IDIOTYPE-ANTI-IDIOTYPE REGULATION

I. Immunization with a Levan-binding Myeloma Protein Leads to the Appearance of Auto-Anti-(Anti-Idiotype) Antibodies and to the Activation of Silent Clones

By CONSTANTIN A. BONA, ELLEN HEBER-KATZ, AND WILLIAM E. PAUL

From the Department of Microbiology, Mount Sinai School of Medicine, New York 10029; and the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

It is now clear that immune responses are regulated by cells and antibodies that are specific for idiotypic determinants as well as by cells and antibodies specific for conventional antigenic determinants (1–4). Indeed, idiotype (Id)1-based regulatory systems have the capacity to favor the use of certain clones of lymphocytes, quite independently of the capacity of those clones to bind antigen (5, 6). Id-based regulatory systems also offer the possibility of extended chains of complementary members (i.e., Id, anti-Id, anti-[anti-Id], etc.) and might serve to interlink clones that express similar Id even if they share essentially no antigen-binding activity. The implications of such regulatory networks have been pointed out by Jerne and others (7–9).

Efforts to explore the regulatory consequences of immunity to a given Id, anti-Id, or anti-(anti-Id) have recently been initiated. These studies have demonstrated that such immunity can markedly influence which members of the repertoire of antibodies specific for a given antigen are actually used (10–12).

We and our colleagues have studied an immune system particularly well suited to the examination of Id-based regulation. The system involves the response of BALB/c mice to bacterial levan (BL), a β(2→6) fructosan with β(2→1) branch points. The antibodies produced in response to BL consist of two broad families of molecules (13). One group of antibodies is specific for β(2→1) linkages and reacts with inulin (In), a β(2→1) fructosan, as well as with BL. Most of these anti-β(2→1) antibodies express cross-reactive idiotypes (the In-IdX) found on a series of In-binding myeloma proteins (14). The IgG anti-β(2→1) antibodies made in BALB/c mice express great homogeneity (15, 16). They are essentially all IgG3 molecules and their isoelectric focusing (IEF) patterns are identical to that of J606, a BALB/c IgG3 In-binding myeloma protein.2 The expressed anti-In repertoire is controlled by Sr-1, an autosomal gene not linked to the Igh-C gene complex (16), and also by Igh genes (14, 16).

1 Abbreviations used in this paper: Ab1, idiotype of Ab1; Ab2, A48 myeloma protein; Ab3, anti-A48 antibody; Ab4, anti-(anti-A48) antibody; BL, bacterial levan; CFA, complete Freund's adjuvant; FCS, fetal calf serum; HA, hemagglutinin; HI, HA inhibition; Id, idiotype; IdX, cross-reactive idiotype; IEF, isoelectric focusing; IFA, incomplete Freund's adjuvant; In, inulin; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cell; RIA, radioimmunoassay.

2 Stein, K. E., C. Bona, R. Lieberman, and W. E. Paul. Manuscript in preparation.
The other major family of anti-BL antibodies bind β(2→6) fructosan linkages. These antibodies are more heterogeneous by IEF analysis, although still comparatively simple (16). The Id identified on the β(2→6) fructosan-binding myeloma proteins ABPC48 (A48 or Ab1) and UPC10 are not generally detected in the serum of mice immunized with BL (14). However, anti-BL molecules expressing the idiotypic determinant(s) of Ab1 (Ab1 Id) are part of the repertoire. They are found in the anti-BL antibody response of congenitally athymic (nu/nu) BALB/c mice pretreated with anti-In-IdX antibody, although not in the response of “normal” nu/nu mice (17).

In the present experiments, we have immunized BALB/c mice with Ab1, BALB/c anti-A48 (Ab2), and BALB/c anti-(anti-A48) (Ab3), and have examined the effect of such immunity on the total anti-BL response and on the expression of Ab1 in this response. Interestingly, mice immunized with Ab2 that have produced Ab3 express a substantial amount of Ab1 in their anti-BL response. In contrast, mice producing Ab2 and anti-(anti-[anti-A48]) (Ab4) show a generalized inhibition of the total anti-BL response.

Strikingly, Ab3 expresses idiotypic cross-reactivity with Ab1 in that Ab4 binds to Ab3, and Ab1 inhibits the binding of radiolabeled Ab3 to Ab4. Although this cross-reaction is of relatively low affinity, it is a feature of 60–70% of the Ab3 molecules detected in this assay. This implies that the Id-anti-Id chain is not unidirectional and strongly suggests that immunization with Ab2 causes the production of “Ab1-like” molecules (Ab3) because it binds to Ab1-like idiotypic determinants on B lymphocytes.

Finally, we have encountered an immunization procedure in which administration of Ab1 to BALB/c mice leads to the production of Ab3. These Ab3-producing mice resemble Ab2-immunized mice in that, upon immunization with BL, they produce antibodies expressing the Ab1 Id. This suggests that functionally important Id-anti-Id chains may be initiated in the course of “normal” immune responses.

Materials and Methods

**Mice.** BALB/c AnN mice, 8–12 wk old, were used in this study.

**Antigens.** BL from *Aerobacter levanicum* (ATCC 1552) was obtained as previously described (14).

**Myeloma Proteins.** Ab1 and UPC10, β(2→6) fructosan-binding BALB/c myeloma proteins, and MOPC-384, a *Salmonella tyrosina* lipopolysaccharide-binding BALB/c myeloma protein, were the gifts of Dr. Michael Potter, National Cancer Institute, National Institutes of Health, Bethesda, Md. Ab1 and MOPC-384 are IgA, κ-proteins; UPC10 is an IgG2a, κ-protein.

**Preparation of Anti-Id Antisera.** Ab2 antisera were prepared in A/He mice by immunization with Ab1 myeloma protein and in BALB/c mice by immunization with an Ab1-keyhole limpet hemocyanin (KLH) conjugate. Ab3 antisera was prepared in BALB/c mice by immunization with a KLH conjugate of affinity chromatography-purified BALB/c Ab3 Id antibody. Similarly, Ab4 antisera was prepared by immunization of BALB/c mice with a KLH conjugate of purified BALB/c Ab3 antibody. Purification of antibodies on Sepharose 4B anti-Id columns, coupling of affinity chromatography-purified antibodies to KLH, and immunization schedules are as previously described (18).

**Preparation of Coupled Sheep Erythrocytes (SRBC).** An O-steryl derivative of BL was prepared according to the technique of Hämmerling and Westphal (19) and then coated to SRBC as previously described (14). SRBC were coated with myeloma proteins and various affinity chromatography-purified antibodies by the chromic chloride method, using a concentration of 0.6–1.0 mg/ml of appropriate protein.

** Determination of Hemagglutinin (HA) Titters.** HA titers of antibodies specific for BL and for Id were determined in microplates by using SRBC coated with O-steryl BL, myeloma proteins,
or affinity chromatography-purified antibodies. The titer recorded is 1/log2 of the highest dilution of antisera giving agglutination.

**Determination of Serum Id.** An HA-inhibition (HI) method described previously (13, 14) was used to test sera for the presence of Id. The Ab1 Id was studied by using both A/He and BALB/c Ab2 antisera and Ab1-coated SRBC. BALB/c Ab3 antisera and affinity chromatography-purified Ab2 antibody-coated SRBC were used to detect Id of Ab2 antibodies. BALB/c Ab4 antisera and affinity chromatography-purified Ab3 antibody-coated SRBC were used to detect Id of Ab3 antibodies.

**Radioimmunoassay (RIA).** Purified Ab3 antibodies and Ab1 myeloma protein were tritiated according to the method of Wilder et al. (20). Ab3 antibodies were labeled with 125I by the chloramine T method (21). The ability of BL to bind to 3H-Ab1 myeloma protein was determined in microtiter plates that had been incubated for 18 h with 50 μg BL, followed by three washings with saline. The plates were incubated for 1 h with 50% fetal calf serum (FCS) and, after three washings, incubated for 3 h with 3H-Ab1 (10,000 cpm/50 μl). The ability of 3H-Ab1, 3H-Ab3, and 125I-Ab3 to bind Ab3 antibodies or Ab4 immunoglobulin (Ig) was determined with the use of microplates that had been incubated with various amounts of purified Ab2 antibodies or with the Ig fraction of Ab2 antisera for 18 h at 4°C. After washing with FCS as described above, the plates were incubated for 3 h with 3H-Ab1, 3H-Ab3 (~5,000 cpm/50 μl), or 125I-Ab3 (39,000 cpm/50 μl). To determine the capacity of various proteins or sera to inhibit the binding of radiolabeled Ab2 antibodies or of 3H-Ab1 to Ab2 or Ab4, plates coated with Ab2 antibodies or with Ab4 Ig were incubated for at least 3 h with various dilutions of inhibitor and washed before the addition of radiolabeled antibodies (22). Radioactivity on plates was measured in a liquid-scintillation spectrometer.

**Plaque-forming Cell (PFC) Assay.** The number of PFC-secreting antibodies specific for BL was determined according to a previously described technique (13). PFC secreting anti-BL antibodies carrying the Ab1 Id were enumerated by the addition of BALB/c (1:300) or A/He (1:150) anti-A48 Id antisera to agarose. The number of PFC obtained in the presence of these sera was subtracted from that obtained if no inhibitory serum was present to give the number of Ab1 Id* anti-BL PFC.

### Results

**Characterization of Id.** A series of anti-Id antibodies mimicking an Id-anti-Id chain, or pathway, comprising Ab2, Ab3, and Ab4 antibodies was induced by immunization of syngeneic mice with KLH conjugates of Abx, Ab2, and Ab3 antibodies, respectively. The presence of these anti-Id antibodies was determined by HA and RIA assays. Thus, Ab2 antisera agglutinated both Ab1-SRBC and Ab2-SRBC (Table I). The Ab1-SRBC agglutinating activity of Ab2 antisera was completely removed by

### Table I

| Sera                              | Unadsorbed | Ab1 | Ab2 | Ab3 |
|-----------------------------------|------------|-----|-----|-----|
| Nonimmune                         | 0          | ND  | 0   | ND  |
| Anti-A48-KLH (Ab2)                | 10         | 0   | 2   | ND  |
| Anti-(anti-A48)-KLH (Ab2)         | 0          | 0   | ND  | 0   |
| Anti-(anti-[anti-A48])-KLH (Ab4)  | 3          | 2   | 0   | 0   |
| Rabbit anti-mouse κ               | >12        | ND  | ND  | >12 |

*HA titers in log₂ units.

† Sera adsorbed with Sepharose 4B conjugated with Ab1, purified Ab2, or purified Ab3.

§ Not done.
absorption of the antiserum with Ab3-Sepharose beads and largely, although not completely, with Ab2-Sepharose beads. Ab3 antiserum agglutinated Ab2-SRBC.

The antiserum containing Ab4 antibodies agglutinated Ab3-SRBC, as expected. Interestingly, the capacity of Ab4 serum to agglutinate Ab2-SRBC was completely removed by absorption with Ab3-Sepharose beads but only partially removed by Ab2-Sepharose beads (Table I). These results indicate that Ab4 can bind to Ab2 but suggest that this binding is of relatively low affinity. To study this in greater detail, we examined the binding of 125I-Ab2 by both Ab2- and Ab4-coated microplates and compared this with the binding of 3H-Ab1 by similar microplates (Table II). We observed that 125I-Ab2 and 3H-Ab1 bound to both Ab2 and Ab4 plates. Controls using BALB/c Ig and Ab3 plates indicated the specificity of this binding. The ratio of amount of 3H-Ab1 bound by the Ab4 and Ab2 plates and the ratio of 125I-Ab3 bound by these plates were similar. Because all Ab1 and Ab3 molecules should be specific for Ab2, the similarity of this ratio suggests that the fraction of the Ab1 molecules specific for Ab4 is similar to the fraction of Ab3 molecules specific for Ab4. Furthermore, Ab1 can inhibit the binding of 60–70% of radiolabeled Ab2 to Ab4 plates. Control proteins such as MOPC-384 and UPC10 cause little or no inhibition. One such inhibition experiment is illustrated in Fig. 1. Comparable results have been obtained in three additional experiments, using 3H-Ab3 as well as 125I-Ab3.

The relative affinity of Ab4 for Ab1 can be estimated from the concentration of Ab1 required to inhibit binding of 125I-Ab3 or of 3H-Ab1 to Ab4 microplates (Table III). We found that 2.2 μg/ml of Ab1 was needed to inhibit 50% of the binding of 125I-Ab3 to Ab4 plates, whereas only 0.006 μg/ml of Ab2 was needed for comparable inhibition. This suggests that Ab4 binds to Ab3 with substantially greater affinity than it binds to Ab1. On the other hand, a concentration of 0.03 μg/ml of Ab1 was sufficient to inhibit 50% of the binding of 3H-Ab1 to Ab4 microplates. This is consistent with a relative affinity of the binding of Ab4 to Ab1, which is greater than that indicated by Ab1 inhibition of binding of Ab4 to Ab3 plates, although still lower than the relative affinity of binding of Ab4 to Ab2. At this time, we cannot fully reconcile these differing estimates of the relative affinity of Ab4 for Ab1.

The interactions of these anti-Id antibodies were also studied by an HI assay. The agglutination of Ab1 SRBC by A/He and BALB/c Ab2 was inhibited by Ab1 myeloma protein (Table IV). The agglutination of Ab1-coated SRBC by BALB/c Ab2 antibod-

---

**Table II**

| Bound ligand       | 3H-Ab1 | 125I-Ab3 |
|--------------------|--------|----------|
| cpm                |        |          |
| BALB/c Ig (10 μg/ml) | 105    | 1,130 ± 111 |
| Ab2 (10 μg/ml)      | 110 ± 8 | ND*      |
| Ab4 (10 μg/ml)      | 1,386 ± 142 | 22,944 ± 1,374 |
| Ab4 (150 μg Ig/ml)  | 524 ± 19 | 8,173 ± 271 |
| Specific binding to Ab4, specific to Ab2† | 0.33  | 0.32     |

* Not done.
† Binding to Ab4 plate − binding to control plates/binding to Ab2 plate − binding to control plates.
CONSTANTIN A. BONA, ELLEN HEBER-KATZ, AND WILLIAM E. PAUL

Fig. 1. Inhibition of binding of $^3$H-Ab$_1$ and $^{125}$I-Ab$_3$ to Ab$_2$ and Ab$_4$ microplates. Microplates were coated with Ab$_2$ (10 #g/ml purified antibody) and Ab$_4$ (150 #g/ml Ig) as described in Materials and Methods. Plates were preincubated with various concentrations of Ab$_2$, Ab$_3$, or MOPC-384, and washed. $^{125}$I-Ab$_3$ or $^3$H-Ab$_1$ were then added and binding measured. Percent inhibition was calculated as follows:

$$\% \text{ inhibition} = 100 \left(1 - \frac{\text{cpm bound after addition of inhibitor} - \text{cpm bound to control plate}}{\text{cpm bound with no inhibitor} - \text{cpm bound to control plate}}\right).$$

Control plates for $^3$H-Ab$_1$ were coated with BALB/c Ig and with Ab$_2$ Ig; control plates for $^{125}$I-Ab$_3$ were coated with BALB/c Ig. The cpm bound to control plates and to Ab$_2$ and Ab$_4$ plates are presented in Table II.

### Table III

| Inhibition of Binding of Radioactive Ab$_1$ and Ab$_3$ to Plates Coated with Ab$_2$ and Ab$_4$ |
|-----------------------------------------------|
| Microplate coated with | Ligand | Inhibitor for 50% inhibition |
|--------------------------|--------|-----------------------------|
|                          |        | $^{125}$I-Ab$_3$ | $^3$H-Ab$_1$ | Ab$_2$ | Ab$_3$ | MOPC-384 |
| Ab$_2$ (10 #g/ml)        | $^{125}$I-Ab$_3$ | 0.007 | ND* | >100 |
| Ab$_2$                   | $^3$H-Ab$_1$ | 0.003 | ND* | >100 |
| Ab$_2$ (150 #g Ig/ml)    | $^{125}$I-Ab$_3$ | 2.2  | 0.006 | >100 |
| Ab$_4$                   | $^3$H-Ab$_1$ | 0.03  | ND | >100 |

* Not done.

ies was also inhibited by Ab$_3$. On the other hand, Ab$_3$ did not inhibit the ability of A/He anti-Ab$_1$ to agglutinate Ab$_1$-SRBC. These results indicate that A/He Ab$_2$ lacks idotypic determinant(s) borne by BALB/c Ab$_2$ antibodies or, as discussed later, that A/He Ab$_2$ is directed to different idiotypic determinants on Ab$_1$ than are BALB/c Ab$_2$ antibodies.

The agglutination of BALB/c Ab$_2$-SRBC by Ab$_3$ was inhibited by BALB/c Ab$_2$ and by Ab$_4$ (Table IV). The inhibitory ability of BALB/c Ab$_2$ was shared by BAB.14 and C.B20 Ab$_2$ antibodies but not by A/He and AL/N antibodies (data not shown).
**Table IV**

*HI Titer of Ab2 Id, Ab3 Id, and Ab4 Id Antisera*

| Inhibitory serum | A/He Ab2 + Ab3 SRBC | BALB/c Ab2 + Ab3 SRBC | Ab4 + Ab3 SRBC |
|------------------|---------------------|-----------------------|----------------|
| Nonimmune serum  | 0                   | 0                     | 0              |
| Ab1 (3 mg/ml)    | 8                   | 8                     | 0              |
| Anti-A48-KLH (Ab2) | 0           | 8                     | 0              |
| Anti-(anti-A48)-KLH (Ab3) | 0 | 8                     | 0              |
| Anti-(anti-[anti-A48]-KLH) (Ab4) | 0 | 6                     | 0              |

The agglutination of Ab3-SRBC by Ab4 was inhibited by Ab3, as anticipated. In addition, it was inhibited by high concentrations of Ab1. This is similar to the finding that relatively high concentrations of Ab1 are required to inhibit the binding of radiolabeled Ab3 to Ab4-coated plates. Both results indicate that Ab4 recognizes Ab3 and Ab1, although its affinity for Ab1 is lower than that for Ab3.

The Ab2, Ab3, and Ab4 antisera did not react with BL to any greater extent than did normal serum (Table V). The low anti-BL HA titer of these antisera and of the sera of normal nonimmunized BALB/c mice presumably represents the presence of "natural" anti-BL antibodies due to immunization with environmental antigens (22, 23). This phenomenon is common for several polysaccharide antigens borne by saprophytic intestinal flora.

These results indicate that through intentional immunization a set of complementary anti-Id antibodies can be produced and that each differs from the others. In particular, Ab2 differs from Ab3 in that it lacks the capacity to agglutinate BL-SRBC (Table V) and to bind to BL-coated plates (data not shown). Ab4 differs from Ab2 in that its ability to agglutinate Ab4-SRBC is ineffectively removed by Ab1-Sepharose and its ability to bind both radiolabeled Ab3 and Ab3-SRBC requires high concentrations of Ab1 for inhibition. Nonetheless, this analysis indicates that the majority of Ab3 molecules express an IdX which cross-reacts with an Id found on Ab1. This result is quite unexpected in a "linear" Ab1-Ab2-Ab3-Ab4 system, but is comparable to a previous result of Wikler et al. (24) in a similar rabbit system.

**Table V**

*Anti-BL HA Titer*

| Sera                  | Number of mice | HA titer (BL-coated SRBC) |
|-----------------------|----------------|---------------------------|
| Normal                | 2              | 2.5                       |
| Ab1 (3 mg/ml)         |                | 11.0                      |
| Anti-A48-KLH (Ab2)   | 5              | 1.8                       |
| Anti-(anti-A48)-KLH (Ab3) | 9          | 2.1                       |
| Anti-(anti-[anti-A48]-KLH) (Ab4) | 3          | 2.3                       |

**Immunization with Ab1 Causes the Production of Ab3.** The previous experiments demonstrate the characteristics of members of an Id-anti-Id chain induced by intentional immunization with a KLH conjugate of the immediately preceding member of that
chain. In order to determine whether the chain normally develops beyond the first step after immunization with native Ab₁, we immunized BALB/c mice with Ab₁, using two different protocols, and tested for the appearance of both Ab₂ and Ab₃.

One group of seven mice was immunized eight times with Ab₁ not conjugated to KLH. The immunization protocol consisted of an initial immunization in complete Freund's adjuvant (CFA), followed by one immunization in incomplete Freund's adjuvant (IFA), and then by six weekly immunizations in saline. These mice were bled weekly after the completion of the entire protocol. Each of the mice of this group made Ab₂, which was found over an 8-wk period after completion of immunization. Ab₃ was not detected in any of these mice during this period (Fig. 2).

In five of the mice of this group, the Ab₂ observed differed from the Ab₂ found in BALB/c mice after immunization with Ab₁ conjugated to KLH (see Table I). The Ab₂ of these five mice failed to agglutinate Ab₂-SRBC. In two of the seven mice, the sera could agglutinate both Ab₁-SRBC and Ab₂-SRBC, although the titers varied substantially during the 8-wk period. Furthermore, these two mice may also have produced Ab₄. We suggest this because adsorption of their sera with Ab₁-Sepharose only partially removed the ability to agglutinate Ab₂-SRBC, whereas absorption with Ab₃-Sepharose removed it completely (Table VI).

A second group consisting of four mice was immunized twice with Ab₁, once in CFA and once in IFA. These mice expressed a low serum titer of hemagglutinating Ab₂ antibodies 1 wk after the completion of immunization (Fig. 3). By 3 wk after completion of immunization, three of these mice had no detectable Ab₂ serum titer and one mouse had a very low titer in its serum. At this time, each of these mice displayed a significant Ab₃ titer. By 5 wk, the Ab₃ titer had fallen in all but one of the mice and was replaced by Ab₂, but by 8 wk, Ab₃ was again found in the absence of detectable Ab₂. Thus, mice immunized in this way with Ab₁ show a prompt appearance of Ab₃ and then show an inverse fluctuation in Ab₂ and Ab₃ levels. However, we have not examined the second peak (at 5 wk) of Ab₂ HA activity to determine whether it represents an Ab₂ or Ab₄, or a mixture of Ab₂ and Ab₄.

Our data indicate the appearance of auto-Ab₃ and possibly of auto-Ab₄ in mice immunized with Ab₁. This is consistent with the development of an extended chain of anti-Id reactions in individual animals.

**Activation of Ab₁ Id⁺ Anti-BL Precursors in Mice Immune to Ab₂.** Normal BALB/c mice, as well as BALB/c mice that had been immunized with Ab₂ Id antibodies, have low serum anti-BL HA titers and a small number of anti-BL PFC (Table VII). No Ab₃

![Fig. 2](image-url)  
**Fig. 2.** HA titer of sera of BALB/c mice immunized with Ab₁ not conjugated to KLH, according to the eight-immunization protocol. HA titer with A₄₈-SRBC (Ab₁) is interpreted to represent Ab₁ titer; HA titer with anti-A₄₈-SRBC (Ab₂-SRBC) is interpreted to represent Ab₂ titer.
Table VI

| Time after completion of immunization | Serum | Adsorption† | Ab1-SRBC | Ab2-SRBC |
|--------------------------------------|-------|-------------|-----------|-----------|
| wk                                   |       |             |           |
| 1                                    | —     | 4           | 6         |
| 5                                    | Ab1   | 1           | 4         |
|                                       |       | 4           | 4         |
|                                       | Ab1   | 1           | 3         |
|                                       | Ab2   | 2           | 0         |
|                                       | MOPC-384 | 3        | 3         |

* Sera from two mice that produced Ab2 antibody that agglutinated Ab2-SRBC were tested.

† Serum (0.2 ml) was adsorbed with 0.1 ml of Sepharose 4B beads to which the indicated protein had been conjugated.

Table VII

| Pretreatment | Immunization with BL* | Number of mice | HA (BL-SRBC) | HI Ab1-SRBC + BALB/c Ab2 | RIA‡ Ab1 | BL PFC | Total PFC/ spleen | Ab1 Id |
|--------------|-----------------------|----------------|--------------|--------------------------|----------|--------|------------------|--------|
| None         | –                     | 2              | 2.5 ± 0.7    | 0                        | <0.1     | 24 ± 11 | 4 ± 5            |
| Ab2-KLH      | –                     | 4              | 3.8 ± 0.3    | 0                        | <0.1     | 44 ± 33 | 5 ± 3            |
| None         | +                     | 5              | 6.0 ± 1.2    | 0                        | <0.1     | 330 ± 34| 9 ± 6            |
| Ab2-KLH      | +                     | 9              | 7.8 ± 3.2    | 1.8 ± 0.7                | 4.8 ± 1.6| 765 ± 433| 32 ± 20          |
| Ab1 (8 immunizations) | + | 6 | 3.3 ± 1.1 | 0.9 ± 1.2 | <0.1 | 435 ± 102 | 18 ± 10 |
| Ab1 (2 immunizations) | + | 4 | 10.8 ± 1.3 | 2.8 ± 0.5 | 10.3 ± 1.8 | 1,192 ± 180 | 47 ± 13 |

* Mice were immunized with 10 μg BL 10 wk after completion of pretreatment. Responses were measured 5 d after BL immunization.

‡ Results calculated based on the capacity of various concentrations of Ab1 to inhibit binding of 3H-Ab1 to plates coated with purified Ab2 (10 μg/ml).
Id* antibodies could be detected by either HI, RIA, or PFC assays in these mice. Immunization with BL leads to the development of a significant titer of anti-BL antibodies and anti-BL PFC. As we have previously shown, Ab1 Id* anti-BL antibodies are not detected in this response (17). BALB/c mice that had been immunized with Ab2-KLH developed a vigorous response upon immunization with BL, and a substantial fraction of their anti-BL antibodies expressed the Ab1 Id. Immune mice producing Ab2 (and possibly Ab4), as a result of immunization with Ab1 using the eight-injection protocol, developed an anti-BL response of lower magnitude than did normal mice immunized with BL. They failed to express Ab1 Id. In contrast, mice that had produced Ab3 as a result of immunization with Ab1 using the two-injection protocol expressed a marked increase in their total anti-BL response and developed a considerable amount of Ab1 Id* anti-BL antibodies upon immunization with BL. Thus, in these animals, the presence of immunity to Ab2 before immunization with BL was associated with the expression of the Ab1 Id in the anti-BL response. In contrast, the presence of Ab2 itself was associated with a failure of expression of Ab1 Id and a decrease in the total anti-BL response.

Further, we examined the kinetics of the response to BL of mice producing Ab2, Ab3, and Ab4 as a result of immunization with KLH conjugates of Ab1, Ab2, and Ab3, respectively. As noted above, Ab2 mice immunized with BL developed a lower anti-BL response compared to BL-immunized normal BALB/c mice. The titer in such Ab2 mice did not approach normal levels until 20 d after immunization (Fig. 4). Ab3 mice immunized with BL exhibited a total anti-BL response quite similar in amount to that of normal mice. Most interestingly, Ab4 mice showed a degree of suppression of their total anti-BL response that was at least as profound as that of Ab2 mice. These results suggest that Ab2 and Ab4 express a functional similarity and reinforce serologic evidence that indicates that Ab4 resembles Ab2 in that it binds Ab1.

Discussion

Our results demonstrate that a chain of complementary anti-Id antibodies comprising four members (Ab1, Ab2, Ab3, and Ab4) can be generated by immunization of syngeneic mice with KLH conjugates of the immediately preceding member of the chain. Thus, these results formally demonstrate that in syngeneic animals an extended Id-anti-Id regulatory system is possible. A similar four-member chain (Ab1–Ab4) has

![Fig. 4. Anti-BL HA titers of sera of BALB/c mice preimmunized with Ab1 KLH (∆-producing anti-A48Id [Ab2 Id] antibodies); anti-A48-KLH (● producing anti-[anti-A48Id] [Ab3] antibodies); or anti-(anti-A48) [Ab3]-KLH (●-producing anti-[anti-(anti-A48)] [Ab4] antibodies. Titters of nonpreimmunized mice designated with ○. All mice were immunized with 10 µg BL 10 wk after completion of preimmunization. Each point represents average ±SD of three mice.](image-url)
been described by Wikler et al. (24) in allotype-matched rabbits, and three-member chains (Ab1–Ab3) have been described previously in syngeneic mice (18) and allotype-matched rabbits (10).

We observed that immunization with unconjugated Ab1, under certain circumstances, leads to the occurrence of auto-Ab3, as well as auto-Ab2. Furthermore, our results suggest that such immunization may occasionally lead to the production of auto-Ab4. These observations indicate that the chain developed by stepwise immunization with KLH conjugates can be observed under normal conditions. Prior evidence that initial events in chain formation occur has been obtained. Thus, it has been reported that the immunization with conventional antigens leads to the occurrence of auto-anti-Id antibodies (25–27), auto-anti-Id PFC (13, 28), and auto-anti-Id-binding cells (29–31). Naturally occurring Id-specific T cells (2), as well as Id-specific T cells generated after immunization with Id-bearing antibodies (30, 32), with Id-bearing antibodies coupled to syngeneic cells (31, 33), or with anti-Id antibodies (30, 34), have also been described.

The presence of immunity to Ab1, Ab2, or Ab3 in mice has a significant effect on the amount and nature of the antibody response to BL, which is the antigen that is the putative initiator of the Id-anti-Id chain. Thus, in mice that have produced Ab3, either because of immunization with KLH-Ab2 or after responses to Ab1, we observed that the primary response to BL contained a major fraction of antibodies that express the Ab1 Id. This Id is not detected in the response to BL of normal or nu/nu mice of the Igha type, nor is it found in the response of intact BALB/c mice that have been pretreated with antibody to the In-IdX (35). The only previous situation in which we observed the appearance of measurable amounts of the Ab1 Id in anti-BL antibodies was in the response to BL of nu/nu BALB/c mice that had previously been treated with anti-In-IdX (17). If such mice are given T lymphocytes at the time of BL immunization, the Ab1 Id is not observed in their antibody response. This suggests that the expression of Ab1 Id is under some type of T lymphocyte-dependent control and that the Ab1 Id is probably not a major member of the anti-BL repertoire, because suppression of the anti-β(2→1) component of the anti-BL response is required for its expression even in the absence of T cells.

In this context, it is interesting to consider why immunity to Ab2 should lead to the development of an Ab1 Id* anti-BL response. One obvious possibility is that Ab3, which is anti-(anti-A48Id), could eliminate suppressor T lymphocytes that express Ab2 (anti-A48Id) specificity. Indeed, we have shown in the MOPC-460-trinitrophenyl (TNP) system that mice immunized with Ab2 (anti-MOPC-460) lack 460Id-specific suppressor T cells, which are found in normal mice and which regulate the activation of the precursors of 460Id* anti-TNP antibody-secreting cells (12). However, if elimination of suppressor T cells specific for Ab1 Id (i.e., Ab2-bearing suppressors) is the only reason that immunity to Ab2 leads to expression of Ab1, one might anticipate that nu/nu mice immunized with BL would express substantial Ab1 Id in their anti-BL response. As we noted above, this is not the case. A second possibility is that Ab3 could be regarded as consisting of the entire set of antibodies that can react with Ab2. Because Ab2 is anti-A48Id, Ab3 would be expected to include Ab1. However, mice immunized with Ab2-KLH, but not with BL, have no greater anti-BL serum antibody titer than do normal mice. Thus, Ab3 cannot be a very important component of Ab3. Nonetheless, precursors of Ab1 (i.e., A48Id* anti-BL) antibody-secreting cells appear
to have been primed by immunization with \( \text{Ab}_2 \), because BL immunization leads to their activation and to the appearance of \( \text{Ab}_1 \) Id in the anti-BL response of \( \text{Ab}_2 \)-KLH primed mice, and of those \( \text{Ab}_1 \)-immunized mice that produced \( \text{Ab}_3 \). It seems likely to us that both of these mechanisms contribute to the regulation of the \( \text{Ab}_1 \) Id\(^+ \) anti-BL response in mice that have produced \( \text{Ab}_3 \).

One of the most interesting results to emerge from these studies and those of Wikler et al. (24) is the apparent asymmetry of the chain of anti-Id. Thus, we observed that \( \text{Ab}_4 \) resembles \( \text{Ab}_2 \) in that both bind to \( \text{Ab}_1 \) and to \( \text{Ab}_3 \), and that both lead to a suppression of anti-BL antibody response. Although \( \text{Ab}_4 \) and \( \text{Ab}_2 \) are not identical, because their affinities for \( \text{Ab}_1 \) are different, it is particularly important to note that the majority of the \( \text{Ab}_4 \) molecules tested in our assays bind \( \text{Ab}_1 \). This was shown by the fact the \( \text{Ab}_1 \) inhibited the binding of \( >60\% \) of radioactive \( \text{Ab}_3 \) by an \( \text{Ab}_4 \) plate and by the capacity of high concentrations of \( \text{Ab}_1 \) to inhibit hemagglutination of \( \text{Ab}_3 \)-SRBC by \( \text{Ab}_4 \). In contrast, \( \text{Ab}_2 \) and \( \text{Ab}_1 \) differ in that \( \text{Ab}_2 \) fails to recognize BL, whereas \( \text{Ab}_1 \) is a BL-binding myeloma protein. In the studies of Wikler et al. (24), a four-member chain was initiated with antibody to the polysaccharide from \( \text{Micrococcus lysodeikticus} \). In these experiments, \( \text{Ab}_4 \) and \( \text{Ab}_2 \) both bound to \( \text{Ab}_1 \), but \( \text{Ab}_3 \) failed to bind to \( \text{Ab}_1 \).

It is possible that the chain is only seemingly asymmetrical and the appearance of asymmetry is due to a relatively low affinity of \( \text{Ab}_1 \) for the polysaccharide antigens (BL and \( \text{M. lysodeikticus} \) polysaccharide) used in these systems. If \( \text{Ab}_4 \) were cross-reactive with \( \text{Ab}_1 \) but had an energy of binding which was 1 or 2 kcal lower than \( \text{Ab}_1 \), it might fail to bind antigen under the conditions used. In contrast, it is likely that the \( \text{Ab}_2 \)-\( \text{Ab}_1 \) interactions, representing antibody-protein antigen interactions, are of relatively high affinity, and that the binding by cross-reactive \( \text{Ab}_4 \) would be detectable even if the energy of interaction of \( \text{Ab}_4 \) for \( \text{Ab}_1 \) was considerably less than that of \( \text{Ab}_2 \) for \( \text{Ab}_1 \). In fact, there are three reported instances of \( \text{Ab}_2 \) displaying some \( \text{Ab}_1 \)-like antigen-binding activity. These are in systems in which the initiating antigens are insulin-binding receptor (36), retinol-binding receptor (36), and tobacco mosaic virus capsid protein (37). However, our observation that the majority of \( \text{Ab}_4 \) molecules bind \( \text{Ab}_1 \) suggests that this is an important feature of Id-anti-Id chains.

Consequently, we wish to suggest an explanation for the observed lack of symmetry in this chain of anti-Id which represents a distinctive view of the Id-anti-Id regulatory system. In considering this proposal, we first wish to outline what we believe to be a major difficulty in certain of the network concepts. In the most simplified and extreme case, one imagines a physiologic network to be initiated by the antibody produced in response to antigenic determinants. Such antigenic determinants, in accordance with the Jerne nomenclature, are termed epitopes (7). Anti-epitope antibodies (\( \text{Ab}_1 \)) express Id that are currently recognized to actually consist of a series of distinctive determinants located on different portions of the variable region. These individual idiotypic determinants have been designated idiotopes. Because each \( \text{Ab}_1 \) may express at least two and probably several idiotopes (38), there should be at least two and probably several \( \text{Ab}_2 \) generated in response to each \( \text{Ab}_1 \) (39). Similarly, each \( \text{Ab}_2 \) should stimulate the appearance of several \( \text{Ab}_3 \) and each of these, in turn, should stimulate the appearance of several \( \text{Ab}_4 \). This would suggest that the number of distinct molecules in the set of \( \text{Ab}_2 \), \( \text{Ab}_3 \), ... \( \text{Ab}_n \) antibodies must be very much larger than the set of distinct \( \text{Ab}_1 \) antibodies, and that the bulk of the antibody repertoire is
devoted to recognition of idiotopes. This concentration on idiotopes might be more illusory than real if most anti-idiotope antibodies also recognized some epitope, presumably because of a structural similarity between the particular idiotope and the particular epitope (40). Thus, the set of anti-idiotope antibodies and anti-epitope antibodies would be the same set. This concept, in its extreme form, is tenable only if the expressed idiotopes of Ig cross-react with virtually every possible antigenic determinant, including polysaccharides, lipids, and simple organic haptens. We regard this as quite unlikely in chemical terms. A second finding not well explained by linear or expanding idiotope-anti-idiotope chains is the capacity of Ab4 to bind Ab1.

We wish to propose a very different idea to explain idiotope-anti-idiotope interactions (Fig. 5). In our model, the set of anti-epitopes (Ab1) and anti-idiotopes (Ab2) are functionally distinct. Thus, we suggest that, although antibodies to conventional antigenic determinants bear many idiotopes, only a limited number of these idiotopes function in eliciting responses in autologous or syngeneic animals. We designate such determinants “regulatory idiotopes.” Thus, the anti-idiotope antibodies that develop in autologous or syngeneic systems will be directed to a limited number of determinants. It should be obvious that Ab2 idiotopes will be similar to each other in that each binds the Ab1 Id. They may be sufficiently heterogeneous to display a large number of regulatory idiotopes, each expressed on only a small fraction of the Ab2 molecules. Alternatively, the set of Ab2 may differ from the set of Ab1 in that it fails to express regulatory idiotopes. In either case, intentional or natural immunization with Ab2 will be relatively efficient in activating those B cells that bear the regulatory

Fig. 5. The regulatory idiotope concept. Ab1 of the a type (a Ab1) represents an anti-epitope antibody which possesses a regulatory idiotope (the a Ab1 idiotope). Immunization of syngeneic animals with a Ab1 causes the production of the complementary anti-idiotope antibody a Ab2, which displays any of its individual idiotopes at low concentration because a Ab2 lacks a regulatory idiotope. Immunization of syngeneic mice with a Ab2 activates B lymphocytes that bear receptors that express the a Ab1 idiotope or related idiotopes. Immunization with Ab2 fails to cause activation of anti-(anti-idiotope) antibodies. Thus, a Ab2 is really the collection of molecules that bear the a Ab1 and related idiotopes. Immunization with a Ab2 thus elicits the production of antibodies specific for the a Ab1 family of regulatory idiotopes. Thus, a Ab2 and a Ab1 will resemble each other. The a Ab1-a Ab2 system is one of a series of distinct complementary systems which include the b Ab1-b Ab2, c Ab1-c Ab2, ... z Ab1-z Ab2 systems.
idiotopes for which Ab$_2$ is specific (the Ab$_1$ regulatory Id), but will cause limited induction of anti-Ab$_2$, either because no single Ab$_2$ Id is present at sufficiently high concentration to stimulate anti-Id antibodies, or because Ab$_2$ molecules do not display regulatory Id. Therefore, for purposes of immunization, Ab$_2$ functions mainly as an anti-Id antibody rather than as an antigen. The antibodies raised against Ab$_2$ should resemble each other in that each will express the Ab$_1$ regulatory idiotope or determinants that cross-react with this idiotope. We suggest that the number of such idiotopes is relatively small and that antibodies of very different specificities may share cross-reactive regulatory idiotopes. Thus, Ab$_3$ should more correctly be regarded as a collection of molecules possessing the Ab$_1$-type regulatory idiotope (i.e., Ab$_1$, Ab$_1^\prime$, ..., Ab$_1^n$), of which Ab$_1$ will be only one member of a relatively large family. Thus, the antigen-binding activity of Ab$_3$ might be difficult to detect. On the other hand, immunization with antigen (e.g., BL) should reveal that Ab$_1$-bearing epitope-specific precursors have been “primed” by Ab$_2$ immunization. That is, Ab$_1$ should be represented in the anti-epitope response to a larger extent than in animals not immunized with Ab$_2$. When Ab$_3$ antibodies are used in immunization, the resultant Ab$_4$ should bind Ab$_1$ because Ab$_1$ and Ab$_3$ express cross-reactive regulatory idiotopes. Thus, Ab$_4$ should be similar to Ab$_4$, because both are directed against the Ab$_1$ regulatory idiotope.

This model is consistent with several findings that have been reported previously. First, the concept that in syngeneic immunizations only the regulatory idiotopes are antigenic is consistent with the Lieberman-Potter rule (42). This states that it is much more difficult to produce anti-Id antibodies against myeloma proteins when the mouse immunized is of the same Igh-C type as the donor of the myeloma protein than when the immunized animal and the donor are of different Igh-C types. Second, the concept that Ab$_3$ consist of molecules bearing idiotopes cross-reactive with Ab$_1$ but that the serum of Ab$_3$ mice has little antigen (e.g., BL)-binding activity is consistent with reports that Id, such as the A5AId, may be found on molecules capable of binding a given antigen (e.g., Streptococcus A carbohydrate), as well as molecules that have no detectable capacity to bind that antigen (43).

Obviously, a model of this sort is best stated in extreme terms both for ease of description and precision of predictions. Nonetheless, one must consider certain problems. First, Ab$_4$ binds Ab$_3$ with much higher affinity than it binds Ab$_1$. Thus, the Ab$_1$ family (i.e., those molecules that bear regulatory idiotopes cross-reactive with that of Ab$_1$) must be moderately heterogeneous. For simplicity, one would prefer a very limited degree of heterogeneity in the Ab$_1$ idiotope. However, this heterogeneity might be explained by influences on the conformation of the regulatory idiotope by the structure of other portions of the hypervariable regions. Second, we demonstrated that Ab$_1$ could inhibit the binding of 60–70% of radioactive Ab$_3$ to Ab$_4$ plates. This suggests that a fraction of the Ab$_3$ molecules may lack the Ab$_1$ regulatory idiotopes, and that immunization with Ab$_2$ actually elicits some anti-Ab$_2$ antibodies. However, it should be pointed out that the immunization scheme, involving Ab$_2$-KLH conjugates, is not physiologic and may allow responses to idiotopes not normally immunogenic in spontaneous autoimmunization.

Finally, one might speculate about whether regulatory idiotopes are a feature of all anti-epitope antibodies (i.e., all Ab$_1$) or only of a subset of Ab$_1$. In particular, regulatory idiotopes might be a feature only of those Ab$_1$ idiotopes that are capable
of becoming dominant Id, possibly because it is these determinants that call forth Id-specific T cell regulatory responses, as well as anti-idiotope (Ab2) antibodies. Indeed, Ab1 bearing anti-BL antibodies appear to be under the regulatory control of T lymphocytes because their expression in the anti-BL response of nu/nu BALB/c mice, pretreated with antibody to the IdX of anti-In, is inhibited by T lymphocytes. Thus, if the number of distinct regulatory idiotopes is relatively small, the existence of relatively large clones of regulatory T lymphocytes specific for these idiotopes might explain the powerful effects of Id-determined T lymphocyte regulation which have been increasingly observed in recent years.

Finally, this anti-epitope-anti-idiotope concept or, more simply, this +,- concept of the Id-anti-Id regulatory system does have some network features in that many apparently unrelated Ab1 idiotopes can be affected by the action of a single Ab2. However, in the model we propose, the effect of a single Ab1-Ab2 system (i.e., the a Ab1-a Ab2 system) should be limited to the Ab1 possessing a common regulatory idiotope (the a Ab1 idiotope) and as such should be relatively isolated from other Ab1-Ab2 systems (e.g., the b Ab1-b Ab2, c Ab1-c Ab2, ... z Ab1-z Ab2 systems) (Fig. 5). One might describe this system as a “ping-pong” effect, in which the Ab1 side has many players and the Ab2 side has few.

Summary

BALB/c mice immunized multiple times with ABPC48 (A48 or Ab1), a BALB/c bacterial levan (BL)-binding myeloma protein, produce anti-Ab1 antibodies (Ab2). Immunization with only two doses of Ab1 often leads to the production of anti-(antiA48) (Ab3) as does immunization with hemocyanin conjugates of Ab2. Finally, immunization with hemocyanin conjugates of Ab3 leads to the production of anti-(anti-[anti-A48]) (Ab4). Normal BALB/c mice immunized with BL produce an anti-BL antibody response containing no detectable Ab1 idiotype (Id)-bearing molecules. Mice producing Ab3 express substantial amounts of Ab1 Id in their anti-BL response whereas mice producing Ab2 and Ab4 show a generalized inhibition in their anti-BL response. These results indicate that states of immunity within an idiotypic chain may have marked effects on antibody responses to the antigen (i.e., BL) which is the putative initiator of the chain. Strikingly, the chain itself has an interesting feature. That is, Ab2 and Ab1 share a cross-reactive Id in that both are bound by Ab4 and Ab2. We propose a model of Id-anti-Id systems to explain this unexpected result. This is based on the concept of regulatory idiotopes on Ab1 molecules which initiate Ab2 (anti-idiotope) responses. In contrast, Ab2 molecules generally fail to initiate anti-Ab2 Id responses either because any individual idiotope is present at very low concentration or because Ab2 molecules tend to lack regulatory idiotopes. Thus, Ab2 molecules immunize syngeneic animals because they interact with cells bearing Ab1-like regulatory idiotopes. Thus, Ab3 will share regulatory idiotopes with Ab1, Ab4 and Ab2 will share the ability to bind the Ab1-like regulatory idiotope.

We thank Ms. Rose Lieberman for helpful discussions and generous gifts of materials.

Received for publication 10 December 1980.
References

1. Hart, D. A., A. L. Wang, L. L. Pawlak, and A. Nisonoff. 1972. Suppression of idiootypic specificities in adult mice by administration of anti-idiootypic antibodies. J. Exp. Med. 135:1283.

2. Bona, C., and W. E. Paul. 1979. Cellular basis of expression of idiotypes. I. Suppressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting antitrinitrophenyl antibodies bearing 460 idiotype. J. Exp. Med. 149:592.

3. Gershon, R. K., P. Cohen, R. Hencin, and S. A. Liebhaber. 1972. Suppressor T cells. J. Immunol. 108:586.

4. Uhr, J. V., and G. Möller. 1968. Regulatory effect of antibody on the immune response. Adv. Immunol. 8:31.

5. Ward, K., H. Cantor, and E. A. Boyse. 1977. Clonally restricted interactions among T and B cell subclasses. In Immune System: Genetics and Regulation. E. Sercarz, L. A. Herzenberg, and C. F. Fox, editors. Academic Press, Inc., New York. 337.

6. Hetzelberger, D., and K. Eichmann. 1978. Recognition of idiotypes in lymphocyte interactions. I. Idiotype selectivity in the cooperation between T and B lymphocytes. J. Eur. Immunol. 8:846.

7. Jerne, N. K. 1974. Towards a network theory of the immune response. Ann. Immunol. (Paris). 125C:373.

8. Urbain, J. 1980. Idiotype network, restrictive recognition and regulation in the immune system. In Strategies of Immune Regulation. E. Sercarz and A. J. Cunningham, editors. Academic Press, Inc., New York. 813.

9. Bona, C., and J. Hiernaux. 1981. Immune response: idiootype-anti-idiotype network. Crit. Rev. Immunol. In press.

10. Cazenave, P. A. 1977. Idiotype-anti-idiotype regulation of antibody synthesis in rabbits. Proc. Natl. Acad. Sci. U. S. A. 74:5122.

11. Urbain, J., M. Wikler, J. D. Frenssen, and C. Collignon. 1977. Idiotype regulation of the immune system by the induction of antibodies against anti-idiotype antibodies. Proc. Natl. Acad. Sci. U. S. A. 74:5126.

12. Bona, C., and W. E. Paul. 1980. The appearance of 460-idiotype (460Id) bearing anti-TNP antibodies is regulated by suppressor T cells specific for 460Id. In Regulatory T Cells. B. Pernis and H. Vogel, editors. Academic Press, Inc., New York. 231.

13. Bona, C., R. Lieberman, C. C. Chien, J. Mond, S. House, I. Green, and W. E. Paul. 1978. Immune response to levan. I. Kinetics and ontogeny of anti-levan and anti-inulin antibody response and expression of crossreactive idiotype. J. Immunol. 120:1436.

14. Lieberman, R., M. Potter, H. Humphrey, and C. C. Chien. 1976. Idiotypes of inulin binding antibodies and myeloma proteins controlled by genes linked to the allotype locus of the mouse. J. Immunol. 117:2111.

15. Bona, C., K. E. Stein, R. Lieberman, and W. E. Paul. 1979. Direct and indirect suppression induced by anti-idiotype antibody in the inulin-bacterial levan antigenic system. Mol. Immunol. 16:1093.

16. Stein, K. E., C. Bona, R. Lieberman, C. C. Chien, and W. E. Paul. 1980. Regulation of clonotype expression by non-allotype linked genes. J. Exp. Med. 151:1088.

17. Lieberman, R., C. Bona, C. C. Chien, K. E. Stein, and W. E. Paul. 1979. Genetic and cellular regulation of the expression of specific antibody idiotypes in the antipolyfructosan immune response. Ann. Immunol. (Paris). 1306:247.

18. Bona, C., R. Hooghe, P. A. Cazenave, C. Le Guern, and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. II. Immunity to anti-MOPC460 idiotype antibodies increases the level of anti-trinitrophenyl antibodies bearing 460 idiotypes. J. Exp. Med. 149:815.
19. Hämmerling, U., and O. Westphal. 1977. Synthesis and use of O-steroyl polysaccharides in passive hemagglutination and hemolysis. *Eur. J. Biochem.* 1:46.

20. Wilder, R., C. C. Yuen, B. Subbarao, V. L. Woods, C. B. Alexander, and R. G. Mage. 1979. Tritium (^3H) radiolabeling of protein A and antibody to high specific activity. Application to cell surface antigen radioimmunoassays. *J. Immunol. Methods.* 28:255.

21. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^131I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* 89:114.

22. Bona, C., P. K. A. Mongini, K. E. Stein, and W. E. Paul. 1980. Anti-immunoglobulin antibodies. I. Expression of crossreactive idiotypes and Ir gene control of the response to IgG_{as} of the b allotype. *J. Exp. Med.* 151:1334.

23. Potter, M. 1979. Mouse myeloma proteins. *Adv. Immunol.* 25:141.

24. Goidl, E. A., A. F. Schrater, G. W. Siskind, and G. J. Thorbecke. 1979. Production of auto-antiidiotypic antibody during the normal immune response to TNP-FicolI. II. Hapten-reversible inhibition of anti-TNP plaque forming cells by immune serum as an assay for auto-antiidiotypic antibodies. *J. Exp. Med.* 150:159.

25. Brown, J. C., and L. S. Rodkey. 1979. Autoregulation of an antibody response via network-induced auto-antiidiotype. *J. Exp. Med.* 150:67.

26. Cosenza, H. 1976. Detection of anti-idiotype reactive cells in the immune response to phosphocholine. *Eur. J. Immunol.* 6:114.

27. Jackson, S., and J. Mestecky. 1979. Presence of plasma cells binding autologous antibody during an immune response. *J. Exp. Med.* 150:1265.

28. Tasiaux, N., R. Lewenkroon, C. Bruyns, and J. Urbain. 1978. Possible occurrence and meaning of lymphocytes bearing auto-anti-idiotype receptors during the immune response. *Eur. J. Immunol.* 8:464.

29. Kelsoe, G., and J. Cerny. 1979. Reciprocal expansions of idiotypic and anti-idiotypic clones following antigen stimulation. *Nature (Lond.)* 279:333.

30. Bona, C., P. A. Cazenave, and W. E. Paul. 1973. Regulation of anti-TNP response by anti-idiotypic and anti-(anti-idiotypic) antibodies. *Ann. Immunol. (Paris).* 130C:303.

31. Dohi, Y., and A. Nisonoff. 1979. Suppression of idiotype and generation of suppressor T cells with idiotype-conjugated thymocytes. *J. Exp. Med.* 150:909.

32. Owen, F. L., S.-T. Ju, and A. Nisonoff. 1977. Presence on idiotype-specific suppressor T cells of receptors that interact with molecules bearing the idiotype. *J. Exp. Med.* 145:1559.

33. Bona, C., R. Lieberman, S. House, I. Green, and W. E. Paul. 1979. Immune response to levan. II. T-independence of suppression of cross-reactive idiotypes by anti-idiotype antibodies. *J. Immunol.* 122:1614.

34. Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra. 1973. Cross idiotypic specificity among monoclonal IgM proteins with anti-y globulin activity. *J. Exp. Med.* 137:331.

35. Bona, C. 1979. Regulation of lymphocyte function by antiidiotype antibody. In *Molecular Basis of Immune Cell Functions.* J. G. Kaplan, editor. Elsevier North-Holland Biomedical Press, Amsterdam. 161.

36. Lindemann, J. 1979. Homobodies: do they exist? *Ann. Immunol. (Paris).* 130C:311.

37. Oudin, J., and M. Michel. 1963. Une nouvelle forme d'allotypie des globulines du serum
de lapin apparent lié à la function et à la spécificité anticorps. C.R. Acad. Sci. (Paris). 257:805.
42. Potter, M., and R. Lieberman. 1967. Genetics of immunoglobulins in the mouse. Adv. Immunol. 7:91.
43. Eichmann, K., A. Coutinho, and F. Melchers. 1977. Absolute frequencies of lipopolysaccharide-reactive B cells producing A5A idiotype in unprimed, anti-A5A idiotype sensitized and anti-A5A idiotype-suppressed A/J mice. J. Exp. Med. 146:1436.