotypic reagents, Leanne DeNenno and Monica Violet for their care and bleeding of experimental animals, and David Stephany and Susan Sharrow for FACS analysis.

*Received for publication 22 November 1982.*

### References

1. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris)*. **125c**:373.
2. Bluestone, J. A., S. O. Sharrow, S. L. Epstein, K. Ozato, and D. H. Sachs. 1981. Induction of anti-H-2 antibodies in the absence of alloantigen exposure by *in vivo* administration of anti-idiotypic. *Nature (Lond.)*. **291**:233.
3. Bluestone, J. A., S. L. Epstein, K. Ozato, S. O. Sharrow, and D. H. Sachs. 1981. Anti-idiotypes to monoclonal anti-H-2 antibodies. II. Expression of anti-H-2K<sup>k</sup> idiotypes induced by anti-idiotypic or H-2K<sup>k</sup> antigen. *J. Exp. Med.* **145**:1305.
4. Epstein, S. L., V. R. Masakowski, J. A. Bluestone, K. Ozato, and D. H. Sachs. 1982. Idiotypes of anti-Ia antibodies. II. Effects of *in vivo* treatment with xenogeneic anti-idiotypic. *J. Immunol.* **129**:1545.
5. Sachs, D. H., J. A. Bluestone, S. L. Epstein, and K. Ozato. 1981. Anti-idiotypes to monoclonal anti-H-2 and anti-Ia hybridoma antibodies. *Transplant. Proc.* **13**:953.
6. Arn, J. S., Riordan, S. E., Pearson, D. and D. H. Sachs. 1983. Strain-restricted typing sera (SRTS) for use in monitoring the genetic integrity of congenic strains. *J. Immunol. Methods*. In press.
7. Oi, V. T., P. P. Jones, L. A. Goding, L. A. Herzenberg, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. *Curr. Top. Microbiol. Immunol.* **81**:115.
8. Cuatrecasas, P. 1970. Protein purification by affinity chromatography. Derivatization of agarose and polyacrylamide beads. *J. Biol. Chem.* **245**:3059.
9. Sachs, D. H., and J. L. Cone. 1973. A mouse B-cell alloantigen determined by gene(s) linked to the major histocompatibility complex. *J. Exp. Med.* **138**:1289.
10. Sharrow, S. O., K. Ozato, and D. H. Sachs. 1980. Phenotypic expression of I-A and I-E/C subregion determinants on murine thymocytes. *J. Immunol.* **125**:2263.
11. Binz, H., and H. Wigzell. 1977. Antigen-binding idiotypic T cell receptors. *Contemp. Top. Immunobiol.* **2**:113.
12. Singer, A., K. S. Hathcock, and R. J. Hodes. 1979. Cellular and genetic control of antibody

- responses. V. Helper T cell recognition of H-2 determinants on accessory cells but not B cells. *J. Exp. Med.* **149**:1208.
13. Bluestone, J. A., H. Auchincloss, Jr., P.-A. Cazenave, K. Ozato, and D. H. Sachs. 1982. Anti-idiotypes to monoclonal anti-H-2 antibodies. III. Syngeneic anti-idiotypes detect idiotopes on antibodies induced by *in vivo* administration of xenogeneic anti-idiotypes. *J. Immunol.* **129**:2066.
  14. Bluestone, J. A., H. C. Krutzsch, H. Auchincloss, Jr., P.-A. Cazenave, and D. H. Sachs. 1982. Comparative analysis of monoclonal anti-H-2K<sup>k</sup> idiotypic and idiotypic-positive molecules induced by *in vivo* anti-idiotypic treatment. *Proc. Natl. Acad. Sci. USA.* **79**:7847.
  15. Bluestone, J. A., H. Auchincloss, Jr., S. L. Epstein, and D. H. Sachs. 1983. Idiotypes of anti-MHC antibodies. In, *Idiotypic Manipulations in Biological Systems*. H. Kohler, P.-A. Cazenave, and J. Urbain, editors. Academic Press, Inc., New York. In press.
  16. Flood, P., K. Yamauchi, A. Singer, and R. K. Gershon. 1982. Homologies between cell interaction molecules controlled by major histocompatibility complex- and Igh-V-linked genes that T cells use for communication. Tandem "adaptive" differentiation of producer and acceptor cells. *J. Exp. Med.* **156**:1390.
  17. Juy, D., D. Primi, P. Sanchez, and P.-A. Cazenave. 1982. Idiotypic regulation. Evidence for the involvement of IgH-C-restricted T cells in the M460 idiotypic suppressive pathway. *Eur. J. Immunol.* **12**:24.
  18. Primi, D., D. Juy, and P.-A. Cazenave. 1981. Induction and regulation of silent idiotypic clones. *Eur. J. Immunol.* **11**:393.
  19. Miller, G. G. P., P. I. Nadler, R. J. Hodes, and D. H. Sachs. 1982. Modification of T cell antinuclear idiotypic expression by an *in vivo* administration of anti-idiotypic. *J. Exp. Med.* **155**:190.
  20. 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.
  21. L'Age-Stehr, J. 1981. Priming of T helper cells by antigen-activated B cells. B cell-primed Lyt-1<sup>+</sup> helper cells are restricted to cooperate with B cells expressing the Igh<sub>H</sub> phenotype of the priming B cells. *J. Exp. Med.* **153**:1236.
  22. Woodland, R., and H. Cantor. 1978. Idiotypic-specific T helper cells are required to induce idiotypic-positive B memory cells to secrete antibody. *Eur. J. Immunol.* **8**:600.
  23. Bottomly, K., B. J. Mathieson, and D. E. Mosier. 1978. Anti-idiotypic-induced regulation of helper cells for the response to phosphorylcholine in adult BALB/c mice. *J. Exp. Med.* **148**:1216.