MODIFICATION OF T CELL ANTINUCLEASE IDIOTYPE EXPRESSION BY IN VIVO ADMINISTRATION OF ANTI-IDIOTYPE

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The immune response to Staphylococcal nuclease (nuclease) is under the control of H-2- and non-H-2-linked Ir genes (1). Antinuclease antibodies from inbred strains of mice have been shown (2) to bear characteristic cross-reactive idiotypes (CRI) that are expressed by all individuals of the same strain. Expression of these idiotypes (Id) is linked to the heavy chain allotype locus (Igh-C) (3), although some of these Id are also shared by more than one mouse strain.

Administration of exogenous anti-Id to mice has been used to study the control of Id expression in several experimental model systems (reviewed in 4). We recently demonstrated that administration of purified heterologous anti-Id to mice leads to the appearance of Id-bearing Ig in the serum of the treated animals in the absence of detectable antigen (nuclease)-binding activity (Id'). This phenomenon was observed after treatment of A/J animals with pig anti-A/J Id (5) and after treatment of BALB/c animals with pig anti-BALB/c Id (6). In each case, treatment with anti-Id was also found to result in the appearance of antigen-specific helper T (T_H) cells in the spleen. These T_H cells as well as those from nuclease-primed animals (22) were shown to bear idiotypic determinants by abrogation of T cell help after treatment with anti-Id plus complement (C) before culture.

In the previous studies, anti-Id was administered to strains that were known to produce the corresponding Id after immunization with nuclease. In the present experiments, we examined the effects of administration of anti-BALB/c Id to B10.D2 mice, a strain not normally expressing the predominant BALB/c CRI. Our results indicate that after administration of anti-Id in complete Freund's adjuvant (CFA), these recipients also produce Id'. Mice producing such "inappropriate" Id' also have been found to possess antigen-specific primed T_H cells. These T cells, as opposed to T

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Abbreviations used in this paper: ARS, azobenzenearsonate; C, complement; CFA, complete Freund's adjuvant; CRI, cross-reactive idiotype; ELISA, enzyme-linked immunosorbent assay; HAI, hemagglutination inhibition assay; Id', idiotype-positive nonantigen-binding Ig induced by treatment with anti-Id; KLH, keyhole limpet hemocyanin; nuclease, Staphylococcal nuclease; PBS, 0.05 M NaH2PO4, 0.15 M NaCl, pH 7.2; PFC, plaque-forming cell; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SRBC, sheep erythrocytes; T_H, helper T cell; TNP, trinitrophenyl; TNBS, trinitrobenzene sulfonic acid; VH, variable region of Ig.
cells from nuclease-primed B10.D2 mice, bear the inappropriate BALB/c idiotypic determinants as indicated by their functional elimination by treatment with anti-BALB/c Id plus C. These results therefore indicate that it is possible to manipulate the proportions of Id-bearing antigen-specific T\(\text{H}\) cells by treatment with anti-Id.

Materials and Methods

**Animals.** B10.D2/SgSn mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. BALB/c mice were either purchased from The Jackson Laboratory or were obtained through the Mammalian Genetics Section, National Institutes of Health, from the Charles River Breeding Laboratories, Wilmington, Mass. Miniature swine were bred and housed at the National Institutes of Health Animal Facility, Poolesville, Md.

**Antigens.** Nuclease was purified by published methods (7, 8) or was purchased from Boehringer-Mannheim Biochemicals, Indianapolis, Ind. Although no major contaminants were found on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the commercial preparation of nuclease, the specific activity of this material was approximately one-half that of enzyme prepared in our laboratory. We therefore conjugated the commercial preparation to trinitrobenzene sulfonic acid (TNBS) for use in in vitro assays, but we used nuclease purified in our laboratory for all immunizations of animals in vivo. Keyhole limpet hemocyanin (KLH; lot 530195) was purchased from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif. Both proteins were conjugated with TNBS as previously described (9) at levels of 13 and 19 TNBS groups per 100,000 mol wt of protein for KLH and for nuclease, respectively.

**Antibodies.** BALB/c antinuclease antibodies (Id) were affinity purified on nuclease coupled to Sepharose 4B, as previously described (2). Anti-Id sera from miniature swine were prepared and affinity purified as previously described (5). Pig anti-BALB/c Ig was derived from the anti-Id serum by affinity chromatography on normal BALB/c Ig during the purification of the anti-Id. Elution conditions were the same for anti-BALB/c Ig and anti-Id.

**Serologic Assays for Id and Antinuclease Activity.** Id was detected by a hemagglutination inhibition (HAI) assay using Id-coated sheep erythrocyte (SRBC) and an appropriate dilution of pig anti-Id (5). An enzyme-linked immunosorbent assay (ELISA) was also used to detect Id. For this assay, 96-well flat bottomed microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated with purified BALB/c anti-nuclease (1 \(\mu\)g/ml, 200 \(\mu\)l/well in phosphate-buffered saline [PBS]) overnight at 4°C. Unadsorbed Id was removed from the wells by washing three times with 0.025% Tween 20 in PBS (PBS-Tween) before use. Serial twofold dilutions of test sera were preincubated with purified pig anti-BALB/c Id (anti-Id) at 2.5 \(\mu\)g/ml in PBS-Tween for 1 h at room temperature. 200 \(\mu\)l of this mixture was transferred to each well of the washed Id-coated ELISA plate and incubated for 1 h at room temperature. The plates were then washed with PBS-Tween and 200 \(\mu\)l of a 1:500 dilution of horse radish peroxidase-conjugated rabbit anti-porcine Ig (Miles Laboratories, Inc., Elkhart, Ind.) was added to each well for 1 h. The plates were washed with PBS-Tween, and 200 \(\mu\)l substrate (0.1 mg/ml o-phenylenediamine plus 0.075% hydrogen peroxide) was added. The reaction was stopped after 30 min by addition of 50 \(\mu\)l 8 N HCl. Absorbance was read at 492 nm in a Titertek Multiskan apparatus (Flow Laboratories, Inc., Rockville, Md.). Antinuclease activity was determined by passive hemagglutination of nuclease-coated SRBC (5).

**Immunizations.** Mice were immunized with 100 \(\mu\)g nuclease i.p. in 0.2 ml of a 1:1 emulsion with CFA (H37Ra; Difco Laboratories, Detroit, Mich.). Anti-Id-treated mice received 20 \(\mu\)g purified anti-Id intraperitoneally in CFA on day 0 unless otherwise indicated. All animals were 2–6 mo old at immunization, and spleens were harvested from 3 to 12 wk thereafter. Mice were immunized with 100 \(\mu\)g KLH or TNP-KLH in 0.1 ml saline emulsified with an equal volume of CFA for in vitro secondary plaque-forming cell (PFC) responses.

**Preparation of Cell Populations.** Splenic T cells were isolated by nylon wool filtration (10). T cell-depleted spleen cells (B plus accessory cells) were prepared by treatment of spleen cells with rabbit anti-mouse brain serum and guinea pig C or monoclonal anti-Thy-1.2 and rabbit C (9, 11).

**Culture Conditions.** Culture conditions for in vitro primary antibody responses to TNP-KLH
and for augmented in vitro responses to TNP-nuclease have been described in detail elsewhere (9, 12). Briefly, the in vitro response to TNP-nuclease required the presence of nuclease-primed T cells; unprimed spleen cells or T cells did not generate significant responses. IgM anti-TNP antibody forming cells (PFC) were generated by nuclease-primed spleen cells or coculture of nuclease-primed T cells and unprimed B plus accessory cells with TNP-nuclease. No IgG- or IgA-secreting anti-TNP PFC were detected (12). Cells were cultured in 200-μl flat bottomed Falcon microtiter plates (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) at a final cell density of 1.5 × 10⁶ cells/ml and a final antigen concentration of 10 μg/ml for TNP-nuclease and 5 μg/ml for TNP-KLH. Cultures were harvested on day 4 or day 5. Direct anti-TNP PFC were detected on TNP-coated SRBC as previously described (12, 13). Conditions for the in vitro secondary IgG responses to TNP-KLH and TNP-nuclease are described in detail elsewhere (6). Briefly, 3 × 10⁶ TNP-primed B cells (TNP-KLH-primed spleen cells treated with anti-Thy-1.2 plus C) were cocultured with graded numbers of unprimed or primed (nuclease, anti-Id, or KLH) T cells, and responses to TNP-nuclease and TNP-KLH were assessed. For these secondary responses, antigen concentrations were 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.).

Results are expressed as the geometric mean of PFC from triplicate cultures calculated as PFC/10⁶ cultured cells. The geometric SE is given in parentheses after the geometric mean.

Anti-Id Plus C Treatment of Cells. T cells at a final concentration of 2.5 × 10⁶ cells/ml were incubated with purified pig anti-BALB/c Id or purified normal pig Ig or pig anti-BALB/c Ig at a final concentration of 250 μg/ml in minimum essential medium containing 10% FCS for 30 min at 37°C. These cells were then washed three times before incubation with a 1:7 dilution of screened rabbit C for 30 min at 37°C. The cells were then washed three times, resuspended in culture medium, and recounted before addition to in vitro cultures. Cell yields after treatment of nuclease-primed T cells with these reagents ranged from 50 to 90%. No significant difference was noted in the yields after treatment with C alone, pig anti-BALB/c Ig plus C, or pig anti-BALB/c Id plus C.

Results

Id Production in BALB/c and B10.D2 Mice in Response to Nuclease or Anti-Id. As shown in Table I, four independently derived pools of ascites from B10.D2 mice hyperimmunized with 12.5 μg purified pig anti-BALB/c Id in 0.2 ml emulsion injected intraperitoneally with CFA, day 0.

| Table I |
| --- |
| *Id in Serum and Ascites of Nuclease and Pig Anti-BALB/c Id-primed BALB/c and B10.D2 Mice* |
| --- |
| Strain | Priming | Antinuclease (logs HA titer on nuclease-SRBC) | BALB/c Id* (logs HA titer on Id-SRBC) |
| --- | --- | --- | --- |
| BALB/c | Nuclease | 10 | 7 |
| B10.D2 1016‡ | Nuclease | 11 | <1 |
| B10.D2 1027‡ | Nuclease | 11 | <1 |
| B10.D2 26‡ | Nuclease | 10 | <1 |
| B10.D2 1439‡ | Nuclease | 7 | <1 |
| BALB/c§ | Pig αBALB/c Id-CFA‖ | <1 | 7 |
| B10.D2§ | Pig αBALB/c Id-CFA‖ | <1 | 4 |

* Pig anti-BALB/c Id was used at a dilution of 1:4,000.
‡ Ascites pooled from 10 to 20 hyperimmunized mice.
§ Serum pooled from five animals and absorbed on normal pig Ig coupled to Sepharose.
‖ One dose 12.5 μg purified pig anti-BALB/c Id in 0.2 ml emulsion injected intraperitoneally with CFA, day 0.
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Fig. 1. ELISA assay of B10.D2 antinuclease antibodies for BALB/c cross-reactive Id. The ordinate shows the absorbance at 492 nm. Absorbance of uninhibited control was 0.75. Absorbance of peroxidase-conjugated rabbit anti-porcine Ig to purified BALB/c Id on the plate was 0.1. Normal BALB/c serum, O; BALB/c antinuclease ascites, V; B10.D2 antinuclease ascites, ▼; uninhibited anti-Id control, □.

Vaccinated with nuclease were tested for expression of the BALB/c Id by HAI. Despite antinuclease titers approximately equivalent to those of BALB/c antinuclease sera, none of these ascites demonstrated significant expression of the BALB/c Id. B10.D2 antinuclease ascites were also tested for expression of the BALB/c Id by an ELISA inhibition assay in which partial cross-reactivity might be seen more readily (Fig. 1). In this assay, B10.D2 antinuclease showed inhibition slightly greater than the normal BALB/c serum control; however, complete inhibition as seen with BALB/c antinuclease was never reached, suggesting the absence of the majority of BALB/c Id in the B10.D2 response. In addition, as will be shown below (Fig. 4 A), antigen-specific helper T cells from nuclease-primed B10.D2, as opposed to those from nuclease-primed BALB/c, were not eliminated by anti-BALB/c Id plus C. These results suggested that the normal B10.D2 humoral and cellular responses to nuclease do not include the BALB/c Id in substantial amounts. We therefore chose B10.D2 mice to examine the effects of anti-BALB/c Id administration in a high responder strain similar to BALB/c at the H-2 locus but not normally expressing the BALB/c Id. BALB/c and B10.D2 mice were immunized with pig anti-BALB/c Id in CFA. Both strains produced Id-bearing Ig that did not bind nuclease (Id') (Table I); however, the titer of the BALB/c Id' induced in the B10.D2 mice was lower than in similarly treated BALB/c animals. We have designated the appearance of BALB/c Id' in B10.D2 as expression of inappropriate Id'.

Anti-BALB/c Id Treatment of B10.D2 Animals Leads to Generation of Antigen-specific Helper T Cells. Previous studies showed that treatment of BALB/c mice with nuclease (12, 22) or pig anti-BALB/c Id (6) led to appearance of nuclease-specific Th cells in the spleens of these animals and a consequent ability of these whole spleen cell populations to respond to TNP-nuclease in vitro. We therefore examined spleen cells
from BALB/c and B10.D2 mice primed with either nuclease or anti-BALB/c Id. As shown in Table II, unprimed spleen cells did not produce a significant response to TNP-nuclease, although they were functional as shown by the in vitro primary response to TNP-KLH. However, both nuclease-primed and anti-Id-primed spleen cells from BALB/c and B10.D2 mice were able to generate significant anti-TNP-nuclease responses.

We have shown previously (12) that the in vitro responses of whole spleen cell populations to TNP-nuclease are dependent on the presence of primed TH cells specific for nuclease. The antigenic specificity of TH cells from BALB/c mice primed

**Table II**

*Anti-BALB/c Id Treatment of Both BALB/c and B10.D2 Mice Primes for Responses to TNP-Nuclease*

| Source of spleen cells | Treatment | In vitro antigen challenge |
|------------------------|-----------|----------------------------|
|                        |           | No Ag | TNP-Nase* | TNP-KLH† |
| BALB/c                 | None      | 8 (1.26)§ | 5 (1.26) | 162 (1.11) |
| BALB/c                 | Nuclease  | 5 (1.44) | 89 (1.24) | 151 (1.03) |
| BALB/c                 | Anti-Id   | 10 (1.22) | 147 (1.54) | 175 (1.23) |
| B10.D2                 | None      | 5 (1.44) | 12 (1.10) | 96 (1.19) |
| B10.D2                 | Nuclease  | 5 (1.26) | 103 (1.14) | 114 (1.04) |
| B10.D2                 | Anti-Id   | 8 (1.53) | 189 (1.64) | 198 (1.47) |

* TNP-Nase was added to cultures at 5 μg/ml final concentration.
† TNP-KLH was added to cultures at 10 μg/ml final concentration.
§ Figures are geometric mean with geometric SE in parentheses.
∥ Animals were primed with 20 μg pig anti-BALB/c Id in CFA.

**Fig. 2.** Helper T cells from anti-Id-primed and nuclease-primed B10.D2 are specific for nuclease is secondary IgG responses. Each culture contained $3 \times 10^6$ anti-Thy-1.2 plus C-treated TNP-KLH-primed (B plus accessory cells) (B10.D2). Graded numbers of T cells were added as indicated on the abscissa. In (A), cultures were challenged with TNP-nuclease; in (B), cultures were challenged with TNP-KLH. ● indicates nuclease-primed B10.D2 T cells; ○, anti-BALB/c Id-primed T cells; ▲, KLH-primed B10.D2 T cells.
FIG. 3. Id of nuclease-primed (A, B) and anti-Id-primed (C, D) BALB/c T cells. T cells from nuclease-primed (closed symbols) or anti-Id-primed (open symbols) BALB/c mice were added to 3 × 10⁴ unprimed BALB/c B plus accessory cells and challenged in vitro with TNP-nuclease (A, C) or TNP-KLH (B, D). Before culture, T cells were untreated (○, □) or treated with pig anti-BALB/c Ig plus C (△, △) or with pig anti-BALB/c Id plus C (■, ■). X indicates PFC/10⁶ cultured cells with no added T cells.

with anti-BALB/c Id has previously been demonstrated (6) using an in vitro secondary IgG PFC response. Because treatment of B10.D2 mice with anti-BALB/c Id might produce unexpected activation of T cells other than those specific for nuclease, we examined the antigenic specificity of the anti-Id-primed B10.D2 T cells using the IgG secondary PFC response. As seen in Fig. 2, anti-Id and nuclease priming of B10.D2 mice generated TH cells for TNP-nuclease IgG responses (Fig. 2 A) but not TNP-KLH secondary responses (Fig. 2 B). These results suggested that anti-BALB/c Id could prime antigen-specific helper T cells in a strain in which that Id does not comprise the dominant humoral or cellular response to nuclease.

*Nuclease-specific Helper T Cells from B10.D2 Mice Primed with Pig Anti-BALB/c Id Bear BALB/c Id.* We found previously (6, 12) that nuclease-primed T cells from BALB/c and B10.D2 mice cooperate equally well with either unprimed BALB/c or unprimed B10.D2 B cells for the anti-hapten response. Because these strains are both H-2d and Mlsb, allogeneic effects were not observed (22). We therefore examined the Id expressed on nuclease and anti-Id-primed BALB/c and B10.D2 T cells by coculturing a single population of unprimed BALB/c (B plus accessory) cells with the various primed T cells. In this manner, potential differences between B cell populations
Figure 4. Id of nuclease-primed (A, B) and anti-Id-primed (C, D) B10.D2 T cells. T cells from nuclease-primed (closed symbols) or anti-Id-primed (open symbols) B10.D2 mice were added to 3 × 10^5 unprimed BALB/c B plus accessory cells and challenged in vitro with TNP-nuclease (A, C) or TNP-KLH (B, D). Before culture, T cells were either untreated (●, ○) or treated with pig anti-BALB/c Ig plus C (▲, △) or with pig anti-BALB/c Id plus C (□, □).

responding to the hapten TNP in each culture were avoided. As shown in Fig. 3 A, C, both nuclease-primed and anti-Id-primed BALB/c T cells provided help for the TNP-nuclease response. Treatment of these T cells with pig anti-Id plus C before culture significantly diminished the responses to TNP-nuclease but had no effect on responses to TNP-KLH (Fig. 2 B, D). Control treatment with pig anti-BALB/c Ig derived from the anti-Id serum during its purification had no effect on either response. As seen in Figure 4 A, C, T cells from B10.D2 mice primed either with nuclease or with anti-Id also generated help for anti-TNP-nuclease responses. However, whereas nuclease-primed B10.D2 T cells were unaffected by treatment with pig anti-BALB/c Id and C, similar treatment of anti-BALB/c Id-primed B10.D2 T cells significantly diminished responses to TNP nuclease. Responses to TNP-KLH were again unaffected by anti-Id plus C treatment (Fig. 4 B, D). Thus, nuclease-specific T_H cells from B10.D2 mice primed with anti-BALB/c Id appeared to express BALB/c idiotypic determinants not detected on helper T cells from B10.D2 mice immunized with nuclease.

Discussion

The experiments described here indicate that in vivo treatment of B10.D2 mice with pig anti-BALB/c Id leads to the appearance of both nuclease-specific helper T cells and non-nuclease-binding Ig molecules expressing determinants similar to those
detectable on BALB/c antinuclease antibodies. B10.D2 mice normally do not express predominant amounts of BALB/c antinuclease Id, as evidenced by the weak inhibition of the binding of anti-Id to Id by B10.D2 antinuclease antibodies. The majority of nuclease-specific B10.D2 TH cells generated normally during the response to antigen also fail to express BALB/c idiotypic determinants, as evidenced by the lack of effect of anti-Id plus C on nuclease-primed B10.D2 T cells. Thus, it appears that a strain that does not normally express a particular Id (or does so at low levels) in response to antigens can nevertheless be induced to express this Id on a substantial proportion of TH cells and on Ig molecules (Id') by treatment with anti-Id. We have called this phenomenon inappropriate Id expression.

A result that may be analogous to those reported here has been found by LeGuern et al. (14), who demonstrated that DBA/2 mice could be induced to express MOPC 460 Id on anti-dinitrophenyl antibodies if they were immunized with anti-MOPC 460 before immunization with dinitrophenyl-ovalbumin.

In contrast to these findings, Eichmann and colleagues (15) were able to induce helper T cells in mice with anti-Id only if the recipients expressed that Id in response to the antigen A.CHO; thus, the administration of anti-A5A could prime TH cells from A/J mice but not BALB/c mice, whereas anti-S117 could prime TH cells of BALB/c mice but not those of A/J mice. One possible explanation for the discrepancies among these results may involve differences in the method used to prime with anti-Id. In previous experiments, we found that anti-Id was equally effective in inducing Id' when given in saline or CFA to recipients that normally expressed that Id during the antinuclease response. In the current experiments, however, B10.D2 mice produced inappropriate Id' only when anti-BALB/c Id was given in CFA (data not shown). Although we have not tested T cells in vitro from animals primed with inappropriate anti-Id in saline, it seems likely that the induction of Id' may correlate with the appearance of primed helper T cells. The experiments of LeGuern et al. cited above (14), which demonstrated production of Id in an otherwise negative strain, also used a regimen that included CFA for priming with anti-Id. In contrast, immunization with anti-A5A has been reported with saline but not with CFA (15). It seems possible, therefore, that priming of T cells and subsequently B cells by anti-Id in a strain that does not normally express the Id requires that the treatment be given in CFA. This requirement may be a reflection of the existence of very small numbers of T cells bearing the BALB/c Id in B10.D2 mice and the effect of adjuvant in enhancing the probability that anti-Id present at the site of administration will interact with appropriate T cells.

Another possible explanation for the discrepancy involves differences in Id repertoires present in the genome. It is clear from suppression of the CRI-positive azobenzenearsionate (ARS) response with anti-CRI in A/J mice that genes encoding multiple Id for anti-ARS antibodies are present in the genome but are usually not expressed (16). Similarly, the expression of individual idiotopes in the anti-4-hydroxy-3-nitrophenyl acetyl response can be regulated by administration of monoclonal anti-idiotopes such that individual idiotopes may be expressed in greater quantity than normal (17).

Viewed in this context, the inhibition of anti-BALB/c Id binding seen with B10.D2 antinuclease and normal BALB/c serum (Fig. 1) may represent low level expression of some BALB/c antinuclease idiotopes. The ability to detect BALB/c Id on TH cells
and Id' in B10.D2 mice after treatment with anti-BALB/c Id might reflect perturbation of mechanisms regulating idiotopes already represented in the genome. Indeed, it might be suggested that such enhanced expression of idiotopes could not be induced by anti-Id unless the appropriate information encoding such VH sequences were present in the genome. If this is the case, the inability to induce anti-A.CHO helper T cells in A/J mice with anti-S117 might be a reflection of the true absence of a germ line gene(s) needed to generate the appropriate amino acid sequence.

In either case, the ability in several systems to expand the Id repertoire expressed by B cells and T cells has several important implications for the study of antibody and receptor diversity. First, it indicates the existence of mechanisms for regulating the expression of multiple V gene products. In the response to ARS, for example, one level of regulation for the predominant Id may occur at the level of cellular interactions involving Id-specific suppressor cells (18). However, the mechanisms by which the expression of other Id are regulated in the absence of suppression of the CRI remain unknown. Another mechanism operating at the cellular interaction level has been proposed by Eichmann et al. (19), who suggested that expression of one Id in a response may be increased through additional help provided by idiotypically restricted helper T cells. In addition to regulatory mechanisms operating at the level of cellular interactions, other potential points of regulation, such as at the DNA level of transcription or RNA translation, could also exist, but these have yet to be described.

A second implication of these studies is that genetic maps of the VH region based on Id expression may not be a true reflection of the organization of VH structural genes. The expression of most Id has been shown to be linked to genes coding for heavy chain allotypes (4, 20). Such studies have usually been performed by analysis of the segregation of Id expression and allotype markers in backcross populations. The simplest interpretation of these studies has been that Id expression reflects the presence of structural genes linked to genes encoding heavy chain allotype determinants. However, the finding that VH structural genes may exist but not be expressed indicates that such mapping studies may detect regulatory rather than structural genes.

Finally, these results are consistent with regulatory effects of the Id-anti-Id interactions predicted by Jerne's network hypothesis (21). The relationship between the observed effects of anti-Id administered experimentally and events occurring normally in vivo remains to be elucidated. However, these data are consistent with the hypothesis that anti-Id administration in vivo leads to perturbation of the Id-anti-Id network normally existing in the immune response to nuclease. Such perturbation may afford a means of specifically manipulating this immune response.

**Summary**

Immunization of BALB/c mice with nuclease leads to the production of antinuclease antibodies bearing a set of cross-reactive idiotypes (Id) distinct from those produced by B10.D2 mice after similar immunization. In both strains, such immunization with nuclease also leads to the production of splenic T helper cells (T\(_H\)), which provide nuclease-specific help in an in vitro plaque-forming cell response to nuclease-TNP. Pig anti-(BALB/c antinuclease) anti-idiotypic antibodies (pig anti-BALB/c Id) react only with T\(_H\) of nuclease-primed BALB/c and not with B10.D2 animals.
After administration of pig anti-BALB/c Id in complete Freund's adjuvant to BALB/c and B10.D2 mice, Id-bearing nonantigen-binding molecules were induced in both strains. Such treatment also resulted in the induction of nuclease-specific splenic T_H cells in both strains. BALB/c T_H cells induced by anti-Id, like the majority of nuclease-primed BALB/c T_H cells, bore BALB/c Id, as shown by their functional elimination with anti-Id plus complement. B10.D2 T_H cells induced by anti-Id, unlike T_H cells from nuclease-primed B10.D2 mice, also bore BALB/c idiotypic determinants by the same criterion. Thus, it appears that one can manipulate the expression of Id on serum immunoglobulins and on antigen-specific T_H cells by administration of exogenous anti-Id reagents. These results have implications both for network interactions in the immune response and for the genetic basis of Igh-C linked Id expression.

Received for publication 31 August 1981.

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