ANTIIDIOTYPE STIMULATION OF ANTIGEN-SPECIFIC ANTIGEN-INDEPENDENT ANTIBODY RESPONSES IN VITRO

I. Evidence for Stimulation of Helper T Lymphocyte Function

BY HANNA Z. WEISSBERGER, ROBERT R. SHENK, AND HOWARD B. DICKLER

From the Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Since the proposal of the network theory by Jerne (1), considerable evidence consistent with regulation of the immune response by a network of idiotypes and antiidiotypes has accumulated. Published data indicate that: (a) auto-antiidiotypes are produced in the course of the immune response to antigen (2-5); (b) administration of antiidiotypes can result in suppression of idiotype expression and generation of idiotype-specific suppressor cells (6-10); and (c) administration of antiidiotype can result in enhanced expression of idiotype, which in some cases binds antigen, and in generation of antigen-specific helper T lymphocytes (11-16). However, the mechanism(s) by which antiidiotypes trigger lymphocyte function remains largely unknown. This lack of knowledge results, at least in part, from a paucity of experimental systems in which antiidiotype triggers lymphocyte function in vitro.

Recently, we have described a microculture system for the elicitation and detection of in vitro secreted antibody responses to the synthetic polypeptide poly-L-(Tyr, Glu)-poly-D,L-Ala-poly-L-Lys [(T,G)-A--L]1 (17). Responses were antigen dependent, antigen specific, and as in the case for in vivo responses to this antigen (18), under the control of H-2-linked Ir genes. Using this system, the current studies have evaluated the effects of antibodies specific for the idiotypes of B10 anti-(T,G)-A--L antibodies (anti-Id). The anti-Id recognize idiotypes on the majority of B10 anti-(T,G)-A--L antibodies (19) and idiotype expression is linked to the IgCH locus (20). The experiments reported here show that the anti-Id induced (T,G)-A--L-primed lymphocytes to secrete anti-(T,G)-A--L antibody in the absence of antigen. Prior antigen priming of T lymphocytes but not B lymphocytes was required and responses were entirely specific. Moreover, direct evidence was obtained for triggering of helper T lymphocyte function by the anti-Id. Finally, data were obtained that suggest that responses to the anti-Id, in contrast to responses to the antigen, were not regulated by H-2-linked Ir genes.

1 Abbreviations used in this paper: C', complement; CFA, complete Freund's adjuvant; ELISA, enzyme-linked immunosorbent assay; Id, idiotype; (T,G)-A--L, poly-L-(Tyr,Glu)-poly-D,L-Ala-poly-L-Lys.

Journal of Experimental Medicine - Volume 158, August 1983 465-475 465
Materials and Methods

Mice. C57BL/10 (B10), B10.A, and (B10 × B10.A)F1, mice of both sexes were obtained from The Jackson Laboratory, Bar Harbor, ME, and were used at 8–16 wk of age. Each experiment was conducted with pooled spleen or lymph node cells from at least five syngeneic mice.

Antigens. (T,G)-A--L (lot MC-9) was obtained from Miles Yeda Research and Development Co., Ltd., Rehovot, Israel. Ovalbumin (grade VI, lot 396-8055) was obtained from Sigma Chemical Co., St. Louis, MO. Complete Freund’s adjuvant (CFA) was obtained from Difco Laboratories, Detroit, MI.

Immunizations. Mice were immunized with (T,G)-A--L, ovalbumin, or only CFA as previously described (17).

Cell Preparation. Single-cell suspensions from spleen, lymph node, or thymus were prepared as previously described (17). (T,G)-A--L-primed cells and CFA-primed cells were from inguinal and popliteal lymph nodes, while ovalbumin-primed cells and unprimed cells were from spleens. B lymphocyte plus accessory cell populations (T lymphocyte-depleted) were prepared by two-stage complement-mediated lysis of T lymphocytes using a mixture of monoclonal anti-Thy-1 (T 24/40.7, produced by Dr. Ian Trowbridge, The Salk Institute, La Jolla, CA), and monoclonal anti-Lyt-1.2 (New England Nuclear, Boston, MA). Such populations contained 90% surface IgM-positive cells and <0.5% Thy-1-positive cells as evaluated by flow microfluorometry, and were completely unresponsive to concanavalin A. T lymphocytes were prepared by nonadherence to plastic petri dishes coated with affinity-purified goat anti-mouse Ig antibodies (generously provided by Dr. Richard Asofsky, National Institutes of Health), as described by Mage and colleagues (21). This population contained <1% surface IgM-positive cells and ~90% Thy-1-positive cells as evaluated using flow microfluorometry. For some experiments, Lyt-1-positive or Lyt-2-positive cells were prepared from these T cells by two-stage complement-mediated lysis using monoclonal anti-Lyt-2.2 or monoclonal anti-Lyt-1.2 (New England Nuclear), respectively. Unprimed T lymphocytes, thymocytes, or B plus accessory cells were used as filler cells in some experiments (see Results) after irradiation with 2,000 rad.

Microcultures. The microculture system has been previously described in detail (17). Briefly, B10 (unless otherwise noted in Results) cells (lymph node or cell mixtures, see Results) were cultured in triplicate at 10^6/200 #l of supplemented medium for 3 d in the presence of antigen and/or antibodies (see Results for concentrations). The initial medium was then removed and replaced with fresh medium that did not contain antigen or antibodies. After three additional days in culture the supernatants were harvested and analyzed for secreted antibodies.

Enzyme-Linked Immunosorbent Assay (ELISA). Supernatants were analyzed for secreted IgM anti-(T,G)-A--L (or IgM anti-ovalbumin) using antigen-coated plates in an ELISA as previously described (17). Each experimental point in each experiment was run in triplicate and the culture wells assayed individually. The results are presented as the arithmetic mean ± the standard error. Standardized IgM anti-(T,G)-A--L antibodies were titered on each plate so that the results could be converted to ng/ml. Negative controls were normal mouse serum or medium cultured in the absence of cells.

Antibodies. The preparation and characterization of rat antibodies specific for the idiotypes of B10 anti-(T,G)-A--L antibodies (Id) have been described previously (19). Briefly, Lewis rats were immunized with antigen affinity-purified B10 anti-(T,G)-A--L antibodies. The resulting antisera were exhaustively absorbed with normal B10 IgG-Sepharose, and are referred to as anti-Id. For certain experiments the anti-(T,G)-A--L antidiotypic antibodies were affinity purified on Id-Sepharose columns. They were eluted with 3.5 M MgCl₂. Rat anti-B10 IgG was obtained from the same antiserum by elution of the material bound to the normal B10 IgG-Sepharose with 3.5 M MgCl₂. Rat anti-mouse IgM was obtained from a rat immunized with TEPC-185 (µ, λ; Litton Bionetics, Kensington, MD) by an identical protocol and affinity purified on MOPC-104E (µ, λ; Litton Bionetics) linked to Sepharose. Rat serum specific for the idiotypes of A/J antistaphylococcal nuclease (anti-Id nuclease), prepared similarly to the anti-(T,G)-A--L anti-Id, was the generous gift of Dr. David H. Sachs, National Institutes of Health. Normal serum,
obtained from Lewis rats, was used either unabsorbed or absorbed identically to anti-Id on normal B10 IgG-Sepharose. All sera were heat inactivated and sterilized by filtration.

**T Lymphocyte Supernatants.** In vivo antigen-primed T lymphocytes were cultured $(5 \times 10^5)$ together with irradiated filler cells $(5 \times 10^5)$ in $200 \mu l$ cultures for $3$ d in the presence of $1\%$ anti-Id, $1\%$ normal serum, or medium only. The supernatants were harvested and assayed for helper activity as described in Results.

**Results**

_Effect of Antiidiotype Antibodies on (T,G)-A-L-primed Lymphocytes In Vitro._ Lymph node cells from B10 mice primed in vivo with the antigen (T,G)-A-L were cultured in vitro with antigen, rat serum specific for the idiotypes of B10 anti-(T,G)-A-L antibodies (anti-Id) or both (Table I). Somewhat to our surprise, anti-Id did not inhibit the production of IgM anti-(T,G)-A-L antibodies in response to antigen, as has been reported for some other systems $(7, 20, 22)$. Moreover, the primed lymph node cells produced IgM anti-(T,G)-A-L antibodies in response to the anti-Id in the absence of antigen. Responses to anti-Id and antigen were comparable and had the same time course (data not shown), and normal rat serum was without effect. Several other points are worth noting. The antigen-primed lymph node cells that were used spontaneously produce high levels of IgG anti-(T,G)-A-L in vitro when cultured with medium alone. Addition of antigen or anti-Id marginally enhances the production of IgG anti-(T,G)-A-L $(17$ and data not shown). Therefore, only the IgM anti-(T,G)-A-L response was further evaluated. If antigen or anti-Id is not removed from the cultures at the end of the 3rd d, the IgM anti-(T,G)-A-L antibodies are not

| Experiment No.* | (T,G)-A-L | Serum† | IgM Anti-(T,G)-A-L ng/ml |
|-----------------|-----------|--------|------------------------|
| 1               | 0.05 μg/ml| None   | 221 ± 4                |
|                 | 0.05 μg/ml| Normal | 192 ± 11               |
|                 | 0.05 μg/ml| Anti-Id| 206 ± 10               |
|                 | None      | None   | 20 ± 2                 |
|                 | None      | Normal | 53 ± 4                 |
|                 | None      | Anti-Id| 252 ± 11               |
| 2               | 0.05 μg/ml| None   | 279 ± 8                |
|                 | 0.05 μg/ml| Normal | 280 ± 11               |
|                 | 0.05 μg/ml| Anti-Id| 280 ± 22               |
|                 | None      | None   | 61 ± 1                 |
|                 | None      | Normal | 54 ± 1                 |
|                 | None      | Anti-Id| 199 ± 5                |

* In vivo (T,G)-A-L-primed B10 lymph node cells were cultured $(10^6$ cells/200 μl in triplicate) for $3$ d in the presence of (T,G)-A-L and/or serum, and then $3$ d in medium only, the supernatant harvested, and IgM anti-(T,G)-A-L assayed by ELISA.

† 1% (vol/vol). Normal = unimmunized rat serum. Anti-Id = serum from rats immunized with antigen affinity-purified B10 anti-(T,G)-A-L and exhaustively absorbed with normal B10 Ig $(19)$.  

---

**Table I**

Anti-Id Antibodies Induce IgM Anti-(T,G)-A-L Antibody Production by (T,G)-A-L-primed Lymph Node Cells in the Absence of Antigen
detected in the supernatants (17 and data not shown). This is presumably because the secreted antibodies are inhibited from binding to the antigen on the ELISA plate by the soluble antigen or anti-Id. The next series of experiments was designed to evaluate the antigen specificity of the in vitro response to anti-Id.

*Antigen-independent Response to Anti-Id Is Antigen Specific.* The antigen specificity of the response to anti-Id was evaluated in terms of priming, the specificity of the antibodies that stimulate the response, and the specificity of the antibodies secreted in culture. In vivo priming with (T,G)-A--L was required for responses to antigen or anti-Id (Table II). Neither CFA-primed cells nor unprimed cells responded. Only anti-Id antibodies stimulated secretion of IgM anti-(T,G)-A--L antibodies (Table III). The rat anti-Id serum and the same antibodies affinity purified on an Id-Sepharose column induced comparable responses. This result argues against the possibility that a nonspecific substance in the serum plays a role in stimulating responses. A variety of control rat sera and antibodies failed to stimulate IgM anti-(T,G)-A--L production. These included normal rat serum unabsorbed and absorbed identically to the anti-Id, rat antibodies specific for mouse IgG or IgM, and antiserum specific for the idiotypes of mouse

### Table II

**Antibody Responses to Anti-Id Antibodies Require Antigen-primed Cells**

| In vivo priming* | IgM anti-(T,G)-A--L in response to: |
|------------------|-------------------------------------|
|                  | Medium | (T,G)-A--L | Anti-Id | Normal serum |
| (T,G)-A--L       | 23 ± 3 | 109 ± 14  | 114 ± 14| 30 ± 4       |
| CFA              | 6 ± 1  | 0         | 0       | 0            |
| None             | 0      | 0         | 0       | 0            |

* (T,G)-A--L and CFA-primed cells were from lymph nodes, unprimed cells were from spleens. Culture conditions and reagents are as described in footnotes to Table I. (T,G)-A--L at 5 μg/ml and sera at 1% (vol/vol).

### Table III

**Only Antibodies Specific for the Idiotypes of Anti-(T,G)-A--L Antibodies Stimulate IgM Anti-(T,G)-A--L Production**

| Reagent* | IgM Anti-(T,G)-A--L |
|----------|---------------------|
| None     | 8 ± 1               |
| (T,G)-A--L | 108 ± 18         |
| Anti-Id-(T,G)-A--L (absorbed) | 111 ± 13 |
| Anti-Id-(T,G)-A--L (affinity purified) | 97 ± 18 |
| Normal serum | 2 ± 0            |
| Normal serum (absorbed) | 3 ± 0 |
| Anti-mouse IgG (affinity purified) | 8 ± 1 |
| Anti-mouse IgM (affinity purified) | 7 ± 2 |
| Anti-Id nuclease (absorbed) | 13 ± 1 |

* Antigen-primed B10 lymph node cells cultured as described in footnote to Table I. (T,G)-A--L at 0.5 μg/ml. All sera and antibodies were rat and were used at 1% (vol/vol). Affinity-purified antibodies were reconstituted to the original serum volume before use.
antinuclease antibodies. These other sera and antibodies were also tested at a
wide variety of concentrations and never stimulated responses (data not shown).
Thus, antibodies specific for the idiotypes of B10 anti-\((T,G)\)-A--L antibodies
were required. Finally, the specificity of the antibodies secreted in vitro was
evaluated in a crisscross experiment using cells primed in vivo with either \((T,G)-\)
A--L or ovalbumin (Table IV). \((T,G)\)-A--L-primed cells secreted IgM anti-\((T,G)\)A--L but not anti-ovalbumin in response to \((T,G)\)-A--L or anti-Id and did not
respond to ovalbumin. Conversely, ovalbumin-primed cells secreted IgM anti-
ovalbumin but not anti-\((T,G)\)-A--L in response to ovalbumin, and did not
respond to either \((T,G)\)-A--L or anti-Id. It was concluded from this series of
experiments that the antigen-independent response of \((T,G)\)-A--L-primed cells
to anti-Id was entirely specific.

**Evidence for Stimulation of Helper T Lymphocyte Function by Anti-Id.** To ascertain
the mechanism(s) by which anti-Id specifically stimulates antibody production in
the absence of antigen, the cell populations affected by anti-Id must first be
determined. Studies on the effects of anti-Id on antigen-primed B lymphocytes
in the absence of T lymphocytes will be reported separately (R. R. Shenk, H. Z.
Weissberger, and H. B. Dickler, manuscript in preparation). As a first step in
determining whether the anti-Id was affecting antigen-primed T lymphocytes,
experiments were conducted with mixtures of \((T,G)\)-A--L-primed T lymphocytes
and unprimed B plus accessory cells (Table V). Mixtures of \(2 \times 10^5\) \((T,G)\)-A--L-
primed T lymphocytes and \(5 \times 10^5\) unprimed B plus accessory cells responded
compactly to either antigen or anti-Id. B plus accessory cells alone or mixed
with CFA-primed T lymphocytes did not respond. \((T,G)-\)A--L-primed T
lymphocytes alone did not respond either at 2 or \(5 \times 10^5\) per culture, confirming
functionally the purity of this population. Moreover, cultures that contained \(5 \times
10^5\) antigen-primed T lymphocytes and \(5 \times 10^5\) irradiated unprimed B plus
accessory cells also did not respond to anti-Id (data not shown). This confirmed
that the anti-\((T,G)\)-A--L response seen in the mixture of primed T lymphocytes

\[
\text{TABLE IV}
\]

**Antibody Responses to Anti-Id Antibodies are Antigen Specific**

| Priming in vivo | Reagent in vitro* | IgM antibodies specific for: | (T,G)-A--L | Ovalbumin |
|-----------------|-------------------|----------------------------|------------|----------|
|                 |                   | ng/ml OD 492(\(\times 10^3\)) | OD 492(\(\times 10^3\)) |
| (T,G)-A--L      | None              | 9 ± 0 136 ± 1                 | 153 ± 16   |
|                 | (T,G)-A--L        | 171 ± 20 579 ± 68            | 160 ± 13   |
|                 | Anti-Id           | 125 ± 8 455 ± 28             | 115 ± 6    |
|                 | Ovalbumin         | 18 ± 1 162 ± 7               | 147 ± 5    |
| Ovalbumin       | None              | 40 ± 4 128 ± 13              | 55 ± 3     |
|                 | (T,G)-A--L        | 41 ± 1 130 ± 4               | 70 ± 8     |
|                 | Anti-Id           | 51 ± 11 165 ± 55             | 88 ± 1     |
|                 | Ovalbumin         | 46 ± 5 149 ± 9               | 1025 ± 205 |

* Culture conditions as described in footnote to Table I. \((T,G)\)-A--L at 0.5 \(\mu g/ml\), ovalbumin at 0.5
\(\mu g/ml\), and anti-Id at 1% (vol/vol). Because a standard IgM anti-ovalbumin was not available, the
data are presented as OD 492(\(\times 10^3\)) and this format is shown for IgM anti-\((T,G)\)-A--L as well as
ng/ml.
### Table V

**Anti-Id Antibodies Stimulate IgM Anti-(T,G)-A--L Production by Mixtures of (T,G)-A--L-Primed T Cells and Unprimed B Plus Accessory Cells**

| Cell mixture* | T cells Priming | Unprimed B + accessory cells Number | IgM Anti-(T,G)-A--L produced in response to: | Anti-Id ng/ml |
|---------------|------------------|------------------------------------|---------------------------------------------|--------------|
|               |                  |                                    | (T,G)-A--L | Anti-Id |
| —             | 0                | 5 x 10^5                           | 0            | 0            |
| CFA           | 2 x 10^5         | 5 x 10^5                           | 8 ± 3        | 0            |
| (T,G)-A--L    | 2 x 10^5         | 5 x 10^5                           | 216 ± 23     | 195 ± 29     |
| (T,G)-A--L    | 2 x 10^5         | 0                                  | 0            | 0            |
| (T,G)-A--L    | 5 x 10^5         | 0                                  | 0            | 0            |

* 2,000 rad-irradiated unprimed T cells were used as filler cells so that the number of cells per culture was constant at 10^6. Conditions are as described in footnotes to Table I. Responses of the mixtures in medium alone were 5 ng/ml or less. (T,G)-A--L at 0.05 μg/ml. Anti-Id at 1% (vol/vol).

and unprimed B plus accessory cells was from the unprimed B cells and not from primed B cells contaminating the primed T cells that responded in the presence of additional accessory cells. A series of experiments, similar to those shown in Tables II–IV, were conducted with mixtures of (T,G)-A--L-primed T lymphocytes and unprimed B plus accessory cells. The results (not shown) demonstrated specificity in terms of priming, the anti-Id, and the secreted antibodies. Thus, these experiments indicated that antigen-priming of T lymphocytes alone was sufficient to allow antigen-independent, antigen-specific antibody responses to anti-Id, and suggested, but did not prove, that anti-Id was directly affecting T helper lymphocytes. These results also suggested that there was no requirement for IgG anti-(T,G)-A--L in these responses since the unprimed B cells (in contrast to the antigen-primed B cells, see above) did not secrete IgG anti-(T,G)-A--L.

The Lyt phenotype of the T lymphocytes required for responses to anti-Id was determined in experiments in which Lyt-1-positive or Lyt-2-positive cells were prepared by complement-mediated cell lysis using monoclonal anti-Lyt-2 or anti-Lyt-1 antibodies, respectively (Table VI). After anti-Lyt-1 plus complement (C') treatment, the remaining cells (10%) no longer provided help for responses to anti-Id or (T,G)-A--L. In contrast, after anti-Lyt-2 + C' treatment, the remaining cells were significantly enriched for helper activity (compare C' only at 0.2 x 10^-5 vs. anti-Lyt-2 plus C' at 0.2 x 10^-5). Thus, the T cells required for responses to anti-Id were Lyt-1-positive, Lyt-2-negative by this criterion, i.e., phenotypically identical to the T lymphocytes required for responses to the antigen. These experiments also suggested that the effect of the anti-Id was a stimulation of helper T cells and not an inhibition of suppressor T cells or their factors. Thus, if the only effect of the anti-Id were inhibition of suppressor T cell function then the (anti-Lyt-2 plus C')-treated population should have responded in the absence of anti-Id, and this was not the case (Table VI).

To determine if the anti-Id was directly affecting helper T lymphocytes, in vivo (T,G)-A--L-primed T lymphocytes were incubated in vitro for 3 d in the
**TABLE VI**

*T Lymphocyte Required for Anti-Id Antibody Stimulation of IgM Anti-*(T,G)-A-L* Production is Lyt-1-Positive, Lyt-2-Negative

| Treatment* | Number (× 10^5) | IgM Anti-*(T,G)-A-L | Anti-Id |
|------------|----------------|---------------------|---------|
| None       | 0 2 ± 1         | 4 ± 1               | 0       |
| None       | 20 0           | 310 ± 10            | 65 ± 16 |
| C' only    | 2.0 10 ± 2      | 350 ± 5             | 111 ± 16|
| C' only    | 0.2 0          | 28 ± 9              | 0       |
| Anti-Lyt-1 + C' | 0.2 0 | 194 ± 24           | 58 ± 10 |
| Anti-Lyt-2 + C' | 2.0 0 | 322 ± 111          | 100 ± 8 |

Recoveries after treatments were: C' only, 90%; anti-Lyt-1 + C', 10%; anti-Lyt-2 + C', 60%. All cultures received 5 × 10^5 unprimed B plus accessory cells, and 2,000 rad-irradiated normal thymocytes were used as filler cells so that the number of cells per culture was constant at 10^6. *(T,G)-A-L* at 0.05 μg/ml; anti-Id at 1% (vol/vol).

**TABLE VII**

*In Vivo Antigen-primed T Lymphocytes Produce Soluble Help When Stimulated by Anti-Id Antibodies*

| Experiment No. | Unprimed B plus accessory cells incubated with:* | IgM anti-*(T,G)-A-L |
|----------------|-----------------------------------------------|---------------------|
|                | Supernatants from primed T cells stimulated by:* | Serum               |
|                | Medium                                         | Anti-Id             | 30 ± 5 |
|                | Normal Serum                                   | Anti-Id             | 6 ± 2  |
|                | Anti-Id                                        | Anti-Id             | 122 ± 18|
|                | Anti-Id                                        | None                | 14 ± 1 |
| 2              | Medium                                         | Anti-Id             | 32 ± 3 |
|                | Normal Serum                                   | Anti-Id             | 20 ± 0 |
|                | Anti-Id                                        | Anti-Id             | 126 ± 7|
|                | Anti-Id                                        | None                | 37 ± 6 |

* Unprimed B plus accessory cells (5 × 10^5/200 μl) were cultured with the T cell supernatant (75%, vol/vol) and with or without anti-Id for 3 d, then 3 d in medium only. Anti-Id at 1% (vol/vol).

* In vivo antigen-primed T cells (5 × 10^5/200 μl) plus 2,000 rad-irradiated filler cells (5 × 10^5/200 μl) were incubated for 3 d in the presence or absence of serum (1%, vol/vol). In experiment 1 the fillers were unprimed B plus accessory cells. In experiment 2 the fillers were unprimed thymocytes.

The presence of anti-Id or as controls medium or normal serum. The supernatants were collected and added to cultures of unprimed B plus accessory cells (which also contained anti-Id) to assess the supernatants for helper factors that would help IgM anti-*(T,G)-A-L* responses (Table VII). Supernatants from primed T
lymphocytes incubated with anti-Id provided help to the B lymphocytes whereas those incubated in medium or normal serum did not. The supernatants from anti-Id-incubated T lymphocytes did not contain detectable anti-Id activity (assayed independently, data not shown) and in order to obtain responses anti-Id was necessary in the B lymphocyte cultures. Thus, these experiments provide direct evidence that the anti-Id stimulates helper function from T lymphocytes, and also is a necessary signal for the responding B lymphocytes. Initial time course experiments indicated that anti-Id-stimulated T cells produced helper factors as early as 24 h and that helper activity in the supernatants peaks at 72 h. Experiments are in progress to determine if the helper factors are antigen specific, and if their production can be stimulated by antigen.

Responses to Anti-Id Appear Not To Be Regulated by H-2-linked Ir Genes. Antibody responses to (T,G)-A-L in vivo and in the in vitro system used in the current experiments are regulated by H-2-linked Ir genes that map in the I-A region (17, 18, 23). It was therefore of interest to determine if responses to the anti-Id were similarly regulated. Mixtures of antigen-primed T lymphocytes from (responder × nonresponder)F1 with unprimed B plus accessory cells from (responder × nonresponder)F1, responder, or nonresponder were analyzed for responses to (T,G)-A-L or anti-Id. As expected, responses to (T,G)-A-L were obtained with B plus accessory cells from the F1 and the responder, but not from the nonresponder. In contrast, anti-Id stimulated responses even from the nonresponder B plus accessory cells. This latter result was not simply due to the possibility that I region antigens of the a haplotype plus anti-Id mimicked allogeneic determinants, because the (responder × nonresponder)F1 T cells needed to be antigen primed (data not shown). This result suggests that responses to anti-Id are not regulated by H-2-linked Ir genes, and that the requirements for activation by anti-Id may differ from those for antigen.

Discussion

The experiments reported here demonstrate a highly reproducible system in which antiidiotype antibodies trigger antigen-specific antibody responses in the absence of antigen from antigen-primed lymphocytes. In vivo priming of T but not B lymphocytes with (T,G)-A-L was required, only antibodies specific for the idiotypes of (T,G)-A-L antibodies evoked responses, and only antibodies specific for (T,G)-A-L were secreted. Responses to antigen and anti-Id were quantitatively comparable, and anti-Id triggered T lymphocytes to release (secrete?) helper factor(s). These studies are the first direct demonstration of triggering of T lymphocyte helper function by antiidiotype antibodies, and extend our knowledge of the regulatory capabilities of such antibodies. This system should be a powerful tool for delineating the mechanisms by which antiidiotype antibodies trigger lymphocyte function. Additional studies (R. R. Shenk, H. Z. Weissberger, and H. B. Dickler, manuscript in preparation) have shown that antiidiotype antibodies will trigger antigen-primed B lymphocytes (but not unprimed B lymphocytes) to secrete specific antibody in the absence of both antigen and T lymphocytes. The formation of idiotype-antiidiotype complexes appears to play a key role in this triggering. It should be noted that these latter observations do not affect the interpretation of the current experiments on T helper lymphocytes.
because unprimed B lymphocytes were used in the mixture experiments.

Antigen-independent responses have been reported by two laboratories previously (24, 25). Trenker and Riblet (24) have reported induction of idiotype-positive antiphosphorylcholine antibodies by rabbit anti-S107 in vitro. However, in this system, help was dependent on recognition of the xenogeneic rabbit antibodies, and no evidence for stimulation of T cell function via idiotypic determinants was obtained. In the present studies, the absolute requirement for antigen priming of the T lymphocytes (Table V), and the failure of normal rat antibodies to trigger helper factor release (Table VII) argue strongly that triggering of helper function is via idiotypic determinants. Eichmann, Falk, and Rajewsky (25) were able to obtain antibody responses in vitro in the absence of antigen or antiidiotypic by mixing T lymphocytes primed with idiotype and B lymphocytes primed with either antiidiotypic or antigen. In the same report, the authors showed one experiment in which antiidiotypic antibodies stimulated antigen-primed B and T lymphocytes to produce antibody in the absence of antigen, but this was apparently not pursued further. In addition to the stimulation of T lymphocyte helper function by antiidiotypes reported here, other laboratories have reported stimulation of T lymphocytes by antiidiotypic reagents in vitro. These include stimulation of proliferation of antigen-primed T lymphocytes (26) and antigen-specific T cell clones (27), triggering of cytotoxic T lymphocyte function (28), and induction of T suppressor cells (29).

It was clear in the present studies that anti-Id triggered function in both helper T lymphocytes and B lymphocytes (Table VIII). Studies that use antiidiotypes raised against antibody molecules to affect T lymphocytes can be (and have been) interpreted to suggest that T lymphocytes use the same genes (encoded in IgVH) as B lymphocytes to synthesize antigen receptors. To date, molecular genetics studies do not support this interpretation (30). It seems equally plausible that the antiidiotypes raised against antibody molecules might simply cross-react with the T lymphocyte receptors for the same antigen. This is particularly true where the antibody response is of limited heterogeneity, as is the case for (T,G)-A-L (unpublished data). A third possibility is that the antigen affinity-purified antibody used as the immunogen for anti-Id contained T cell receptor molecules specific for (T,G)-A-L. This interpretation would lead to the prediction that T

| B plus accessory cells* | IgM anti-(T,G)-A-L |
|------------------------|--------------------|
|                        | Medium | (T,G)-A-L | Anti-Id |
| (Responder × Nonresponder)F1 | 2 ± 0   | 234 ± 27  | 143 ± 50 |
| Responder               | 5 ± 0   | 318 ± 18  | 76 ± 20  |
| Nonresponder            | 12 ± 0  | 0         | 88 ± 3   |

* Responder is B10. Nonresponder is B10.A. (Responder × Nonresponder)F1 is (B10 × B10.A)F1. Unprimed B plus accessory cells from the indicated strains (5 × 10^5) were cultured with in vivo antigen-primed (B10 × B10.A)F1 T cells (2 × 10^5) and unprimed (B10 × B10.A)F1 2,000 radi-irradiated thymocytes (3 × 10^5) as fillers. (T,G)-A-L at 0.5 μg/ml; anti-Id at 1% (vol/vol). Cultures containing the primed T cells (plus fillers) only, or unprimed B plus accessory cells of the indicated strain (plus fillers) only, did not respond to either (T,G)-A-L or anti-Id (0 ng/ml).
and B cells were triggered by different antibodies in the anti-Id. We plan to test this prediction with monoclonal anti-Id antibodies. Whichever interpretation is correct, the functional consequences and mechanisms of triggering, which are the focus of the current studies, would be similar.

It is of interest to consider by what mechanism(s) anti-Id antibodies trigger T lymphocyte helper function. It has been proposed by Eichmann and colleagues (25) that one mechanism would be physical cross-linking of B and T helper lymphocytes by divalent anti-Id antibody. Since the current studies show that antiidiotype induces secretion of soluble help that will replace the T lymphocytes (Table VII), this mechanism, while not excluded, is not obligatory. It remains possible that the antiidiotype might cross-link B lymphocytes with soluble helper factor. Studies are in progress to determine if the helper factor(s) released by (T,G)-A--L-primed T lymphocytes upon incubation with antiidiotype antibodies is antigen specific and bears idiotypic determinants.

A second possible mechanism is antigen mimicry. The initial evaluation of Ir gene regulation (Table VIII) clearly distinguished the response to (T,G)-A--L from that to antiidiotype antibodies, arguing against antigen mimicry. Indeed, if H-2-linked Ir gene regulation is simply a special case of T cell recognition of antigen in the context of Ia antigens (reviewed in reference 31), one might speculate that antiidiotype triggering of helper T lymphocyte function will not require recognition of Ia antigens. This question is being more rigorously approached in experiments in which antiidiotype triggering of helper factor production from (T,G)-A--L-primed T lymphocytes depleted of Ia-positive cells by complement-mediated cytotoxicity is being evaluated. However, we favor the view that antiidiotypes trigger helper T lymphocytes directly and that variables such as affinity and valence (ability to cross-link receptors) will be of critical importance.

Finally, it is of interest to consider the physiologic significance of anti-Id antibody triggering of antigen-specific antibody responses from primed lymphocytes in the absence of antigen. It is attractive to hypothesize that such responses play an important role in the perpetuation of humoral immunity after disappearance of the antigen.

Summary

Antibodies specific for the idiotypes of B10 anti-(T,G)-A--L antibodies (anti-Id) induced in vivo (T,G)-A--L-primed lymphocytes to secrete anti-(T,G)-A--L antibodies in vitro in the absence of antigen. Responses to anti-Id were quantitatively and qualitatively similar to responses to antigen. Responses were specific in that: (a) only lymphocytes primed in vivo with (T,G)-A--L (but not other antigens or unprimed cells) were inducible; (b) only anti-Id (but not a variety of control antibodies) induced responses; and (c) only anti-(T,G)-A--L antibodies were secreted. Antigen-primed T lymphocytes mixed with unprimed B plus accessory cells also responded to anti-Id and the phenotype of the responding T cells was Lyt-1*, Lyt-2-. Supernatants obtained from antigen-primed T cells incubated for 3 d with anti-Id (but not when incubated with controls) provided help to unprimed B plus accessory cells in the presence of anti-Id, thus providing direct evidence for induction of T lymphocyte helper function by anti-Id. In
contrast to responses to (T,G)-A–L, responses to anti-Id did not appear to be regulated by H-2-linked Ir genes. The system described is a powerful tool for delineation of the mechanisms whereby antiidiotype antibodies affect lymphocyte function.

We wish to thank Ms. Joy Britten for excellent technical assistance, and Drs. Jeffrey Bluestone, Richard Hodes, Ronald Schwartz, Alfred Singer, and David Sachs for critical review of the manuscript.

Received for publication 11 April 1983 and in revised form 18 May 1983.

References

1. Jerne, N. K. 1974. Towards a network theory of the immune system. Ann. Immunol. (Paris). 125C:373.
2. Schrater, A. F., E. A. Goidl, G. J. Thorbecke, and G. W. Siskind. 1979. Production of auto-anti-idiotypic antibody during the normal immune response to TNP-Ficoll. I. Occurrence in AKR/J and BALB/c mice of hapten-augmentable, anti-TNP plaque-forming cells and their accelerated appearance in recipients of immune spleen cells. J. Exp. Med. 150:138.
3. Brown, J. C., and L. S. Rodkey. 1979. Autoregulation of an antibody response via network-induced auto-anti-idiotype. J. Exp. Med. 150:67.
4. Cunningham-Rundles, C. 1982. Naturally occurring autologous anti-idiotypic antibodies. Participation in immune complex formation in selective IgA deficiency. J. Exp. Med. 155:711.
5. Cohen, P. L., and R. A. Eisenberg. 1982. Anti-idiotypic antibodies to the coombs antibody in NZB F1 mice. J. Exp. Med. 156:173.
6. Hart, D. A., A. L. Wang, L. L. Pawlak, and A. Nisonoff. 1972. Suppression of idiotypic specificities in adult mice by administration of anti-idiotypic antibody. J. Exp. Med. 135:1293.
7. Cosenza, H., and H. Kohler. 1972. Specific suppression of the antibody response by antibodies to receptors. Proc. Natl. Acad. Sci. USA. 69:2701.
8. Eichmann, K. 1974. Idiotype suppression. I. Influence of the dose and of the effector functions of anti-idiotypic antibody on the production of an idiotype. Eur. J. Immunol. 4:296.
9. Eichmann, K. 1975. Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. Eur. J. Immunol. 5:511.
10. 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.
11. Eichmann, K., and K. Rajewsky. 1975. Induction of T and B cell immunity by anti-idiotypic antibody. Eur. J. Immunol. 5:661.
12. Black, S. J., G. J. Hammerling, C. Berek, K. Rajewsky, and K. Eichmann. 1976. Idiotypic analysis of lymphocytes in vitro. I. Specificity and heterogeneity of B and T lymphocytes reactive with anti-idiotypic antibody. J. Exp. Med. 143:846.
13. Julius, M. H., H. Cosenza, and A. A. Augustin. 1978. Evidence for the endogenous production of T cell receptors bearing idiotype determinants. Eur. J. Immunol. 8:484.
14. Sachs, D. H., M. El-Gamil, and G. Miller. 1981. Genetic control of the immune response to staphylococcal nuclease. XI. Effects of in vivo administration of anti-idiotypic antibodies. Eur. J. Immunol. 11:509.
15. Miller, G. G., P. I. Nadler, Y. Asano, R. J. Hodes, and D. H. Sachs. 1981. Induction of idiotype-bearing, nuclease-specific helper T cells by in vivo treatment with anti-
idiotypic J. Exp. Med. 154:24.
16. Takemori, T., H. Tesch, M. Reth, and K. Rajewsky. 1982. The immune response against anti-idiotypic antibodies. I. Induction of idiotypic-bearing antibodies and analysis of the idiotypic repertoire. Eur. J. Immunol. 12:1040.
17. Weissberger, H. Z., and H. B. Dickler. 1983. Elicitation and detection of in vitro H-2-linked Ir gene regulated antibody responses to poly-L-(Tyr, Glu)-poly-dL-Ala-poly-L-Lys. J. Immunol. Methods. 58:183.
18. McDevitt, H. O., B. D. Deak, D. C. Shreffler, J. Klein, J. H. Stimpflying, and G. D. Snell. 1972. Genetic control of the immune response. Mapping of the Ir-1 locus. J. Exp. Med. 135:1259.
19. Pincus, S. H., D. H. Sachs, and H. B. Dickler. 1978. Production of antisera specific for idiotypic(s) of murine anti-(T,G)-A-L antibodies. J. Immunol. 121:1422.
20. Pincus, S. H., A. Singer, R. J. Hodes, and H. B. Dickler. 1979. Anti-(T,G)-A-L idiotypes: initial studies of genetic control and cellular expression. In Cells of Immunoglobulin Synthesis. H. Vogel and B. Pernis, editors. Academic Press, Inc., New York. 371-380.
21. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. J. Immunol. Methods. 15:47.
22. Nadler, P. I., G. G. Miller, D. H. Sachs, and R. J. Hodes. 1982. The expression and functional involvement of nuclease-specific idiotypic on nuclease-primed helper T cells. Eur. J. Immunol. 12:113.
23. Marksman, M., and H. B. Dickler. 1980. Definitive mapping of the immune response gene(s) for (T,G)-A-L to the I-A subregion. J. Immunol. 124:2999.
24. Trenker, E., and R. Ribet. 1975. Induction of antiphosphorylcholine antibody formation by anti-idiotypic antibodies. J. Exp. Med. 142:1121.
25. Eichmann, K., I. Falk, and K. Rajewsky. 1978. Recognition of idiotypic determinants on T-cell subpopulations. I. Antigen-independent cooperation between T and B lymphocytes that possess similar and complementary idiotypes. Eur. J. Immunol. 8:853.
26. Lifshitz, R., B. Parhami, and E. Mozes. 1981. Enhancing effect of murine antidiotypic serum on the proliferative responses specific for poly (Tyr, t. Glu)-poly (dt. Ala)-poly (t. Lys) [(T,G)-A-L]. Eur. J. Immunol. 11:27.
27. Infante, A. J., P. D. Infante, S. Gillis, and C. G. Fathman. 1982. Definition of T cell idiotypes using anti-idiotypic antisera produced by immunization with T cell clones. J. Exp. Med. 155:1100.
28. Binz, H., H. Frischknecht, F. W. Shen, and H. Wigzell. 1979. Idiotypic determinants on T-cell subpopulations. J. Exp. Med. 149:910.
29. Kim, B. S. 1979. Mechanisms of idiotype suppression. I. In vitro generation of idiotype-specific suppressor T cells by anti-idiotypic antibodies and specific antigen. J. Exp. Med. 149:1371.
30. Kemp, D. J., J. M. Adams, P. L. Mottram, W. R. Thomas, I. D. Walker, and J. F. A. P. Miller. 1982. A search for messenger RNA molecules bearing immunoglobulin V_{H} nucleotide sequences in T cells. J. Exp. Med. 156:1848.
31. Klein, J., A. Juretic, C. N. Coxevanis, and Z. A. Nagy. 1981. The traditional and a new version of the mouse H-2 complex. Nature (Lond.) 291:455.