INDUCTION OF IDIOTYPE-BEARING, NUCLEASE-SPECIFIC HELPER T CELLS BY IN VIVO TREATMENT WITH ANTI-IDIOTYPE

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Staphylococcal nuclease (nuclease) is a naturally occurring bacterial enzyme, the sequence and structure of which are known (1, 2), and for which a wealth of biochemical and immunochemical data is available (3–4). The initial antibody response to nuclease is under the control of an *H-2*-linked immune response (*Ir*) gene (or genes) mapping to the *I-B* subregion (5, 6). Several strains of mice produce anti-nuclease antibodies of limited heterogeneity, and it has been demonstrated that cross-reactive idiotypes (Id) predominate in these responses (5). In addition, it has recently been demonstrated that nuclease-specific T helper cells from nuclease-primed mice bear cell surface idiotypic determinants as shown by their elimination with anti-Id plus complement (C)[1] or by their functional inhibition with anti-Id alone (Nadler et al., manuscript in preparation). Thus, for the response to nuclease, dominant cross-reactive Id are expressed both on antigen-specific T helper cells and on anti-nuclease antibodies.

Previous experiments from this laboratory have demonstrated that in vivo administration of anti-Id to A/J mice without antigen results in the generation of Id-bearing non-antigen-binding molecules (Id′) in the serum of the recipients (7). The existence of such molecules has been interpreted as evidence for the presence of immunoglobulin (Ig) heavy-chain variable-region gene structures normally associated with anti-nuclease antibodies in other Ig of unrelated specificity. These findings raise questions concerning the mechanism by which antigen and/or anti-Id are capable of triggering immune responses. In a response system in which idiotypic receptors are expressed both on helper T cells and on antibody, the effect of anti-Id could involve a variety of pathways.

In the present report, we have attempted to assess the effects of anti-idiotypic antibodies on both helper T cell function and on Ig Id expression. In doing so, we have attempted to elucidate the mechanisms whereby Id′ is produced and to correlate the cellular events that occur after anti-Id treatment with the humoral responses that have been observed. The experiments described here demonstrate that: (*a*) in vivo

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[1] *Abbreviations used in this paper:* C, complement; CFA, complete Freund's adjuvant; HAI, hemagglutination inhibition assay; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNP, trinitrophenyl.

anti-Id administration leads to the generation of nuclease-specific primed helper T cells; (*b*) these T cells from anti-Id-primed animals, like those from nuclease-primed animals, bear surface idiotypic determinants as shown by their elimination with anti-Id plus C; and (*c*) Id′ is not produced in nude mice, which lack functional T cells, suggesting that the generation of antigen-specific, Id-positive helper T cells and of Id′ may be related rather than independent events.

Materials and Methods

Animals. BALB/cAnN and BALB/cAnN nude mice were obtained from the Animal Production Unit, National Cancer Institute. A/J and A.BY mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). Miniature swine were bred and housed at the National Institutes of Health Animal Facility, Poolesville, Md.

Antigens. Nuclease was purified as previously published (8) or purchased from the Boehringer-Mannheim Biochemical Co. (Elkhart, Ind.). Although no major contaminants were found on sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the commercial preparation of nuclease, the specific activity of this material was approximately half that of enzyme prepared in our laboratory. We therefore used nuclease purified in our laboratory for the immunization of animals and the commerical preparation for conjugation to 2,4,6-trinitrobenzene sulfonic acid (TNBS) and in vitro challenge. Keyhole limpet hemocyanin (KLH) was purchased from Calbiochem-Behring Corp. (lot 530195; San Diego, Calif.). Nuclease and KLH were conjugated with TNBS as previously described (9), at ratios of 17.4 and 13.0 trinitrophenyl (TNP) residues per 100,000 mol wt, respectively.

Immunizations. Mice 2–6 mo of age were immunized intraperitoneally with 100 μg nuclease in 0.1 ml saline emulsified with an equal volume of complete Freund's adjuvant (CFA). Mice receiving anti-Id were treated with 20 μg of purified pig anti-(BALB/c anti-nuclease) (pig anti-BALB/c Id) in 0.2 ml saline on day 0 and day 3, or with one dose of 20 μg pig anti-BALB/c Id in 0.2 ml emulsion with CFA on day 0. Mice were immunized with 100 μg KLH or TNP-KLH in 0.1 ml saline emulsified with an equal volume of CFA. Miniature swine were immunized with affinity-purified BALB/c anti-nuclease antibodies as previously described for production of pig anti-A/J Id (7).

Purification of Antibodies and Anti-Id. BALB/c anti-nuclease antibodies used to immunize miniature swine were purified from pools of high-titered serum and/or ascites by affinity chromatography on nuclease coupled to Sepharose-4B by the cyanogen bromide method, as previously described (10). Serum with anti-Id activity from a single miniature pig immunized with purified BALB/c anti-nuclease was absorbed exhaustively with normal BALB/c Ig coupled to Sepharose-4B. Further absorption was performed with LPC-1, a BALB/c γ2a myeloma protein, coupled to Sepharose-4B. The anti-BALB/c Ig was eluted from these two columns with 6 M guanidine HCl in 0.1 M phosphate buffer, pH 7.0, and dialyzed into normal saline. This pig anti-BALB/c Ig was used as a control pig Ig for specificity of anti-Id immunization. Efficacy of absorption of these anti-Ig antibodies from the anti-Id was demonstrated by removal of passive hemagglutination activity against LPC-1-coated sheep erythrocytes (SRBC) in a hemagglutination assay, as previously described (10). The anti-Id serum was then adsorbed to Sepharose-4B coupled to purified BALB/c anti-nuclease (Id) and eluted with 2 M and then 6 M guanidine HCl, in 0.1 M phosphate buffer, pH 7.0; the eluates were dialyzed into normal saline and then pooled. Anti-Id activity was determined by passive hemagglutination of BALB/c anti-nuclease-coated SRBC as previously described for pig anti-A/J Id or by a site-specific spectrophotometric assay (10).

Measurement of Id and Anti-Nuclease Antibodies. Sera were tested for the presence of Id by a hemagglutination inhibition assay (HAI) as previously described (7). Briefly, serial twofold dilutions of unknown and control sera were tested for their ability to inhibit agglutination of Id-coated SRBC by an appropriate dilution of pig anti-BALB/c Id. Sera from mice immunized with pig anti-BALB/c Id were first absorbed with normal pig Ig coupled to Sepharose-4B to remove reactivity with pig Ig. Anti-nuclease activity was determined by passive hemagglutination of nuclease-coated SRBC.

Preparation of Cell Populations for In Vitro Anti-TNP Plaque-forming Cell (PFC) Responses. Nylon

nonadherent spleen (T) cells were prepared by the method of Julius et al. (11). Suspensions depleted of T cells (B plus accessory cells) were prepared by sequential treatment of whole spleen cell suspensions with either rabbit anti-mouse brain serum and guinea pig C, or hybridoma anti-Thy-1.2 plus rabbit C as previously described (9, 12).

Anti-Id Plus C Treatment of Cells. 5×10^6 T cells were suspended in 1 ml minimum essential medium (Grand Island Biological Co.) containing 10% fetal calf serum and mixed with an equal volume of purified pig anti-BALB/c Id or pig anti-BALB/c Ig as control (final concentration 250 μg/ml) for 30 min at 37°C. The cells were washed, resuspended in 1 ml screened rabbit C (1:7 dilution), incubated at 37°C for 30 min, washed, and resuspended in medium for in vitro culture.

Culture Conditions. Culture conditions for in vitro primary antibody responses to TNP-KLH have been described previously (9), as have conditions for the augmented in vitro primary response to TNP-nuclease (6). Briefly, the generation of anti-TNP PFC in vitro in response to TNP-nuclease required nuclease-primed spleen cells or splenic T cells. Unprimed spleen cells did not generate significant responses, whereas nuclease-primed T cells plus unprimed B and accessory cells sufficed to generate the response. Optimum responses were obtained by culturing 3×10^5 spleen cells in a final volume of 200 μl per well of flat-bottomed microtiter plates. Optimum antigen concentrations were 5–20 μg/ml TNP-nuclease and 10 μg/ml TNP-KLH. Peak responses were seen on days 4 and 5. The anti-TNP PFC responses to TNP-nuclease under these conditions were predominantly IgM. In vitro secondary IgG antibody responses were also studied to further assess the specificity of primed T helper cells. In these experiments, 3×10^5 TNP-primed B cells (TNP-KLH-primed spleen cells treated with anti-Thy-1.2 plus C) were cocultured with graded numbers of either control unprimed or primed (nuclease, anti-Id, or KLH) T cells, and responses to TNP-nuclease and TNP-KLH were assessed. Antigens were used in these secondary responses at optimum concentrations of 0.001 μg/ml for TNP-KLH and 0.1 μg/ml for TNP-nuclease. IgG-secreting anti-TNP PFC were measured using a facilitating rabbit anti-mouse Ig (N. L. Cappel Laboratories, Cochranville, Pa.). For each culture, direct and total plaques were counted and IgG plaques were calculated by subtraction of direct from total plaques.

Results

Id-Positive Non-Antigen-Binding Molecules Are Induced by In Vivo Treatment with Pig Anti-BALB/c Id in Normal BALB/c But Not in Nude BALB/c Mice. Previous experiments had demonstrated that adult A/J mice treated with pig anti-A/J Id produced molecules in their serum that bore A/J Id but had no detectable anti-nuclease activity (7). Such molecules might conceivably be produced by direct stimulation of B cells bearing cross-reactive idiotypic determinants not related to the binding site for nuclease. Alternatively, a more complex pathway, perhaps involving T cells, could be envisioned. To test these possibilities, normal and nude BALB/c mice were compared for their ability to produce these molecules after treatment with pig anti-BALB/c Id. As shown in Fig. 1, normal BALB/c mice treated with pig anti-BALB/c Id produced molecules bearing BALB/c Id. None of these mice showed significant anti-nuclease activity in their serum despite the presence of the anti-nuclease Id, and we have therefore referred to the induced idiotype as Id′ (7). BALB/c nude (*nu/nu*) mice, however, failed to produce Id′ after anti-Id administered either as an emulsion in CFA or in solution in saline. These results suggested that direct B cell activation by anti-Id, if it occurs, is insufficient to generate detectable quantities of these molecules and that functional T cells played a role in the generation of such molecules.

Administration of Anti-Id In Vivo Generates Antigen-specific Helper T Cells. We have recently demonstrated that nuclease-specific T helper cells from antigen-primed mice express idiotopes corresponding to those expressed on anti-nuclease antibodies (Nadler et al., manuscript in preparation). In light of this finding, and in view of the possible

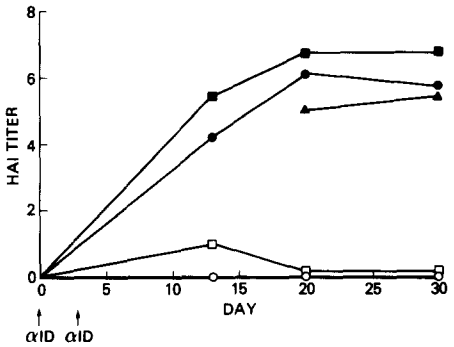

FIG. 1. Induction of Id-positive non-antigen-binding molecules by anti-Id in normal vs. nude BALB/c mice. The ordinate indicates the HAI titer for BALB/c Id. (○), nude mice treated with 20 μg anti-Id in CFA on day 0. (□), nude mice treated with 20 μg anti-Id in saline on day 0 and day 3. (●), normal BALB/c treated with 20 μg anti-Id on CFA on day 0. (■), normal BALB/c treated with 20 μg anti-Id in saline on day 0 and 3. (▲), normal BALB/c immunized with 100 μg nuclease on day 10. All sera were absorbed on normal pig Ig coupled to Sepharose-4B.

importance of T cells in the induction of Id' suggested by the experimental findings in nude mice, we examined the functional properties of T cells from anti-Id-primed mice using the augmented primary in vitro response to TNP-nuclease as an assay system. This system is well suited to studying the properties of specific cell populations because anti-TNP PFC can be generated in vitro with normal, unprimed B plus accessory cells in the presence of antigen-primed T cells, and the *Ir* gene control of the in vitro antibody responses parallels that of initial in vivo antibody responses (6).

The ability of whole spleen cell suspensions from mice primed with anti-Id or nuclease to generate anti-TNP PFC upon in vitro challenge with TNP-nuclease was assessed. As seen in Fig. 2, anti-TNP PFC were generated in spleen cells from both anti-Id-treated and nuclease-primed mice, but not in spleen cells from unimmunized mice or mice immunized with normal pig Ig or CFA alone. The competence of these control cell populations was demonstrated by the ability of each population to generate an in vitro primary response to TNP-KLH (Fig. 2).

The effect of anti-Id treatment was demonstrated to be dependent on activation of T cells as shown in Fig. 3. In these experiments, graded numbers of T cells from nuclease-primed or anti-Id-treated mice were compared with T cells from mice immunized with CFA alone, with normal pig Ig in CFA, or from untreated mice for ability to generate anti-TNP PFC with unprimed B cells and accessory cells when challenged with TNP-nuclease. T cells from anti-Id-treated animals were found to provide help for anti-TNP-nuclease responses equivalent to that provided by T cells from nuclease-primed animals. Nonspecific stimulation with CFA or normal pig Ig administration did not generate primed T cells.

Helper T Cells Induced by Anti-Id Are Specific for Nuclease. Although these experiments indicated that either nuclease immunization or anti-Id treatment could suffice to provide helper T cells for anti-TNP-nuclease responses, they did not fully demonstrate antigen specificity because in vitro primary responses to TNP-KLH only showed that control T cells were functional. To determine whether T cells from nuclease and anti-Id-primed animals were indeed specific for nuclease, a secondary IgG response system for TNP-nuclease and TNP-KLH was used. In general, the optimum generation of

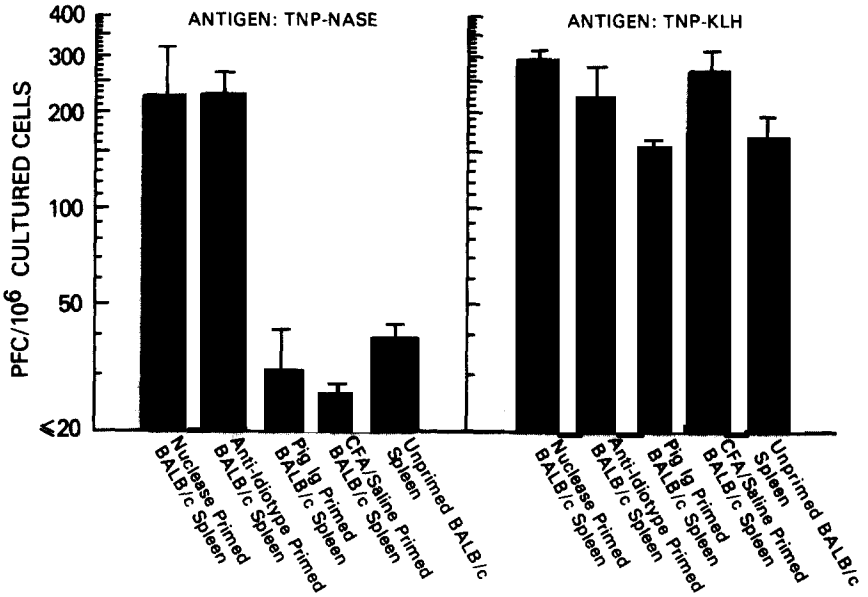

FIG. 2. Treatment with anti-Id in vivo results in priming for anti-TNP-nuclease responses in vitro. Spleen cells from unprimed mice or from mice immunized with nuclease, pig anti-BALB/c Id (20 μg in CFA), normal pig Ig (20 μg in CFA), or CFA alone were challenged in vitro with TNP-nuclease or TNP-KLH. Vertical bars show ± SEM. All responses were abolished by pretreatment of cells with rabbit anti-mouse brain serum plus C; control cultures with no antigen gave <5 PFC/10^6 cultured cells for all populations of cells.

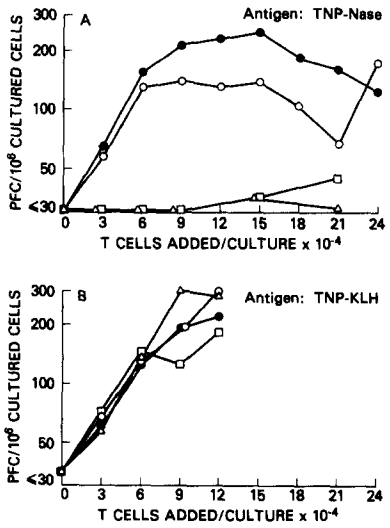

FIG. 3. T cells from nuclease-primed or anti-Id-treated mice function as T_H cells for in vitro TNP-nuclease responses. Graded numbers of T cells from BALB/c mice immunized with nuclease (●), or pig anti-BALB/c Id (○) were added to 3×10^5 unprimed B plus accessory cells (rabbit anti-mouse brain serum plus C-treated normal BALB/c spleen) and challenged in vitro with TNP-nuclease (A) or TNP-KLH (B). Control T cells were from mice immunized with normal pig Ig (□), or CFA alone (△).

IgG-secreting anti-hapten PFC responses has required the coculture of hapten-primed B cells with carrier-primed T cells (13). In the following experiments, therefore, B cells from animals primed with TNP-KLH were cultured with T cells primed with nuclease, anti-Id, or KLH, and challenged in vitro with TNP-nuclease or TNP-KLH. As a more stringent control of the specificity of the anti-Id used for in vivo treatment, T cells were also tested from control mice immunized with pig anti-BALB/c Ig derived from the anti-Id serum during the course of purification of the anti-Id. As shown in Fig. 4 A, only nuclease and anti-Id-primed T cells generated significant IgG PFC responses to TNP-nuclease. Conversely, only KLH-primed T cells provided significant help for an IgG response to TNP-KLH (Fig. 4 B). These experiments, using directly comparable IgG secondary responses, demonstrated that anti-Id treatment of adult animals in vivo generates a population of antigen-specific helper T cells.

Antigen-specific Helper T Cells from Anti-Id-treated Animals Bear Idiotypic Determinants. To examine the expression of idiotypic determinants on nuclease or anti-Id-primed T helper cells, the effects of anti-Id plus C treatment on these populations were determined. As shown in Fig. 5 A and B, anti-Id plus C treatment of T cells from nuclease-primed mice before culture abrogated the response to TNP-nuclease with no apparent effect on the response of these cells to TNP-KLH. Similarly, in Fig. 5 C and D, anti-Id plus C treatment of T cells from anti-Id-primed mice abolished the anti-TNP-nuclease response with no demonstrable effect on the response to TNP-KLH. Treatment with normal pig Ig plus C had no effect on either response. In 10 consecutive experiments, pretreatment of both nuclease and anti-Id-primed T cells with anti-Id plus C consistently resulted in 70–100% decreases in the anti-TNP-nuclease responses without significant effect on the response to TNP-KLH.

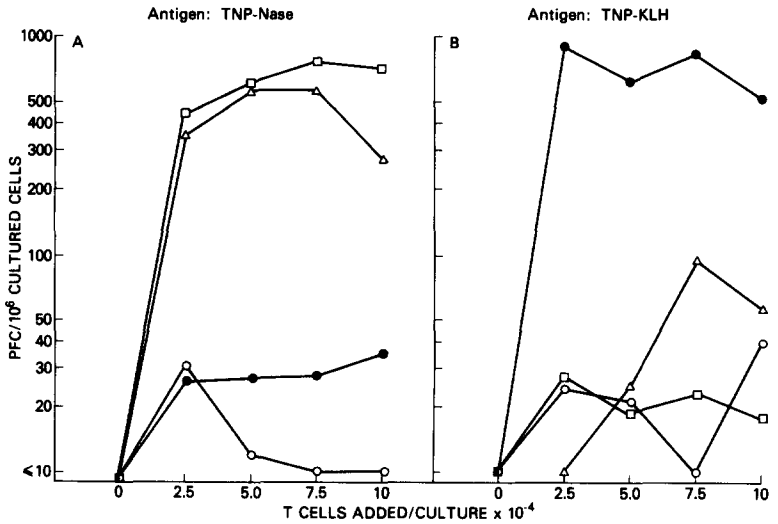

FIG. 4. Helper T cells from anti-Id-primed and nuclease-primed BALB/c mice are specific for nuclease in secondary IgG responses. Each culture contained 3×10^5 anti-Thy-1.2 plus C-treated TNP-KLH-primed (B plus accessory) cells plus graded numbers of T cells as indicated on the abscissa. In (A), cultures were challenged with TNP-nuclease; in (B), cultures were challenged with TNP-KLH. (△), nuclease-primed T cells; (●) KLH-primed cells; (□), anti-Id-primed T cells; (○), pig anti-BALB/c Ig-primed T cells.

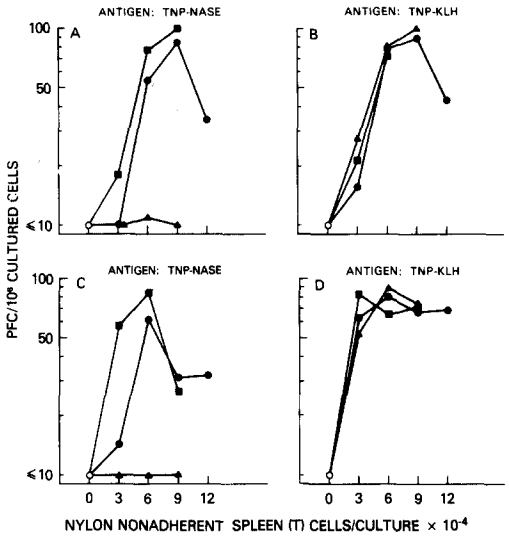

FIG. 5. Helper T cells from nuclease and anti-Id-primed animals bear Id. Nuclease-primed T cells were treated with anti-Id plus C, normal pig Ig plus C, or were untreated before addition to cultures of 3×10^5 unprimed B plus accessory cells challenged with TNP-nuclease (A) or TNP-KLH (B). T cells from anti-Id-primed mice were similarly treated with anti-Id plus C, normal pig Ig plus C, or were untreated before culture with TNP-nuclease (C) or TNP-KLH (D). (●), untreated T cells; (▲), pig anti-Id plus C-treated T cells; (■), normal pig Ig plus C-treated T cells.

Discussion

The experiments described here demonstrate two distinct but probably related occurrences after in vivo administration of anti-Id: the generation of antigen-specific Id-bearing helper T cells and the production in the same animals of Id-bearing serum Ig without detectable antigen-binding activity. Interpretation of these findings as well as of similar observations in other systems therefore requires a reconciliation of these two seemingly paradoxical effects of anti-Id.

Examining effects at the cellular level first, T cells from anti-Id-primed animals appear to be functionally identical to those from nuclease-primed animals in terms of capacity to provide antigen-specific help and presence of idiotypic determinants on the cell surface as shown by elimination with anti-Id and C. However, these experiments do not rule out the possibility that anti-Id may also result in T cells primed for responses other than those examined here. As will be discussed further, the finding that T cells are required for the production of non-nuclease-binding Id-positive Ig suggests the existence of T cells primed by anti-Id that are not specific only for nuclease. Thus, we can say with assurance only that a subpopulation of T cells exists in animals primed with anti-Id that is antigen-specific and bears idiotypic determinants on its surface.

The ability to induce antigen-specific helper T cells with anti-Id has been reported previously by Eichmann and coworkers (14, 15) for the anti-A.CHO response using anti-A5A, and by Julius et al. (16) and Benca et al. (17) for phosphorylcholine-specific T cells, although these authors did not report Id-positive molecules in serum before antigen administration. These findings together with reports of induction of Id-specific suppressor T cells with anti-Id (18–20) and the demonstration of circulating endoge-

nous anti-Id (21–23) suggest that anti-idiotypic regulation of T cell activity may be a commonly occurring phenomenon, as predicted by Jerne's network hypothesis (24).

As concerns the humoral consequences of anti-Id treatment, these experiments confirm and extend our earlier observations on the in vivo effects of treatment with anti-Id to anti-nuclease antibodies (7). For both the previous studies using pig anti-A/J Id treatment of A/J mice and the studies using pig anti-BALB/c Id treatment of BALB/c mice reported here, the administration of anti-Id led to production of Ig molecules bearing idiotypic determinants in the absence of detectable anti-nuclease activity (Id′). The generation of such molecules after anti-Id immunization has previously been reported in rabbits (25–27) and in mice (28, 29), and their induction in the absence of a conventional antigenic stimulus has suggested that antigen-independent mechanisms exist for the expansion of B cell clones bearing these Id. Our results in *nu/nu* mice suggest that B cell stimulation by anti-Id requires functional T cells, and that the experiments previously reported by others (14, 25–29) have occurred in this setting. Thus the production of Id′ and probably the stimulation of idiotypic B cells that sometimes occurs after treatment with anti-Id appear to require T cells and can occur in the absence of antigen.

The mechanism of T helper cell induction by anti-Id remains uncertain. The most straightforward interpretation of the results presented here is that anti-Id acts directly upon the Id-bearing precursors of nuclease-specific T helper cells and activates such cells to proliferate and/or differentiate into the functional helper population detected in vitro. Alternatively, it is possible that anti-Id acts to perturb the Id-anti-Id network in a more complex manner. For example, the appearance of T helper cells could reflect a decrease in network-mediated suppression of helper cell activity as a result of anti-Id administration in vivo. An apparent paradox presented by the findings reported here is the concomitant induction by anti-Id of nuclease-specific Id-bearing T helper cells and of Id-bearing but non-antigen-specific Ig molecules. One interpretation of these results is that they reflect differences between the antigen receptors of T cells and those of B cells or antibodies. The absence of detectable light chain determinants on most T cells suggests one mechanism by which the interactions of T cells with antigens may be different from those of B cells. It seems possible that the presence of one or a few idiotopes detected by the anti-nuclease Id on T cells may be sufficient to allow these T cells to recognize and respond to nuclease. In the case of antibodies, however, this combination of idiotope with a light chain may not give the requisite combining site for antigen. Such antibodies would have the characteristics of Id′ or Ab3 (25, 26). In this sense, T cell specificity may be more "degenerate" than that of Ig.

Although the predictions of the network hypothesis are applicable to our results and to those of others, the molecular and cellular requirements for the component Id-anti-Id interactions at cell surfaces are not yet clear. For example, it is not yet known whether anti-Id can directly activate Id-bearing T cells. Such direct activation would have several implications, because light chain determinants have not yet been demonstrated on T cells and anti-Id binding to isolated heavy chains is usually of much lower affinity than binding to the intact Ig. The ability of anti-Id to activate T cells directly would therefore imply either: (*a*) that there exists a small subset of anti-idiotypic antibodies that bind to heavy chains with high affinity; (*b*) that low affinity

interactions suffice for activation; or (*c*) that another, as yet undefined, molecule exists that stabilizes this interaction.

On the other hand, most experimental data on antigen-specific antivation of T cells indicate that antigen must be presented in association with major histocompatibility complex (MHC) products on the surface of appropriate macrophages or antigen-presenting cells. Indeed, failure of such presentation is currently thought to be a likely basis of *H-2*-linked *Ir* gene defects (30). If activation by anti-Id is similar to that induced by antigen, it may also require such MHC-restricted mediation by presenting cells. If this is the case, *H-2*-linked *Ir* genes may also play a role in the generation of helper T cells by anti-Id as they do for antigen priming. The relationship between the MHC-restricted *Ir* gene function and the triggering of cellular interactions by anti-Id may provide insights with respect to the cellular level of each of these phenomena, and experiments to explore this relationship further are in progress.

Summary

Treatment of BALB/c mice with purified pig anti-(BALB/c anti-nuclease) anti-idiotypic antibodies has been found to induce the appearance of idiotype-bearing immunoglobulins (Id′) in the serum of these mice in the absence of detectable antigen binding activity. This phenomenon appeared to require T cells in the hosts because no Id′ was detected in the serum of nude mice similarly treated. Furthermore, the spleens of BALB/c mice treated with anti-idiotype were found to contain helper T cells capable of providing help in an in vitro plaque-forming cell response to trinitrophenyl-nuclease equivalent to that provided by helper T cells from the spleens of nuclease-primed animals. Helper T cells from both anti-idiotype-treated and nuclease-treated animals were found to be antigen-specific and to be similarly susceptible to elimination by treatment with anti-idiotype plus complement. Therefore, treatment with both antigen and anti-idiotype appeared to prime similar populations of antigen-specific helper T cells, while having different effects on the induction of antibody. These findings are consistent with the network theory of receptor interactions in the immune response, and may provide a means for studying individual cell populations involved in such interactions.

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References

1. Arnone, A., C. J. Bier, F. A. Cotton, V. W. Day, E. E. Hazen, D. C. Richardson, J. S. Richardson, and S. Youath. 1971. A high resolution structure of an inhibitor complex of the extracellular nuclease of *Staphylococcus aureus*. *J. Biol. Chem.* **246:**2302.
2. Cotton, F. A., and E. E. Hazen. 1971. Staphylococcal nuclease X-ray structure. *In* The Enzymes. P. D. Boyer, editor. Academic Press, Inc., New York. 153.
3. Anfinsen, C. B., P. Cuatrecasas, and H. Taniuchi. 1971. Staphylococcal nuclease, chemical properties and catalysis. *In* The Enzymes. P. D. Boyer, editor. Academic Press, Inc., New York. 177.
4. Sachs, D. H., A. N. Schechter, A. Eastlake, and C. B. Anfinsen. 1972. Antibodies to a distinct determinant of staphylococcal nuclease. *J. Immunol.* **109:**1300.
5. Sachs, D. H., J. A. Berzofsky, D. S. Pisetsky, and R. H. Schwartz. 1978. Genetic control of the immune response to staphylococcal nuclease. *Springer Semin. Immunopathol.* **1:**51.

6. Nadler, P. I., G. P. Miller, D. H. Sachs, and R. J. Hodes. 1981. Genetic control of antibody responses to TNP-nuclease in vitro. *J. Immunol.* **126:**1706.
7. Sachs, D. H., M. El-Gamil, and G. Miller. 1981. Genetic control of the immune response to staphylococcal nuclease. XI. Effects of in vivo administration of anti-idiotypic antibodies. *Eur. J. Immunol.* In press.
8. Bohnert, J. L., and H. Taniuchi. 1975. The purification of staphylococcal nuclease by an improved method. *J. Biol. Chem.* **250:**2394.
9. Hodes, R. J., and A. Singer. 1977. Cellular and genetic control of antibody responses in vitro. I. Cellular requirements for the generation of genetically controlled primary IgM responses to soluble antigens. *Eur. J. Immunol.* **7:**892.
10. Fathman, C. G., and D. H. Sachs. 1976. Genetic control of the immune response to Staphylococcal nuclease. II. Detection of idiotypic determinants by the inhibition of antibody-mediated inactivation. *J. Immunol.* **116:**959.
11. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. *Eur. J. Immunol.* **3:**645.
12. Lake, P., E. A. Clark, M. Korshidi, and G. H. Sunshine. 1979. Production and characterization of cytotoxic Thy-1 antibody secreting hybrid cell lines. Detection of T cell subsets. *Eur. J. Immunol.* **9:**875.
13. Cheers, C., J. C. S. Breitner, M. Litle, and J. F. A. P. Miller. 1971. Cooperation between carrier-reactive and hapten sensitive cells *in vitro*. *Nature (Lond.).* **232:**248.
14. Eichman, K., and K. Rajewsky. 1975. Induction of T and B cell immunity by anti-idiotypic antibodies. *Eur. J. Immunol.* **5:**661.
15. Black, S. J., G. J. Hammerling, C. Berek, K. Rajewsky, and K. Eichmann. 1976. Idiotypic analysis of lymphocytes *in vitro*. I. Specificity and heterogeneity of B and T lymphocytes reactive with anti-idiotypic antibody. *J. Exp. Med.* **143:**846.
16. Julius, M. H., H. Cosenza, and A. A. Augustin. 1978. Evidence for the endogenous production of T cell receptors bearing idiotypic determinants. *Eur. J. Immunol.* **8:**484.
17. Benca, R. J., J. Quintas, J. F. Kearney, P. M. Flood, and H. Schreiber. 1980. Studies on phosphorylcholine-specific T cell idiotypes and idiotype specific immunity. *Mol. Immunol.* **17:**823.
18. Eichmann, K. 1975. Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. *Eur. J. Immunol.* **5:**511.
19. Owen, F. L., S.-T. Ju, and A. Nisonoff. 1977. Binding to idiotypic determinants of large proportions of thymus derived lymphocytes in idiotypically suppressed mice. *Proc. Natl. Acad. Sci. U. S. A.* **74:**2084.
20. 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.
21. Kluskens, L., and H. Koehler. 1974. Regulation of immune response by autologous antibody against receptor. *Proc. Natl. Acad. Sci. U. S. A.* **71:**5083.
22. Cosenza, H. 1976. Detection of anti-idiotype reactive cells in the response to phosphorylcholine. *Eur. J. Immunol.* **6:**114.
23. Tasiux, N., R. Leuwenkroon, C. Bruyuns, and J. Urbain. 1978. Possible occurrence and meaning of lymphocytes bearing autoanti-idiotypic receptors during the immune response. *Eur. J. Immunol.* **8:**464.
24. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris).* **125:**373.
25. Cazenave, P. A. 1971. Idiotypic-anti-idiotypic regulation of antibody synthesis in rabbits. *Proc. Natl. Acad. Sci. U. S. A.* **74:**5122.
26. Urbain, J., M. Wikler, J. D. Franssen, and C. Collignon. 1971. Idiotypic regulation of the

immune system by the induction of antibodies against anti-idiotypic antibodies. *Proc. Natl. Acad. Sci. U. S. A.* **74:**5126.

27. Wikler, M., J. D. Franssen, C. Collignon, O. Leo, B. Mariame, P. van de Walle, D. de Groote, and J. Urbain. 1979. Idiotypic regulation of the immune system: common idiotypic specificities between idiotypes and antibodies raised against anti-idiotypic antibodies in rabbits. *J. Exp. Med.* **150:**184.
28. Le Guern, C., F. Ben Aissa, D. Juy, B. Mariame, G. Buttin, and P.-A. Cazenave. 1979. Expression and induction of MOPC-460 idiotypes in different strains of mice. *Ann. Immunol. (Paris).* **130:**293.
29. Bona, C., R. Hooghe, P. A. Cazenave, C. Le Guern, and W. E. Paul. 1979. Cellular basis of regulation 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.
30. Singer, A., C. Cowing, K. S. Hathcock, H. B. Dickler, and R. J. Hodes. 1978. Cellular and genetic control of antibody responses *in vitro*. III. Immune response gene regulation of accessory cell function. *J. Exp. Med.* **147:**1611.