NON-DINITROPHENYL-BINDING IMMUNOGLOBULIN THAT BEARS A DOMINANT IDIOTYPE (Id460) ASSOCIATED WITH ANTIDINITROPHENYL ANTIBODY IS SPECIFIC FOR AN ANTIGEN ON PASTEURELLA PNEUMOTROPICA

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Dominant idiotypes have been described in several different antigenic systems (1). Most dominant idiotypes are associated with immunoglobulins that are specific for bacterial antigens (for a summary see Table III). We have previously characterized a dominant idiotype, Id460, that is produced in response to secondary immunization with 2,4-dinitrophenyl-ovalbumin (DNP-OVA) in mice having appropriate genes encoding Igh-V and Vk (2, 3). Similar to other dominant idiotypes (see Table III), Id460 is also present in normal, preimmune mouse serum; however the normal serum Id460-positive immunoglobulin does not bind DNP (4).

The high levels of Id460 measured in normal sera from conventionally reared mice (4) suggested that the normal serum, non-DNP-binding Id460, might be directed toward a commonly encountered environmental antigen. The lower levels of Id460 detected in sera from germ-free mice supported this idea. To test this hypothesis, three previously described (4) Id460-positive non-DNP-binding monoclonal antibodies have been screened for their ability to agglutinate various bacterial isolates cultured from our mouse colony. Two of the monoclonal antibodies agglutinate an isolate of Pasteurella pneumotropica (Ppneumo), an opportunistic pathogen among the normal flora of mice (5), but no other bacteria that have been tested. Furthermore, Ppneumo not only induces an increase in serum Id460 without increasing serum anti-DNP antibody but also absorbs the majority of Id460 activity out of both normal, preimmune, and Ppneumo immune sera. Therefore, we propose that the Id460 present in normal mouse

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Abbreviations used in this paper: Anti-MIg, rabbit anti-mouse immunoglobulin antibody; AP, alkaline phosphatase; BS, borate saline buffer, pH 8.4; BSA, bovine serum albumin; DNP, 2,4-dinitrophenyl; ELISA, enzyme-linked immunosorbent assay; Id460, idiotype associated with the DNP-binding myeloma protein MOPC 460; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide from E. coli 055:B5; OVA, ovalbumin; PBS, phosphate-buffered saline, pH 7.2; Ppneumo, Pasteurella pneumotropica; Raid460, rabbit antiidiotype prepared against MOPC 460 myeloma protein; RIA, radioimmunoassay.

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serum is induced by an unknown antigenic determinant on Pasteurella pneumotro-
pica and perhaps other microorganisms encountered environmentally.

Materials and Methods

Mice and Immunizations. BALB/cByJ mice were purchased from The Jackson Labora-
tory, Bar Harbor, ME. Immunizations with DNP5-OVA were performed as previously
described (2). Immunizations with either live or formalinized Ppneumo were intraperito-
near. The number of organisms injected was estimated by nephelometry at 550 nm; the
actual numbers injected were determined by plating limiting dilutions of the live innocu-
ulum on sheep blood agar plates.

Bacteria and Slide Agglutinations. The various bacterial isolates used in these experi-
ments were cultured from the nasopharynx and/or gut of normal mice in our colony
during routine necropsy. Pure cultures of each organism were established by standard
protocol. Isolates of gram-negative rods, Escherichia coli, Citrobacter freundii, Pasteurella
pneumotropica, Pasteurella hemolytica, Pasteurella multocida, and Pseudomonas aerugino-
sa were speciated by biochemical and/or fermentative criteria using a standard API-20e (Analytical
Profile Index) system (Analytab Products, Plainview, NY) (6). Streptococcal and staphy-
lococcal isolates were identified by standard methods (7). Different isolates of Ppneumo
were obtained by independent isolation from individual mice.

Bulk cultures of Ppneumo were grown in either tryptase soy or brain-heart-infusion
broth by overnight rotation in a 37°C air shaker (New Brunswick Scientific, Edison, NJ).
The organisms were collected by centrifugation and washed three times in physiological
phosphate-buffered saline, pH 7.2 (PBS). Immunizations and/or absorptions with live
organisms were performed immediately after washing. Formalinized organisms were
incubated in 0.1% formalin saline at 37°C for 1 h, then overnight at 4°C, centrifuged,
and finally resuspended in fresh 0.1% formalinized saline (5). These were stored at 4°C.

Enzyme-linked Immunosorbent Assays (ELISA)

Id460. Serum and supernatant Id460 levels were quantitated in a competitive ELISA
using polystyrene microtitre plates (Dynatech, Alexandria, VA) coated with limiting dilutions
of rabbit anti-Id460 (Raid460) in BS, blocked with 1% BSA in BS, and assayed for
inhibition of binding of alkaline phosphatase- (AP) (type VII, Sigma, St. Louis, MO)
conjugated MOPC 460 to the Raid460-coated plates. This assay is identical to the
radioimmunoassay (RIA) previously described (2). The preparation of the Raid460 was
as previously described (2) with the exception of an additional absorption with MOPC
315-coupled Sepharose. The resulting Raid460 reacted with MOPC 315 myeloma protein
and V1 myeloma proteins other than MOPC 460 1/2,000 as well as with purified MOPC
460 protein. Serial threefold dilutions of sera and supernatants were in 1% BSA in BS.
Titers are expressed as the reciprocal dilution that produced 50% inhibition of binding
of the AP-MOPC 460, and relative titers are the 50% inhibition titers multiplied by the concentration of MOPC 460 protein that produced 50% inhibition of AP-MOPC 460 binding.

**Anti-DNP.** This assay was performed similarly to the RIA previously described (2). Polystyrene microtiter plate wells were coated with 100 μl of 10 μg/ml DNP6-KLH in BB and blocked with 1% BSA in BB. Sera or culture supernatants were diluted and serially, threefold, titered in 1% BSA in BB. Bound antibody was detected with AP-conjugated rabbit anti-mouse immunoglobulin (anti-MIg). The preparation and conjugation of the anti-MIg was as previously described (2, 8). Titers are expressed as reciprocal dilutions that produced 50% of maximum binding of a standard anti-DNP antibody that was added to each plate.

**Anti-Pneumo.** This assay is a modification of an anti-group A streptococci assay (D. Bibles, personal communication). Between 5 × 10⁷ and 5 × 10⁸ Pneumo bacteria in 100 μl of saline were added per well to polystyrene microtiter plates. The plates were then dried at 56°C, and the bacteria were fixed by a 5-min incubation in methanol. The plates were then washed two times by flooding with saline and blocked with a 2-h incubation with 1% BSA in BB. Serum titrations and addition of an AP-anti-MIg were identical to the anti-DNP assay described above. Titers are expressed as reciprocal dilutions that gave 50% of maximum binding compared to a standard anti-Pneumo serum added to each plate.

All of the ELISAs described above were developed by the addition of 100 μl/well of 1 mg/ml phosphatase substrate (Sigma 104; Sigma Chemical Co., St. Louis, MO) in diethanolamine buffer, pH 9.8 (8). Phosphatase reactions were stopped by the addition of 50 μl/well of 3 N NaOH. Developed plates were scanned for absorbances at 405 nm on an automated ELISA plate reader.

**Results**

*Id460-Positive, Non-DNP-binding Monoclonal Antibodies Agglutinate Pasteurella pneumotropica.* Idiotype (Id460)-positive, non-DNP-binding hybridomas have been generated by in vivo stimulation of normal BALB/c mice with lipopolysaccharide from *E. coli* 055:B5 (LPS) and fusion of their spleen cells with SP-2/0 (4). Several of these hybridomas were expanded as ascites tumors after cloning. To determine whether these Id460-positive Ig have specificity for bacterial antigens, the ascites fluids were tested for their ability to agglutinate various bacterial strains isolated from mice in our colony (Table I). Ascites fluids from two of three hybridomas tested, LB8 and LF4d, agglutinated Pneumo but not Pasteurella multocida or Pasteurella hemolytica. Neither of these hybridoma antibodies agglutinated other genera of bacteria such as *E. coli* or *Pseudomonas aeruginosa*.

Pneumo isolates UMRL, N1-1, N5-1, N2-2, N4-4, and 460 were all cultured from mice in our colony. Each isolate was typed as Pneumo by biochemical/fermentative criteria using the standard API system. MOPC 467 myeloma protein (generously provided by Dr. A. Mason Smith, East Carolina University Medical School, Greenville, NC), which is Id460 negative, has been previously shown to bind Pneumo (9) and is capable of agglutinating all of the Pneumo isolates we have tested with the exception of the UMRL isolate (Table I). The Id460-positive monoclonal antibodies from both LB8 and LF4d were able to agglutinate Pneumo 460 but not the other isolates. MOPC 460 myeloma protein (Id460 positive) did not agglutinate any of the Pneumo isolates, nor did the Id460-negative myeloma proteins MOPC 315, TEPC 817, or TEPC 105, the latter two tested as ascites fluids. Pooled BALB/c normal mouse serum also did not agglutinate Pneumo.
Id460-positive monoclonal antibodies from two additional hybridomas, 2AB5 and Ppl, also bind to P pneumotropica 460 (Fig. 1a and b). 2AB5 was obtained by fusing spleen cells from an anti-Id460–immunized BALB/c mouse with SP2/0 (Lee, H.-S., E. A. Lerner, E. A. Dzierzak, and C. A. Janeway, unpublished results). Ppl was obtained by fusing spleen cells from a P pneumotropica 460–immunized BALB/c mouse with P3x63-Ag8.653 (10). Antibodies from both 2AB5 and Ppl were absorbed by P pneumotropica 460, but neither antibody bound DNP by solid-phase ELISA criteria (Fig. 1a and b). Using the same conditions for absorption, the Id460-positive, DNP-specific monoclonal antibody from D35, a hybridoma obtained by fusing spleen cells from a DNP-keyhole limpet hemocyanin (KLH)–immunized mouse (Dzierzak and Janeway, unpublished observation), was not absorbed by P pneumotropica 460 (Fig. 1c). Just as 2AB5, LB8, and LF4d antibodies agglutinated only P pneumotropica 460 and not other P pneumotropica isolates (Table I), Ppl antibody was absorbed by P pneumotropica 460 but not by P pneumotropica UMRL (Fig. 1a).

P pneumotropica Induces the Production of Id460-positive, Non–DNP-binding, Specific Antibody in BALB/c Mice. Since non–DNP-binding, Id460-positive monoclonal antibodies from LPS- or anti-Id460–generated hybridomas were able to bind P pneumotropica, we reasoned that non–DNP-binding, Id460-positive antibody detected in normal mouse serum may be induced by P pneumotropica and other environmentally encountered bacteria bearing the same antigenic determinant(s). We tested this hypothesis by immunizing BALB/c mice with live or formalin-fixed P pneumotropica.
FIGURE 1. Absorption of Id460-positive monoclonal antibodies with Ppneumo. Closed symbols, percent inhibition of binding of AP-MOPC 460 to RaId460 in a competitive ELISA; open symbols, percent binding to DNP-KLH in a direct-binding ELISA; (-----), unabsorbed culture supernatant; (---), culture supernatant absorbed with (a) $10^{10}$ and (b and c) $2 \times 10^{10}$ Ppneumo 460; (-----), culture supernatant absorbed with $5 \times 10^{10}$ Ppneumo UMRL. (a) Pp1 is a hybridoma from a Ppneumo-immunized mouse; (b) 2AB5, a RaId460-immunized mouse; and (c) D35, a DNP-KLH-immunized mouse. D35 has been previously shown to be specific for DNP (Dzierzak and Janeway, unpublished results).
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Figure 2. Induction of Id460-positive but not DNP-binding antibody by Ppneumo immunization. Pooled sera from eight mice were assayed (see footnotes for Table III). (○) Ppneumo binding titers after Ppneumo 460 immunization; (△) relative Id460 titers after Ppneumo immunization; (□) DNP binding titers after Ppneumo immunization; (▼) anti-DNP titers 7 and 14 d after primary DNP-OVA immunization.

460 and then assayed sera for Id460, anti-DNP, and anti-Ppneumo titers (Fig. 2 and Table II). Immunization with live Ppneumo 460 induced a threefold increase in serum Id460 antibody that was accompanied by a marginal increase in serum anti-DNP titer. By comparison, the titers of anti-DNP antibody induced by specific, primary immunization with DNP-OVA are 5-40-fold greater than those induced by Pneumo over the same time periods (Fig. 2). The kinetics of Id460 induction exactly paralleled those for induction of anti-Ppneumo-specific antibody in the same serum pool. These results strongly suggested that Pneumo can stimulate the production of Id460-positive antibody without inducing concomitant increases in serum anti-DNP titers.

These results were confirmed by absorbing both normal, preimmune, and Ppneumo-immune BALB/c mouse sera with live Ppneumo 460 (Fig. 3 and Table II). The majority of Id460-positive antibody in both preimmune and immune mouse sera can be removed by absorption with Ppneumo 460 since absorption of either reduces the Id460 titer to approximately the same level. 50-70% of the Id460-positive Ig in most normal BALB/c mouse sera can be removed by absorption with Ppneumo 460. After immunization with Ppneumo 460, 80-90% of the increase in serum Id460 above the level in Ppneumo-absorbed preimmune
**Table II**

Specific Induction and Absorption of Id460-positive Antibody by *P. pneumotropica*

| Experiment | Days | Relative Id460 titer* | Percent of increase over background absorbed\(^a\) |
|------------|------|-----------------------|-----------------------------------------------|
|            |      | Unabsorbed | Absorbed |                                      |
| 1\(^b\)    | 0    | 87         | 41\(^f\) | —                                        |
|            | 5    | 67         | 23       | 100                                      |
|            | 7    | 111        | 84       | 38                                       |
|            | 10   | 138        | 73       | 73                                       |
|            | 14   | 265        | 72       | 72                                       |
| 2          | 0    | 55 ± 15    | 18 ± 3\(^f\) | —                                        |
|            | 11   | 200 ± 41   | 28 ± 1   | 91 ± 3                                   |
| 3          | 0    | 44 ± 9     | 18 ± 2\(^f\) | —                                        |
|            | 5    | 109 ± 74   | 29 ± 10  | 72 ± 30                                  |
|            | 7    | 118 ± 35   | 33 ± 4   | 80 ± 8                                   |
|            | 10   | 117 ± 19   | 31 ± 6   | 78 ± 13                                  |
|            | 14   | 72 ± 16    | 24 ± 4   | 96 ± 8                                   |

* Sera were diluted 1:100 and 1 ml of each dilution was absorbed with 1.6 \(\times 10^{10}\) live *Ppneumo* 460 in Experiment 1, 10\(^9\) live *Ppneumo* 460 in Experiment 2, and 10\(^9\) – 2 \(\times 10^{10}\) in Experiment 3. Unabsorbed sera were diluted 1:100 before assay for Id460 in a competitive ELISA. Addition of the bacteria used for absorption did not result in further detectable dilution of the 1:100 diluted sera when an irrelevant radioactive tracer was used to measure such dilution. Sera from individual mice were pooled in Experiment 1; the results from Experiments 2 and 3 are the means ± standard errors of sera from individual mice.

\(^a\) The percent of increase in serum Id460 over the normal serum background that could be absorbed by *Ppneumo* 460 was determined as follows: 
\[(\text{Unabsorbed Id460 titer on day } n) - (\text{Absorbed Id460 titer on day } n)] / (\text{Unabsorbed Id460 titer on day 0}) - (\text{Absorbed Id460 titer on day 0})\]

\(^b\) Eight mice were each immunized intraperitoneally on day 0 with 3 \(\times 10^{10}\) live *Ppneumo* 460 in Experiment 1; 5 mice, with 10\(^9\) *Ppneumo* 460 vaccine in Experiment 2; and 8 mice, with 3 \(\times 10^{7}\) live *Ppneumo* 460 in Experiment 3. Immunized mice were bled for sera on the indicated days after immunization.

\(^f\) The normal serum background Id460 is that proportion of Id460 in normal serum that was not absorbed by *Ppneumo* 460.

Serum can be removed by absorption with *Ppneumo* 460 (Table II). The Id460-positive antibody induced by immunization with *Ppneumo* remains specific for *Ppneumo* 460 over the entire time course of the response since the levels of non-*Ppneumo*-specific, Id460-positive Ig were observed to remain fairly constant (Table II). Both the kinetics and final peak levels of the Id460-positive portion of the anti-*Ppneumo* response were quite variable from animal to animal and experiment to experiment; however, the specific induction of Id460-positive antibody by *Ppneumo* was consistently observed.

The absorption of Id460-positive Ig from normal and immune sera appeared to be specific. Very little antigen binding or idiotypic activity could be removed from BALB/c anti-OVA serum, diluted 1:200 for absorption, or MOPC 460 myeloma protein, diluted to 5 \(\mu\)g/ml for absorption, respectively, when either was absorbed with the same bacterial preparations used for the absorptions.
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Figure 3. Absorption of Id460 from normal and Pneumo 460-immune sera (see footnotes to Table III). The bars indicate relative Id460 titers in sera from five individual mice on days 0 and 11 after Pneumo immunization. The stippled portion of each bar represents that proportion of the Id460-positive Ig absorbed by Pneumo 460. As a measure of non-specific absorption, 1 ml each of 5 µg/ml MOPC 460 myeloma protein and 1:200 diluted BALB/cByJ anti-OVA serum were each absorbed with 2 × 10^9 live Pneumo 460. MOPC 460 was assayed for Id460 activity before and after absorption. Anti-OVA serum was assayed before and after absorption for binding to OVA in a solid-phase ELISA. Activity in each case is presented as percent activity of the unabsorbed sample.

Described above. However, slight and quantitatively identical nonspecific absorption did occur in both cases. This demonstrates that, unlike Pneumo-induced, Id460-positive antibody, the Id460-positive, DNP-binding myeloma protein MOPC 460 does not bind Pneumo to a significant extent.

Discussion

The present studies demonstrate that an opportunistic mouse pathogen, Pasteurella pneumotropica, found in the nasopharynx and/or gut of most conventionally reared mice (5, 9), specifically absorbs several Id460-positive, non-DNP-binding monoclonal antibodies and specifically induces the production of Id460-positive antibody that does not bind DNP. 50% of normal serum Id460-positive Ig and 80–90% of the increase in Id460-positive Ig induced by immunization with Pneumo can be removed by absorption with Pneumo; therefore it would seem that two distinct populations of Id460-positive antibodies exist and can be respectively stimulated by immunization with DNP or with Pneumo.

Hybridomas that produce monoclonal Pneumo-binding antibodies that are Id460-positive and non-DNP-binding have been generated by fusing spleen cells from LPS-stimulated (4), RaId460-immunized, and Pneumo-immunized...
### TABLE III

**Antigenic Specificities for Dominant Idiotypes**

| Idiotype family | Hapten specificity | Level in NMS* | NMS Id binds hapten | Environmental form of antigen bound by the idiotype family | References |
|-----------------|-------------------|---------------|---------------------|----------------------------------------------------------|------------|
| NP              | NP, NIP, NIP      | 0.010*       | Yes                 | ?                                                         | 12, 13     |
| CR1             | azaphenylarsonate | 10-20        | No                  | Brucella abortus                                         | 14-16      |
| A5A             | N-acetylglucosamine | 30*         | Yes                 | Group A streptococci                                     | 17, 18     |
| T15             | phosphocholine    | 10-100       | Yes                 | Streptococcus pneumoniae                                | 19         |
| DEX-IdX         | dextran           | 500          | Yes                 | Lactobacillace                                           | 20, 21     |
| 460             | DNP               | 20-100       | No                  | Pasteurella pneumotropica                                | 2-4, This paper |
| E109-IdX        | insulin           | 10           | Yes                 | Lactobacillace                                           | 22         |

Lgh-V region markers:

| U10-175 | Levan, galactan | 10-200 | No | Lactobacillace | 23 |
| J606-GAC | N-acetylglucosamine, levan | 500-500* | Yes | Lactobacillace | 24 |

* Normally detected levels of the indicated idiotypes in normal mouse serum, µg/ml.
* Normal serum idiotype-bearing immunoglobulin binds the hapten for which induced idiotype-positive antibody is specific.
* Bacteria that induce and/or bind the indicated idiotype-positive immunoglobulin.
* T. Imanishi-Kari, personal communication.
* D. Briles, personal communication.
* Basra, P. and D. E. Briles. The mouse H chain variable region marker J606-GAC is not restricted to particular B cell isotypes or subsets. Submitted for publication.

BALB/c mice. Since LPS stimulates B cells in a non-antigen-specific fashion (11), B cells that are programmed to produce Ppneumo-binding, Id460-positive antibody must be part of the normal repertoire of antigen-responsive B cells in BALB/c mice. That these monoclonal antibodies do not bind to DNP (4) further suggests that DNP-binding and Ppneumo-binding, Id460-positive antibodies represent distinct antibody populations. The possibility remains, however, that among Id460-positive Ig there may exist antibodies that bind both antigens. More Ppneumo- and DNP-binding, Id460-positive antibody-producing hybridomas are being generated to address this issue.

Idiotype-positive Ig in normal serum bearing dominant idiotypes, including Id460, does not always appear to be specific for the antigen normally used to induce the particular idiotype (Table III). In such cases, the antiidiotypic reagent that detects idiotype in normal serum may be directed toward idiotopes that are common to Ig with disparate antigenic specificities. A recent hypothesis has been put forth by Paul and Bona (25) that such idiotopes represent regulatory idiotopes. These regulatory idiotopes may be targets for regulatory mechanisms responsible for maintaining a balance among the network of specific and “non-specific, parallel sets” of idiotope-bearing Ig (26). As our interest in this system is to determine the role of idiotypes in immune regulation, it is precisely those idiotopes present in normal serum that most interest us.

These results add more evidence to the increasing body of information that suggests that inherited or dominant idiotypes may represent the products of antibody variable region genes that have been selected and maintained through evolution because of their ability to bind to antigens encountered on potentially pathogenic bacteria, fungi, or parasites (27). Briles et al. (28) have elegantly demonstrated that T15 idiotype-positive, antiphosphocholines antibodies present in normal mouse serum have important survival value for mice by providing a first line of defense against infection with *Streptococcus pneumoniae*. A list of several inherited and/or dominant idiotypes and their antigenic specificities are pre-
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Presented in Table III. Immediately obvious is the fact that for all but one group listed, these antibodies are specific for bacterial antigens. Moreover, given the intensity and unusual characteristics of the primary anti-NP antibody response, it seems likely that the idiotype associated with anti-NP antibodies will also be found on antibody specific for an environmental antigen(s) (27).

All mice maintained in conventional facilities carry Ppneumo as part of their normal flora (references 5, 9, and Marion and Janeway, unpublished observation) and are susceptible to Ppneumo-induced pneumonia if their resistance mechanisms become compromised. Sendai virus-infected mice are quite susceptible to Ppneumo-induced pneumonia (29). Furthermore, the recent description of a genetic mutation (SCID) that leads to severe combined immunodeficiency in mice (30) further suggests that Ppneumo-binding, Id460-positive antibody may serve a protective function. A significant percentage of SCID mice raised in a conventional facility succumb to infections with Ppneumo (30).

The phenomenon of idiotype dominance is well established; however, the mechanisms leading to its establishment are poorly understood. Three sets of hypotheses related to dominant idiotype expression need to be tested: (a) idiotype dominance is due to the selective rearrangement of germline genes that encode an optimal antibody for the test antigen (31); (b) environmental antigen(s) primes B cells that may or may not be antigen cross-reactive for the test antigen but do express the relevant dominant idiotype; and (c) idiotype-specific regulation via antiidiotype antibody or idiotype-specific regulatory cells may control dominant idiotype expression (25-27, 32-39).

A particularly good example of idiotype-specific regulation in the form of an idiotype-recognizing helper T cell (ThId) occurs in the T-dependent antiphosphocholine response (32, 38, 39). T15 idiotype expression in response to T-dependent forms of phosphocholine is influenced by a T15-recognizing ThId. T15-specific suppressor T cells have also been found to be operating in the T15-positive antiphosphocholine response (37). Idiotype regulation of the transiently dominant CRI-positive antiarsonate response has also been observed (40) and appears to involve a ThId specific for CRI (41). Furthermore, immunization with a monoclonal anti-CRI antibody (AD8) has suggested a regulatory idiotypic connectance between CRI-positive, arsonate binding and CRI-positive, non-arsonate-binding antibody populations (14).

The present results in combination with our previous observations (2-4) indicate that a regulatory connectance may exist between Id460-positive, DNP-binding and Id460-positive, non-DNP-binding antibody populations. Moreover, since we have previously demonstrated the requirement for an Ig dependent helper T cell (ThIg) for optimal DNP-specific antibody production to DNP-OVA (42), an Id460-specific ThId may also be involved in the regulation of dominant Id460 expression in responses to DNP-OVA. Ppneumo-binding, normal serum Id460 Ig may provide the initiating stimulus for the ThIg cell that regulates the antibody response to DNP-OVA.

In conclusion, non-DNP-binding, Id460-positive Ig detected in normal mouse serum appears to be antibody induced by the opportunistic mouse pathogen Ppneumo. Immunization of BALB/c mice with Ppneumo induces increased serum levels of Id460 that can be removed by absorption of Ppneumo-immune
sera with Ppneumo. Future efforts to understand the Id460 family of antibodies will concentrate on serological and molecular genetic analyses of the different antigen-binding groups of Id460 antibodies and on the relationship between expression of the Ppneumo-binding, naturally occurring Id460-positive antibody and the DNP-specific, Id460-positive antibody made in response to DNP immunization.

Summary

We have previously described an idiotype (Id460) that transiently dominates anti-2,4-dinitrophenyl (DNP) antibody responses of mice that possess the appropriate Igh-V and Vd genotypes. Normal serum has significant levels of Id460 that does not bind DNP, and hybridomas derived from spleen cell fusions that produce monoclonal antibodies with these characteristics have been generated. Many of these monoclonal, Id460-positive antibodies bind the opportunistic mouse pathogen Pasteurella pneumotropica. P. pneumotropica induces a marked increase in serum Id460 titers without significantly increasing serum anti-DNP titers. Both normal serum and P. pneumotropica-induced Ig460-positive immunoglobulin specifically bind to P. pneumotropica. These results suggest that the normal serum Id460-positive immunoglobulin is induced by environmentally encountered antigens on P. pneumotropica. We propose that this naturally occurring Id460 activates antidiotypic regulatory cells that in turn promote production of Id460-positive anti-DNP antibody following DNP-ovalbumin immunization. These data are compatible with those obtained in several other idiotypic systems that suggest that dominant idiotypes may be associated with antibodies that have been evolutionarily selected for expression because of their specificity for antigens on environmentally encountered pathogens.

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