Sendai virus-specific T-cell clones: Induction of cytolytic T cells by an anti-idiotypic antibody directed against a helper T-cell clone*

(idiotypic network/vaccines/T-cell stimulation)

HILDEGUND C. J. ERTL AND ROBERT W. FINBERG

Dana-Farber Cancer Institute, Laboratory of Infectious Diseases, Jimmy Fund Building, Boston, MA 02115

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ABSTRACT We used a monoclonal anti-idiotypic antibody that is directed against a Sendai virus-specific helper T-cell clone to induce an viral immune response in vivo. Splenocytes primed with the anti-idiotypic antibody mediated an antigen-specific cytolytic response. Preimmunization of mice with the anti-idiotypic antibody resulted in protection against a subsequent lethal infection with Sendai virus.

Jenner’s network theory, initially formulated and experimentally supported for humoral responses (1), seems to apply as well to certain T-cell-mediated immune mechanisms (2). Idiotypic antibodies have been shown to induce anti-idiotypic antibodies with complementary specificity (3, 4). Induction of protective humoral immunity by vaccination of mice with a mixture of several monoclonal anti-idiotypic antibodies has been described recently for a trypanosomiasis model (5).

Protection against many acute viral infections, such as murine influenza A (6) and ectromelia (7) virus infection, seems to depend upon T-cell-mediated immune mechanisms. Antibody-induced anti-idiotypic antibodies have been shown to induce T helper cells (8) (which activate B cells), T cells [which mediate a delayed-type hypersensitivity response (9, 10)], and suppressor T cells (11) (which inhibit generation of effector T cells).

Auto-anti-idiotypic antisera directed against T-cell populations stimulate a mixed leukocyte reaction (12). Several groups have recently reported the generation of monoclonal antibodies by immunization of mice with cloned T-cell lines or hybridomas (13–15). These antibodies bind to the T-cell clones used for immunization and immunoprecipitate a 90,000-dalton molecule that presumably represents the T-cell receptor (14). Functionally, these antibodies inhibit or stimulate interleukin 2 production by these T-cell clones (13–15). We have recently generated a monoclonal antibody against a cloned Sendai virus (SV)-specific T helper cell line. This IgM antibody binds specifically to a fraction of SV-specific T cells. In vivo this anti-idiotypic antibody stimulates an antiviral immune response.

MATERIAL AND METHODS

Mice. Female B10.D2 (H-2d) BALB/c (H-2b), DBA/2 (H-2b), AJ (H-2b), and B10.BR (H-2b) mice were purchased from The Jackson Laboratory and used at the age of 6–10 weeks.

Viruses. SV and influenza A/PR8 virus were prepared, titrated, and inactivated as described (16). Reovirus type 3 was most kindly provided by B. Fields.

Tumor Cell Lines. P815 cells, EL4 cells, BW5147 cells, P3-NS11-Ag4-1 (designated NS-1) myeloma cells, and B14.E6, a B cell hybridoma line (a fusion product between NS-1 cells and B10.D2 splenocytes) that secretes an anti-idiotypic antibody, were maintained in vitro in Dulbecco’s modified Eagle’s medium (DME medium) supplemented with 10% fetal bovine serum. L929 cells were provided by B. Fields.

T-Cell Lines. The methods that were employed to generate SV-specific T-cell lines were described in detail earlier (16). 2H3.E8 (2H3.E8.14 is a subclone) and 2H3.F10.6 refer to cloned T-cell lines; 1G5 and 2G8 are uncloned, long-term T-cell lines that were derived from a time assayed.

Generation of Anti-Idiotypic B-Cell Hybridomas. Over a period of 6 months, B10.D2 mice were immunized intraperitoneally at 14-day intervals with 1–2 × 106 Ficoll-Isopaque-purified 2H3.E8 cells. Mice were sacrificed 1 week after the last immunization and splenocytes were fused with the myeloma line NS-1 (17). Hybridoma culture supernatants were tested for binding to the clone used for immunization. Out of 48 different supernatants, 1 was found to be positive. Cells of this well were subcloned (1 cell per 10 flat-bottom microtiter plate wells). One subclone (designated 1B4.E6) was expanded and used for further studies. Culture supernatant of 1B4.E6 was precipitated, titrated with a saturated ammonium sulfate solution and reconstituted to a final protein concentration of 20 mg/ml (18). The secreted immunoglobulin was identified as IgM by radial immunodiffusion. To ensure that 1B4.E6 was not directed against SV antigens, ammonium sulfate-precipitated culture supernatant was tested by hemagglutination inhibition assay (HIA) and an enzyme-linked immunosorbent assay (ELISA) for anti-viral activity (19). No anti-SV activity could be found by either assay.

Immunization of Mice and in Vitro Stimulation of Effector Cells. Mice were injected twice within 24 hr intraperitoneally with 200 μl of 1B4.E6 antibody or NS-1 supernatant. Control mice received one injection of 105 hemagglutinating units (HAU) of UV-light-inactivated SV (SVuv) or influenza A/PR8 virus or 107 plaque-forming units (pfu) of reovirus type 3 (13). Seven days after the initial injection, 6 × 106 splenocytes were incubated with 100 HAU of SVuv or A/PR8 virus or 105 pfu T3 in 1.6 ml of DME medium supplemented with 2% fetal bovine serum and 0.1 mM 2-mercaptoethanol. To estimate the frequency of antiviral cytolytic T cells, limiting numbers of splenocytes (1 × 105 to 1.4 × 106 per well, 24 wells for each dilution) were incubated with 1 × 106 irradiated SVuv-pretreated (1 HAU/dish) autologous splenocytes in 20 μl of DME medium supplemented with 10% fetal bovine serum and 25% rat concanavalin A supernatant as a lymphokine source in round-bottom microtiter plates (20). Control wells received no responder cells. For analysis in a fluorescence-activated cell sorter (FACS), cells were harvested on day 6 or 7 of the in vitro culture, separated over nylon wool columns (21) to remove B cells, and subsequently centrifuged through a Ficoll-Isoaque gradient (22) to remove erythrocytes and dead cells.

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Abbreviations: FACS, fluorescence-activated cell sorter; HAU, hemagglutinating units; SV, Sendai virus; SVuv, UV-light-inactivated Sendai virus.
*This is paper no. 4 in a series. Paper no. 3 is in preparation.

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Cell Staining Procedures. Lymphocytes (10^6) were incubated for 45 min with 100 μl of 1B4.E6 or HO.134 (anti-Thy-1.2 IgM) or medium (phosphate-buffered saline) and subsequently for 45 min with 100 μl of fluorescein isothiocyanate-conjugated goat antibodies to mouse immunoglobulin. After several washings cells were fixed for 10 min with a 2% (wt/vol) paraformaldehyde solution, washed, and analyzed in a FACS II.

Cytolysis Assay. Effector cells were harvested from bulk culture after 5 days of incubation and purified by centrifugation through a Ficoll-Isopaque gradient. Various dilutions of effector cells were incubated with 2–5 × 10^3 ^51Cr-labeled infected and uninfected target cells. ^51Cr release was measured after a 5-hr incubation. Cells of wells that showed ^51Cr release more than three standard deviations above the mean of ^51Cr release in the presence of cells from control wells were regarded as being positive. The number of input responder cells/wells that yielded 37% negative wells was used to define the frequency of SV-specific cytolytic T cells (22).

Induction of Protective Immunity. Groups of five B10.D2 mice were injected intraperitoneally three times in 4-day intervals with 200 μl of 1B4.E6 or once with 10^4 HAU of SVuv or A/PR8 virus. Six days later these mice and naive control mice were inoculated intranasally with a lethal dose of infectious SV (1–2 × 10^5 HAU) or influenza virus A/PR8 (2 HAU). Five days later the virus in the lungs of infected mice was determined by titration in embryonated chicken eggs as described (23).

RESULTS
We used a cloned T-cell line, designated 2H3.E8, to induce anti-idiotypic antibodies. According to its functions—i.e., secretion of lymphokines such as interferon-γ in response to SV antigen—2H3.E8 was classified as a T helper cell clone (24). Phenotypically 2H3.E8 was Thy-1.2−, Lyt-1−2− as described for allospecific T helper cell clones (20).

Syngeneic B10.D2 mice were used to raise anti-T-cell receptor antibodies. After fusion with NS-1 myeloma cells, the supernatant of cells 1 well (out of 48 wells that contained growing hybridoma cells) was found to bind to 2H3.E8. After subcloning, the specificity of the antibody secreted by one subclone, which was designated 1B4.E6, was further investigated. We tested this antibody for binding to 2H3.E8 and 2H3.E8.14 and three other SV-specific proliferative T-cell lines (such as 1G5 and 2G8). All of these T-cell lines were derived from B10.D2 spleenocytes. The antibody bound to 2H3.E8 (data not shown), 2H3.E8.14 (Fig. 1), and one of the other T-cell clones (1G5) but failed to bind to two other T helper cell clones (2H3.F10.F6 and 2G8 shown in Fig. 1) that were functionally identical to 2H3.E8. Of 16 SV-specific T-cell lines, 6 (37%) bound 1B4.E6 (one positive and one negative example are shown in Fig. 1). Neither an anti-reovirus type 3 (derived from BALB/c mice) nor an anti-azobenzene-arsonate (derived from A/J mice)-specific proliferative T-cell line bound the antibody (data not shown). Supernatants derived from either NS-1 myeloma cells or the 8.79.26 hybridoma line, which secretes an anti-idiotypic IgM directed against a neutralizing antibody that recognizes the hemagglutinin molecule of reovirus type 3 (25), did not stain 2H3.E8 (data not shown). The antibody 1B4.E6 was of the IgM class and had no anti-viral activity.

Because we found that the anti-T-cell antibody (1B4.E6) bound to more than one SV-specific clone while not binding to other T-cell clones, we hypothesized that the determinant recognized by 1B4.E6 might be a common SV-specific idiotype. Therefore we investigated its ability to stain T cells generated from cultures of in vitro stimulated spleen cells.

In the first set of experiments, T cells from naive B10.D2 mice and from B10.D2 mice that had been primed in vivo with SV or an unrelated virus (reovirus type 3) and subsequently restimulated in vitro with SV or reovirus type 3 were tested for binding by 1B4.E6 or anti-Thy-1.2 antibodies (Fig. 2). Neither spleen cells from immunized mice stimulated in vitro with SV nor spleen cells from mice primed in vivo that were restimulated in vitro with reovirus type 3 bound the anti-idiotypic antibody. A significant number of T cells from SV-immune mice that were restimulated in vitro with SV were stained by 1B4.E6. The percentage of cells stained varied from 4% to 30% of all T cells in an SV-specific secondary culture in vitro.

In the next set of experiments we examined stimulation of a virus-specific cytotoxic T-cell response by immunization of mice with the anti-idiotypic antibody (1B4.E6). We primed mice of different haplotypes (which differ in H-2 or IgH-1 genes or both) with the anti-idiotypic antibody, SV, or influenza A/PR8 virus (as a control). Spleenocytes were restimulated in vitro with SVuv or A/PR8 virus and subsequently tested for cytolytic activity on infected and uninfected target cells (Table 1). Spleenocytes that had been primed and restimulated with virus lysed, as expected, the appropriate infected target cells. Unprimed spleenocytes that had only been stimulated with SVuv in vitro failed to cause significant lysis.
cells. Lysis was mediated by Thy-1.2⁺, Lyt-1⁻2⁻ T cells (data not shown).

To better quantitate the immunogenicity of the anti-idiotypic antibody as compared to virus, B10.D2 mice were immunized with 1B4.E6. SVuv (as a positive control), or NS-1 supernatant (as a negative control). Splenocytes were subsequently restimulated under limiting-dilution conditions and tested on SV-infected and uninfected target cells (no lysis of uninfected target cells could be observed; data not shown). Surprisingly, 1B4.E6 induced approximately as many antigen-specific cytolytic T cell clones as did SVuv (Fig. 3 Upper). The high frequency of anti-SV cytolytic T cells in mice that had been injected with the anti-idiotypic antibody should be considered in the context of the high dose of antibody injected and the relatively low dose of inactivated SV we used. The overall lysis mediated by anti-idiotypic primed T cells was, in most experiments, lower than lysis mediated by SV-immune T cells (Fig. 3 Lower), which is consistent with the results of bulk culture cytolytic lymphocytes obtained in Table 1.

T cells recognize foreign antigens in association with self antigens encoded by the major histocompatibility complex. Experiments using bulk culture analysis of spleen cells from animals immunized with SV or anti-idiotypic had indicated that the anti-idiotypic-induced T-cell response was less H-2 restricted—i.e., showed more crossreactivity on SV-infected allogeneic target cells than did cytolytic T lymphocytes induced by SV. To better quantitate this crossreactivity, limiting numbers of cells from SV-immunized mice (1 x 10⁶ cells per well; 48 wells) or 1B4.E6-immunized mice (1 x 10⁸ cells per well; 96 wells) were restimulated in vitro in microtiter wells. After 7 days, cells of each well were tested on three SV-infected target cells [P815(H-2⁰), EL4(H-2⁰), and BW5147(H-2⁰)] which have different haplotypes and (as a control) on one uninfected target cell (EL4) (Fig. 4). A responder cell dilution was chosen at which only 1/4 to 1/5 of the wells showed significant lysis of the SV-infected syngeneic target cells (14 out of 48 wells for SV-immune splenocytes; 36 out of 96 wells for 1B4.E6-immune splenocytes), and thus presumably contained clonally derived T cells. Most of the SV-induced T cells were specific for syngeneic virus-infected target cells. Only 3 of 14 positive wells (i.e., 21%) had

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### Table 1. Induction of SV-specific cytolytic effector cells by anti-idiotypic antibody

<table>
<thead>
<tr>
<th>Mice</th>
<th>Effector cells immunized/ restimulated</th>
<th>Target cells</th>
<th>% specific lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>100:1</td>
</tr>
<tr>
<td>B10.D2 (H-2⁰)</td>
<td>SV/SV</td>
<td>P815(H-2⁰)-SV</td>
<td>68 ± 7 (4 ± 2)</td>
</tr>
<tr>
<td></td>
<td>1B4.E6/SV</td>
<td></td>
<td>39 ± 3 (17 ± 2)</td>
</tr>
<tr>
<td></td>
<td>N/SV</td>
<td></td>
<td>18 ± 4 (1 ± 0)</td>
</tr>
<tr>
<td></td>
<td>SV/SV</td>
<td></td>
<td>37 ± 1 (17 ± 2)</td>
</tr>
<tr>
<td></td>
<td>1B4.E6/SV</td>
<td></td>
<td>35 ± 5 (17 ± 1)</td>
</tr>
<tr>
<td></td>
<td>A/PR8/A/PR8</td>
<td></td>
<td>7 ± 0 (4 ± 4)</td>
</tr>
<tr>
<td>A1 (H-2⁰-kkdd)</td>
<td>N/SV</td>
<td>P815(H-2⁰)-A/PR8</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>SV/SV</td>
<td></td>
<td>14 ± 0</td>
</tr>
<tr>
<td></td>
<td>1B4.E6/SV</td>
<td></td>
<td>4 ± 1</td>
</tr>
<tr>
<td></td>
<td>A/PR8/A/PR8</td>
<td></td>
<td>29 ± 1</td>
</tr>
<tr>
<td>A1 (H-2⁰-kkdd)</td>
<td>N/SV</td>
<td>L929(H-2⁰)-SV</td>
<td>5 ± 1 (6 ± 1)</td>
</tr>
<tr>
<td></td>
<td>SV/SV</td>
<td></td>
<td>35 ± 2 (1 ± 0)</td>
</tr>
<tr>
<td></td>
<td>1B4.E6/SV</td>
<td></td>
<td>37 ± 4 (10 ± 1)</td>
</tr>
<tr>
<td></td>
<td>SV/SV</td>
<td></td>
<td>43 ± 5 (11 ± 2)</td>
</tr>
<tr>
<td></td>
<td>1B4.E6/SV</td>
<td></td>
<td>54 ± 5 (16 ± 1)</td>
</tr>
</tbody>
</table>

Mice of various strains were immunized with SV, influenza A/PR8 virus, or 1B4.E6. Seven days later splenocytes of naive (N) or preimmunized mice were restimulated with either SV or influenza A/PR8. Effector cells were tested at three different effector-to-target cell ratios on infected [P815(H-2⁰)-SV, P815(H-2⁰)-A/PR8, or L929(H-2⁰)-SV] and uninfected (values in parentheses) target cells in a 4-hr ⁵¹Cr-release assay. Results are presented as mean ± SD.
DISCUSSION

The principal goal of the studies reported here was to determine whether an anti-idiotypic antibody made against a virus-specific T-cell clone could induce a virus-specific immune response in vivo. This anti-idiotypic antibody was induced by multiple immunizations of B10.D2 mice with a SV-specific T helper cell clone (23). The anti-idiotypic antibody, which belongs to the IgM class, binds to the T-cell clone used for immunization but fails to react with unrelated T-cell lines that are directed against a different virus or a hapten. The idiotype that is recognized by this antibody is expressed on 4–30% of all SV-specific T cells and thus seems to represent a dominant idiotype of the anti-SV response. Upon in vivo administration of this anti-idiotypic antibody, a cytolytic T-cell response directed against SV-infected target cells could be demonstrated in the spleens of immunized mice.

The frequency of SV-specific cytolytic T cells after immunization of mice with anti-idiotypic antibody was approximately as high as after immunization with a low dose of inactivated SV. T cell clones that were induced with anti-idiotypic antibody exhibited, on the average, less lysis of syngeneic target cells and more crossreactive lysis of infected allogeneic target cells as compared to clones that were induced with viral antigen. The anti-idiotypic antibody may stimulate a subset of cytolytic T lymphocytes that has higher affinity for viral antigen and a lower affinity for H-2 antigen. It is as yet unknown how (or whether) the anti-idiotypic antibody is processed and presented to the T-cell system in vivo.

Viral antigen and anti-idiotypic antibody may stimulate effector cells that crossreactively lysed SV-infected allogeneic (H-2k) target cells. Two other wells (which were negative on the syngeneic target cell) showed a small amount of lysis of infected H-2b target cells. Anti-idiotypic induced effector cells had much more crossreactive lysis of infected allogeneic target cells. Twenty-two of 36 positive wells (i.e., 61%) lysed infected allogeneic target cells, in addition to infected syngeneic targets. (Fig. 4.

To determine if it is possible to protect mice against a lethal SV infection by preimmunization with anti-idiotypic antibody, groups of five mice were immunized either three times in 4-day intervals with IB4.E6 or once with SVuv. Six days later, mice were challenged with a lethal dose of SV. Control mice received no preimmunization. Four out of five of the unvaccinated control mice died within 10 days after the infection. The only mouse that did not die showed clinical symptoms of SV infection but eventually recovered. Mice that were preimmunized with SVuv or IB4.E6 were completely protected against the SV infection (i.e., never displayed any signs of illness). To attempt quantitation of the effects of immunization with anti-idiotypic or inactivated virus, mice were sacrificed 5 days after infection with a lethal dose of either SV or, as a control, influenza A/PR8. The lungs of mice immunized with IB4.E6 had less than 1/103 as much infectious SV as did controls (Table 2). The administration of IB4.E6 antibody had no measurable effect on A/PR8 titers in the same experiment (data not shown).
distinct populations of T cells. The evidence of more cross-reactivity in the anti-idiotype-stimulated mice invites the speculation that the affinity of the anti-idiotypic antibody for the T-cell receptor of some T precursor lymphocytes is high enough to cause direct stimulation—i.e., without processing and presentation in context with H-2. T cell clones that are directly stimulated by the anti-idiotype might have a high enough affinity to the viral antigen to override H-2 restriction. Viral antigen that becomes integrated into the cell membrane and thus associated with H-2 molecules, on the other hand, might stimulate (predominantly) T-cell clones with low affinity for viral antigen and moderate affinity for self H-2.

Anti-idiotypic antibodies that were induced against idiotypic antibodies have been shown previously to induce T-cell-mediated immunity. Auto-anti-idiotypic antibodies directed against allo-specific T-cell populations can stimulate a proliferative T-cell response as well as cytolytic T lymphocytes (12). Anti-idiotypic antibodies, directed against antigen-specific T-cell clones, have been shown to stimulate or inhibit T-cell functions in vitro (13, 14).

We have demonstrated that cytolytic T cells can be stimulated by an anti-idiotypic antibody made to a helper T cell clone. This might suggest that cytolytic T cells and helper T cells share idiotypic structures, indicating that their repertoires are overlapping. The other hypothesis, that the anti-idiotypic antibody stimulates proliferation of antigen-specific (idiotype-bearing) helper T cells, which in turn stimulate the development of cytolytic T cells, cannot be excluded.

Data presented here show conclusively that T-cell-induced anti-idiotypic antibodies can serve as potent immunogens in vivo. We have demonstrated that anti-idiotypic antibodies can induce protective immunity against a subsequent lethal infection and thus have potential as anti-viral vaccines.