NZB/NZW F1, (B/W) mice spontaneously develop an autoimmune disease characterized by lupus erythematosus cell formation, immune complex glomerulonephritis, and production of antibodies to DNA and RNA (1-4). They are considered a laboratory model for human systemic lupus erythematosus. Genetic, immunologic, and viral factors are involved in the pathogenesis of their disease (5). Recent studies implicate a loss of suppressor T cells (6, 7) and a deficiency of thymic hormone (8, 9) as important contributing factors in their disorder.

We reported the development of monoclonal macroglobulinemia in one-third of B/W mice over the age of 12 mo (10). Monoclonal IgM was associated with generalized lymphoproliferation and tumor formation. A malignant lymphoma (141) secreting a monoclonal IgM arose spontaneously in our colony and was established as a transplantable tumor by serial passage into newborn and adult syngeneic recipients. An anti-idiotypic antiserum to the 141 IgM was prepared and used to identify the secreted monoclonal immunoglobulin in the serum of tumor transplant recipients (10).

Idiotypic determinants on the surface of normal B lymphocytes play an important role as receptors for antigens. Anti-idiotypic serum can be specifically immunosuppressive, suggesting that an immune response directed against idiotypic determinants on myeloma proteins are specifically protected against the corresponding plasma cell tumor which contains the idiotype on its cell surface (14).

We have investigated the presence of idiotype on the surface of lymphoma 141
tumor cells and the ability of 141 IgM to confer protective immunity against the

tumor. Our results show that protective immunity can be induced by prior

immunization against the monoclonal IgM and demonstrate cellular mech-

anisms which may contribute to this immunity.

Materials and Methods

Mice. B/W mice were from our colony maintained at the University of California, San Francisco,

Calif. 2- to 6-mo old mice (three to five mice per group) were used in the tumor protection

experiments.

Isolation of IgM 141. IgM was isolated from serum by 10-35% sucrose density gradient ultra-

centrifugation in an SW40 rotor (Beckman Instruments, Inc. Fullerton, Calif.) at 38,000 rpm for

20 h. Ultracentrifugation was preceded by preparative agarose gel electrophoresis in 0.05 Veronal

(pH 8.0) or 20% cold ethanol precipitation of three diluted mouse serum. MOPC 104E IgM is a

λ-type monoclonal protein produced by a mineral oil-induced transplantable plasmacytoma of

BALB/c mice (15). This protein was purified from ascitic fluid in the same way as 141 IgM.

Lymphoid Tumors in B/W Mice. Two spontaneous transplantable lymphomas (141 and 10) are

maintained in serial passage by subcutaneous or intraperitoneal inoculation of 0.2-0.5 ml of minced

cells from nodular tumors or 0.5-1.0 ml of ascites containing tumor cells (10). Cell transfers are made

at about 2-wk intervals. Tumor 141 is histologically a lymphoblastic lymphoma producing IgM which

originated from an enlarged spleen (16). This tumor is now in its 24th transplant generation. Tumor

10 is a mixed lymphoma (lymphoblasts and reticulum cells) which originated from enlarged cortical

and axillary lymph nodes. This tumor is now in its 10th transplant generation.

Membrane Localization of Idiotype by Indirect Immunofluorescence. Fluorescein-conjugated

goat antirabbit 7S IgG (Meloy Laboratories, Inc., Springfield, Va.) was used at a concentration of 1

mg/ml. Rabbit anti-idiotype specific serum to 141 IgM was prepared as previously described (10). 2 × 10^9/ml

of lymphoma 141 cells were suspended in RPMI 1640 medium. (Grand Island Biological Co., Grand

Island, N. Y.). 0.1 ml of cells was incubated with 0.1 ml of diluted rabbit anti-idiotype 141 at 0°C for

30 min and washed in a large volume (12-15 ml) of medium. Washed cells were suspended in 0.1 ml of

fluorescein-conjugated goat antirabbit IgG for 30 min at 0°C. Cells were then washed in a large

volume of medium and resuspended in 50 μl of medium containing 50% glycerol for microscopic

study. After washing some cells were further incubated in culture medium at 37°C for various time

periods in order to allow “capping” to take place.

Fluorescence Microscopy. A Zeiss microscope (Carl Zeiss, Inc., New York) was used with a BG12

excitation filter and a 53/50 barrier filter. The light source was an Osram HBO-200 mercury arc lamp.

Photographs were taken on High Speed Ektachrome 35-mm daylight film.

Immunization of B/W Mice with 141 IgM and Transplantation of Lymphoma 141 Cells. Purified

mouse IgM (141 or 104E), human IgM, or bovine serum albumin in Freund’s complete adjuvant were

injected into B/W mice in multiple sites. The total protein was 1.0 mg given in three weekly or

biweekly injections. 1 wk after the last injection, mice were inoculated subcutaneously with different

doses of 141 tumor cells (1 × 10^4 or 1.5 × 10^5). Every 3 days thereafter, mice were examined for

palpable tumors and the diameter of the tumors was measured. Animals which showed delayed

appearance of tumors were called protected mice, whereas animals regressing tumors were called

regressor mice.

51Cr Labeling of Lymphoma 141 Target Cells. Ascitic tumor cells were harvested by aspiration

and washed three times in RPMI 1640 medium. The cell concentration was adjusted to 2 × 10^8

cells/ml. 1 ml of this suspension was incubated with 200 μCi of 51Cr (as sodium chromate, sp act > 100
μCi/μg; International Chemical & Nuclear Corporation, Burbank, Calif.) at 37°C for 30 min. The

labeled cells were washed twice in 50 ml of RPMI 1640 medium and diluted to a final concentration of

5 × 10^5 cells/ml.

Preparation of Spleen Cells. Spleens were removed from control and immunized mice and finely

minced in RPMI 1640 medium. The resultant single cell suspension was washed three times and cells

adjusted to 1 × 10^7 cells/ml of RPMI 1640 medium containing 10% heat-inactivated human serum.

Viability was more than 90% as determined by trypan blue dye exclusion test.
Assay for Cell-Mediated Cytotoxicity. The cell-mediated cytotoxicity test was performed by the method of Canty and Wunderlich with some modifications (17). 1 ml of control or immune spleen cell suspension (1 x 10^7 cells) and 0.1 ml of 51Cr-labeled tumor target cell suspension (5 x 10^6 cells), was pipetted into 35 x 10-mm plastic petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). The immune cell to target cell ratio was 200:1. Cultures were performed with 2-3 replicates. Dishes were placed in horizontal trays on a rocking platform at 37°C in a moist atmosphere of 90% air and 10% carbon dioxide. Dishes were rocked continuously at 6 complete cycles/min during the incubation period of 7 or 15 h. After incubation, the contents of each petri dish were transferred into tubes with 1 ml of cold medium and centrifuged at 400 g for 10 min. 1 ml of supernate from each tube was assayed for radioactivity (51Cr release) in a Packard γ-Scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The specific cytotoxicity was then calculated as follows:

\[
\text{Specific cytotoxicity} (\%) = \frac{E - C}{T - C} \times 100.
\]

E, cpm of 51Cr released from cells in the petri dish containing experimental cells; C, cpm of 51Cr released from cells in the petri dish containing normal cells; and T, cpm of 51Cr released from cells in the petri dish containing 1 ml of distilled water.

Inhibition of cell-mediated cytotoxicity by 141 IgM or 104E IgM was performed by incubating 1.0 ml of spleen cells (4 x 10^5/ml) with 1.0 ml of 141 IgM or 104E IgM (100 μg/ml or 160 μg/ml) for 30 min at 37°C. After incubation, cells were washed and adjusted to 1 x 10^6/ml viable cells in RPMI 1640 medium.

Assay for Complement-Dependent Cytotoxicity. Complement-dependent humoral cytotoxicity was determined by mixing 0.1 ml of 51Cr-labeled target cells with 0.1 ml of anti-idiotypic serum and 0.1 ml of five times diluted rabbit serum preabsorbed with lymphoma 141 cells. The percent cytotoxicity was calculated the same way as for cell-mediated cytotoxicity.

Lymphocyte Transformation by 141 IgM or 104E IgM. Spleen cells from B/W mice preimmunized with IgM 141 and challenged with the corresponding 141 lymphoma were either pretreated with 141 or 104E IgM before washing and culturing, or cultured in the presence of IgM (5–100 μg/ml). The proteins were first dialyzed against RPMI 1640. Spleen cells from control B/W mice who were neither immunized nor challenged with tumor were handled in the same way. Cultures of 5.0 x 10^5 lymphocytes in a final vol of 0.2 ml were established in microtiter plates, using RPMI 1640 medium containing 10 mM Hepes buffer and 1% antibiotic-antimycotic mixture (GIBCO). They were maintained in 5% CO₂ at 37°C. 1 μCi of tritiated thymidine (3,000 mCi/mM) was added 8–12 h before automated suction harvesting with saline, trichloracetic acid, and methanol on the 4th day (18). Data shown are mean ± standard errors of counts per minute incorporated, usually from three or four replicate cultures. The difference between 141 IgM-stimulated incorporation and 104E IgM-stimulated incorporation in control spleen cells was compared with the corresponding difference in protected spleen cells using the Student’s t test. Statistical significance was determined from the means and standard errors of the logarithms of the incorporated radioactivity, pooling the variance of the logarithms.

Antibody Activity of 141 IgM. Purified 141 IgM was studied for antibody activity against various antigens by the following methods: (a) Filter radioimmunoassay to detect antibodies to DNA or RNA (19), (b) bentonite flocculation test to detect antimouse IgG (20), (c) trypan blue dye exclusion method to detect antithymocyte antibody (21), (d) hemagglutination test to detect antired blood cell antibody, (e) immunodiffusion method to detect anti-α1–3 dextran (courtesy of Dr. Roy Riblett, Salk Institute, San Diego, Calif.), and (f) immunoprecipitation method to detect antimalleus leukemia virus antigens (courtesy of Dr. Richard Lerner, Institute for Cancer Research, Philadelphia, Pa.).

Results

Idiotypic Determinants on Cell Surface Membranes. The presence of idiotypic determinants on the surface of lymphoma 141 cells was demonstrated by indirect immunofluorescence using rabbit anti-idiotypic antiserum (Fig. 1).
tensity of fluorescence diminished with dilution of the anti-idiotype serum, but some staining was still seen even at 1:1,280 dilution (Table I). To confirm the specificity of this staining reaction, inhibition studies were performed using purified monoclonal IgM from lymphoma 141 and from a BALB/c plasmacytoma (MOPC 104E). The 141 IgM inhibited the fluorescent staining completely, whereas the 104E IgM had no effect (Table I).

Two further specificity controls were performed: (a) anti-idiotypic serum prepared against another B/W monoclonal IgM (108) failed to show immunofluorescence against the lymphoma 141 cells, and (b) anti-idiotypic serum against lymphoma 141 failed to show membrane immunofluorescence against another B/W transplantable lymphoma (10) maintained in our laboratory. Lymphoma 10 does have surface immunoglobulin since it shows membrane immunofluorescence with antiserum against μ-heavy chain and κ-light chain.

### Table I

**Detection of Idiotypic Determinants on Membranes of Lymphoma 141 Cells by Treatment with Rabbit Antiserum to Idiotype 141**

<table>
<thead>
<tr>
<th>Titer of anti-141</th>
<th>Indirect immunofluorescence*</th>
<th>Percent</th>
<th>Percent after inhibition by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>141 IgM</td>
</tr>
<tr>
<td>80</td>
<td>+3§</td>
<td>92</td>
<td>4</td>
</tr>
<tr>
<td>160</td>
<td>+3</td>
<td>92</td>
<td>5</td>
</tr>
<tr>
<td>320</td>
<td>+2</td>
<td>91</td>
<td>0</td>
</tr>
<tr>
<td>640</td>
<td>+1</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>1,280</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,560</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* After staining with fluorescein-conjugated, goat antiserum to rabbit IgG, fluorescence was graded on a scale of 0 to +3.

† Specific release of °Cr from 141 cells in the presence of complement, and after incubation of the anti-141 serum with 4 μg/ml of 141 or 104E IgM.

§ Fluorescence was completely eliminated by inhibition with 141 IgM (25 μg/ml) but not reduced by 104E IgM at the same concentration.

We attempted to induce “capping” by exposure of lymphoma 141 cells to anti-idiotypic antiserum followed by incubation at 37°C for various time periods. Only about 10% of cells showed typical “capping” after 3-h incubation; the majority of cells showed membrane localization in a crescent pattern over approximately 30% of the cell surface (Fig. 1). There was no increase in typical capping even when a double antibody method was used (incubation with rabbit anti-idiotype and goat antirabbit sera at 37°C for up to 12 h).

![Fig. 1. Indirect immunofluorescence of lymphoma 141 cells exposed to rabbit anti-idiotypic antiserum and fluorescent goat antirabbit serum at 0°C for 30 min (a) or at 37°C for 3 h (b). Magnification: (a) × 400, (b) × 300.](image-url)
Additional evidence for the presence of idiotypic determinants on lymphoma 141 cells was provided by cytotoxicity studies using tumor cells labeled with radioactive chromium. The rabbit anti-idiotypic serum was highly cytotoxic in the presence of complement. As with the immunofluorescent studies, this cytotoxicity was specifically inhibited by 141 IgM but not by 104E IgM (Table I). These inhibition studies confirm the specificity of the anti-idiotypic serum against cell surface immunoglobulins and indicate the accessibility of the idiotypic determinants on the cell membrane.

Protection Against Lymphoma 141 by Prior Immunization with 141 IgM. Experiments by Lynch and colleagues suggest that idiotypic determinants on induced BALB/c plasma cell tumors behave as tumor-associated transplantation antigens (13). We studied whether the idiotype of 141, a naturally occurring B/W lymphoma, could function in the same way. In the first experiment, 6-mo old B/W male mice were immunized with either 141 IgM or human macroglobulinemic serum in complete Freund's adjuvant. After the third injection, the immunized mice received $1.5 \times 10^7$ lymphoma 141 cells injected subcutaneously. Tumor nodules appeared in all mice 15 days later and grew progressively. Three out of five mice immunized with 141 IgM showed tumor regression starting about day 35 (Fig. 2). One regressor mouse was sacrificed for cytotoxicity studies (see

![Graph](image-url)
The other two showed a gradual regrowth of tumor and died 2 and 3 mo later. Serum of these animals showed a positive immunoprecipitin reaction with anti-idiotypic antiserum on several occasions as well as a small amount of an M protein. Two mice immunized with human macroglobulinemic serum showed no regression of tumors.

In a second experiment, 2-mo old mice were unimmunized or immunized with either 141 IgM, 104E IgM, human monoclonal IgM from a patient with Waldenstrom's macroglobulinemia, or bovine serum albumin. Tumor cells were again injected after the last immunization. A smaller number of lymphoma cells (1 × 10^5) were inoculated, this representing the minimal lethal dose of tumor. 15 of 16 control mice developed progressive tumors and died on the 35th day (Fig. 2). Four out of five mice immunized with 141 IgM had only a small tumor nodule on the 35th day.

Because of the close similarity between 141 IgM and 104E IgM, it is of interest to compare the survival data of mice immunized with these monoclonal mouse macroglobulins. All seven mice immunized with 141 IgM survived past day 35 after tumor grafting, whereas all five mice immunized with 104E IgM died before day 28. These results are highly significant (p < 0.001) by chi square analysis with Yates modification.

**Mechanism of Protection against Lymphoma 141.** Sera from mice regressing tumors were studied for cytotoxic activity against lymphoma 141 cells labeled with radioactive chromium. Whereas rabbit anti-idiotypic antiserum was highly cytotoxic (Table I), regressor sera killed only 7–20% of the tumor cells (compared to control sera from mice with progressive tumors which killed 1–5% of tumor cells). These results suggest that humoral immunity against lymphoma 141 was present but rather weak. We could not detect any antibody-dependent cellular cytotoxicity using regressor sera and normal mouse spleen cells.

By contrast, cellular cytotoxicity against lymphoma 141 was observed in four out of five protected mice studied (Table II). The degree of positive cytotoxicity varied from 26–88%. The specificity of this cytotoxic activity was studied. In two

<table>
<thead>
<tr>
<th>Mouse no.</th>
<th>Specific cytotoxicity*</th>
<th>Percent after inhibition by:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>141 IgM</td>
</tr>
<tr>
<td>477</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>505</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>501</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>536</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>537</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

*Specific release of ^4^Cr from 141 cells in the absence of complement, and after treatment of cytotoxic spleen cells with 141 or 104E IgM.
experiments (mice 536 and 537), spleen cells were cytotoxic against lymphoma 141 but not against lymphoma 10. Moreover, in mice 501 and 536, cytotoxicity by spleen cells could be specifically blocked by prior incubation with 141 IgM but not with 104E IgM (Table II). These results suggest the presence in protected or regressor mice of spleen cells specifically cytotoxic for lymphoma 141, presumably by virtue of idiotypic recognition.

The presence of such spleen cells capable of recognizing idiotype was detected by a second assay procedure. Spleen cells from protected mice were exposed to 141 or 104E IgM in tissue culture and the incorporation of \(^{3}H\)thymidine into DNA was measured. Responses were compared with control B/W mice. In general, neither protein stimulated DNA synthesis in control spleen cells, whereas 141 IgM but not 104E IgM stimulated DNA synthesis in cells from experimental mice (Table III). In the first experiment, the specific proliferative response to 141 IgM was partially dependent upon the concentration of IgM used to stimulate the cells. In the second experiment, a specific response was obtained by leaving the IgM in the cultures but not by removing it before culturing. In the third experiment, metastases to the spleen reduced baseline synthesis 10-fold, but responsiveness specific for 141 IgM was still evident. Thus, in all three experiments, there was stimulation of spleen cells from protected mice by 141 IgM but not by 104E IgM.

**Lack of Detectable Antibody Activity in 141 IgM.** About 5% of monoclonal

### Table III

<table>
<thead>
<tr>
<th>Protocol*</th>
<th>Incorporation of (^{3}H)Tdr (cpm ± SE) after:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of IgM</td>
<td>No IgM</td>
</tr>
<tr>
<td>Exp.</td>
<td>Wash</td>
</tr>
<tr>
<td>I</td>
<td>5 + Control</td>
</tr>
<tr>
<td>5 + 501</td>
<td>6,189 ± 38</td>
</tr>
<tr>
<td>50 + Control</td>
<td>3,877 ± 518</td>
</tr>
<tr>
<td>50 + 501</td>
<td>6,189 ± 38</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>50 + Control</td>
</tr>
<tr>
<td>50 + 536</td>
<td>3,806 ± 121</td>
</tr>
<tr>
<td>83 - 536</td>
<td>4,902</td>
</tr>
<tr>
<td>III</td>
<td>100 - Control</td>
</tr>
<tr>
<td>100 - 535</td>
<td>537 ± 45</td>
</tr>
</tbody>
</table>

* Spleen cells incubated with various concentrations of 141 or 104E IgM were either cultured directly or washed to remove IgM and then cultured.
immunoglobulins have been shown to combine with specific antigens (15, 22). MOPC 104E, for example, reacts with $\alpha_1 \rightarrow 3$ dextrans (15). Many monoclonal IgMs in humans are antigamma globulins and have rheumatoid factor-like properties (22, 23). We have tried to find an antibody activity for the 141 IgM that arose spontaneously in B/W mice. We are unable to detect activity against DNA or RNA, mouse gamma globulin, mouse erythrocytes, mouse thymocytes, $\alpha_1 \rightarrow 3$ dextran, or mouse leukemia viral antigens.

Discussion

This study demonstrates the presence and potential significance of idiotypic determinants on the surface membranes of an IgM-producing lymphoma (141) that arose spontaneously in B/W mice. The presence of idiotypic was shown by immunofluorescence and cytotoxicity using a rabbit anti-idiotypic antiserum. The specificity was confirmed by inhibition with 141 IgM but not with 104E IgM, a monoclonal IgM produced by an oil-induced plasmacytoma of BALB/c origin. Moreover, another B/W lymphoma (10) failed to show membrane fluorescence with anti-idiotypic serum directed against 141.

Surface membrane immunoglobulins are a distinguishing characteristic of normal and malignant B lymphocytes. In normal human peripheral blood, about 25% of circulating lymphocytes show surface immunoglobulin by immunofluorescence with polyvalent antiserum (24). In chronic lymphocytic leukemia, almost 100% of circulating lymphocytes show membrane immunoglobulin and are classified as B lymphocytes. This membrane immunoglobulin is often monoclonal, belonging to either the IgG or IgM immunoglobulin class (25).

In Waldenstrom's macroglobulinemia, the majority of circulating lymphocytes contain the monoclonal IgM on their cell surface (25). Indeed, in Waldenstrom's macroglobulinemia and cold agglutinin disease, the secreted monoclonal IgM and the cell surface IgM have the same idiotype as shown by cytotoxicity (26). This result indicates that the same unique antigenic determinants are present both on the secreted immunoglobulin and on the cell surface immunoglobulin.

The potential significance of finding idiotypic determinants on the surface membranes is shown by their ability to function as tumor-associated transplantation antigens. Our experiments relate to the work of Eisen and associates who first observed that BALB/c mice produced "autoimmune-like" antibodies against unique idiotypic determinants present in the ligand-combining sites of some functional BALB/c myeloma proteins (27). They next observed (13) that such immunized mice could specifically suppress growth of the corresponding transplanted tumor cells (either MOPC-315 or MOPC-460). In all, they found anti-idiotypic antibody responses in BALB/c mice to 6 of 7 myeloma proteins, and in three cases, animals making an anti-idiotypic response suppress growth of the corresponding plasmacytoma (H. N. Eisen, personal communication). They did not look for cell-mediated immunity in their protected mice, but considered cellular immunity directed against idiotypic determinants as a possibility.

Our experiments with a natural tumor of B/W mice extend Eisen's observations. Our results strongly suggest that cellular immunity directed against
idiotype is present in the spleens of regressor and protected mice. Both by cytotoxicity and lymphocyte stimulation, evidence was obtained for cellular sensitization to the syngeneic 141 IgM. Four out of five cytotoxicity experiments were positive, and in each of two experiments, cellular cytotoxicity was inhibited by 141 IgM but not by 104E IgM. Likewise, splenic lymphocytes from protected mice were stimulated to synthesize DNA by 141 IgM but not by 104E IgM. Thus, this phenomenon appears to represent cellular “autoimmunity” directed against idiotype, the counterpart of Eisen’s finding with humoral immunity.

Recognition of antigenic determinants on 141 IgM that are not idiotypic are largely excluded by using the 104E IgM as a control. The lack of another B/W monoclonal IgM prevents further specificity controls at this time. However, since idiotypic determinants are known to function as tumor-associated transplantation antigens conferring protective immunity (13), and since idiotypic determinants are accessible on the surface membranes of lymphoma 141 cells, we interpret our results to suggest cellular recognition of idiotype as the most likely protective mechanism.

Our failure to detect much humoral immunity against idiotype in the protected mice may be due to lack of an assay such as Eisen employed that measures the interaction between monoclonal antibody, anti-idiotype, and specific ligand (27). Such an assay depends upon a known functional activity for the monoclonal protein, which we have been unable to find.

The nature of the cytotoxic cell will be studied in future experiments. We presume it to be a T lymphocyte. Rollinghoff and Wagner have recently shown that T cells are required for both the induction of immunity and the effector phase of cell-mediated cytotoxicity directed against plasmacytomas (28). Furthermore, allotype suppression requiring the active presence of T lymphocytes has been reported (29).

The presence of lymphocytes with receptors capable of recognizing idiotype may also have important implications for immune regulation. Anti-idiotypic serum can be specifically immunosuppressive. For example, anti-idiotypic serum directed against receptors for phosphorylcholine can suppress a normal immune response to phosphorylcholine (11). A theory of regulation based on anti-receptor (idiotype) recognition has been proposed as a means for limiting the extent of an immune response (12, 30). Our findings suggest that cellular as well as humoral mechanisms may participate in such regulation. Indeed, lymphocytes capable of recognizing idiotype receptors on other lymphocytes could function as suppressor cells by releasing cytotoxic or inhibitory factors. If the receptors were those capable of binding autoantigens, such a suppressor mechanism could function normally to prevent the proliferation of autoantibody producing clones and the release of autoantibodies. The lack of such a regulatory mechanism might contribute to some types of autoimmune and lymphoproliferative disorders. Moreover, the generation of lymphocytes capable of recognizing idiotype receptors for autoantigens might have therapeutic potential in these diseases. Since functional monoclonal proteins exist in mice (15), this possibility can be tested experimentally.
Summary

A spontaneous lymphoma (141) producing monoclonal IgM is established in NZB/NZW F1 (B/W) mice who spontaneously develop an autoimmune disease. Idiotypic determinants of 141 IgM are present on the lymphoma cell surface as shown by indirect immunofluorescence and specific cytotoxicity with rabbit anti-idiotype antiserum. Fluorescence and cytotoxicity are inhibited by 141 IgM but not by 104E IgM, a monoclonal IgM produced by a BALB/c plasmacytoma.

Immunization of B/W mice with 141 IgM before transplantation of lymphoma 141 confers protective immunity. No such protection occurs after immunization with 104E IgM or other unrelated proteins. Protected mice contain spleen cells cytotoxic for 141 lymphoma cells. This cytotoxicity is blocked by incubation of spleen cells with 141 IgM but not with 104E IgM. Moreover, splenic lymphocytes from protected mice are stimulated to synthesize DNA by 141 IgM but not by 104E IgM. These results suggest that specific cellular immune responses to idiotypic determinants may participate in the observed protection against challenge with the corresponding B-cell tumor.

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References


