PROTECTIVE AND CELLULAR IMMUNE RESPONSES

TO IDIOTYPIC DETERMINANTS

ON CELLS FROM A SPONTANEOUS

LYMPHOMA OF NZB/NZW F$_1$ MICE*

By SUSUMU SUGAI, DONALD W. PALMER, NORMAN TALAL, AND ISAAC P. WITZ‡

(From the Department of Medicine, University of California at San Francisco, San Francisco,
California 94143 and the Clinical Immunology Section, Veterans Administration, San
Francisco, California 94121)

NZB/NZW F$_1$ (B/W)[1] 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 idiotype cell receptors may be one mechanism for regulation of the antibody response (11, 12). Idiotypic determinants on the surface of malignant plasma cells behave like tumor-associated transplantation antigens (13). Syngeneic mice immunized against the 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 mechanisms 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 ultracentrifugation 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 times 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 cervical 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-idiotypic serum to 141 IgM was prepared as previously described (10). 2×10^7/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 (1×10^5 or 1.5×10^7). 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.

^{51}Cr 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 2×10^7 cells/ml. 1 ml of this suspension was incubated with 200 µCi of ^{51}Cr (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 (1×10^7 cells) and 0.1 ml of ^{51}Cr-labeled tumor target cell suspension (5×10^4 cells), was pipetted into 35×10-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 (^{51}Cr 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 ^{51}Cr released from cells in the petri dish containing experimental cells; C, cpm of ^{51}Cr released from cells in the petri dish containing normal cells; and T, cpm of ^{51}Cr 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 (4×10^7/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 1×10^7/ml viable cells in RPMI 1640 medium.

Assay for Complement-Dependent Cytotoxicity. Complement-dependent humoral cytotoxicity was determined by mixing 0.1 ml of ^{51}Cr-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 5.0×10^5 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% CO_2 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 methonol on the 4th day (18). Data shown are mean $\pm$ 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$\rightarrow$3 dextran (courtesy of Dr. Roy Riblett, Salk Institute, San Diego, Calif.), and (f) immunoprecipitation method to detect antimouse 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).The in-

1a

1b

1550
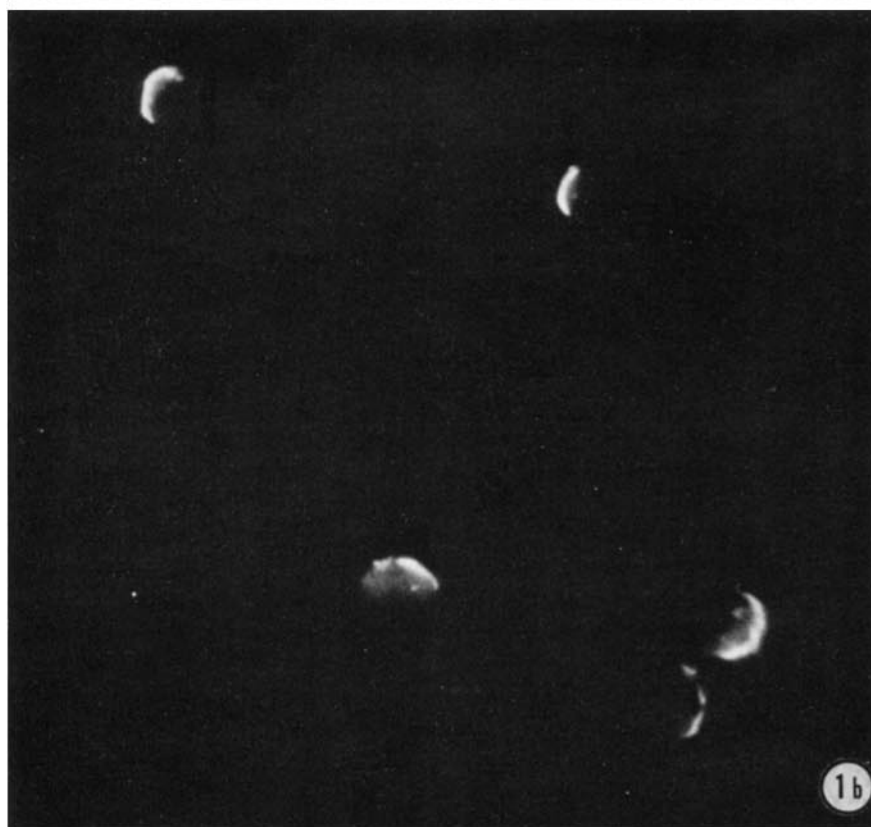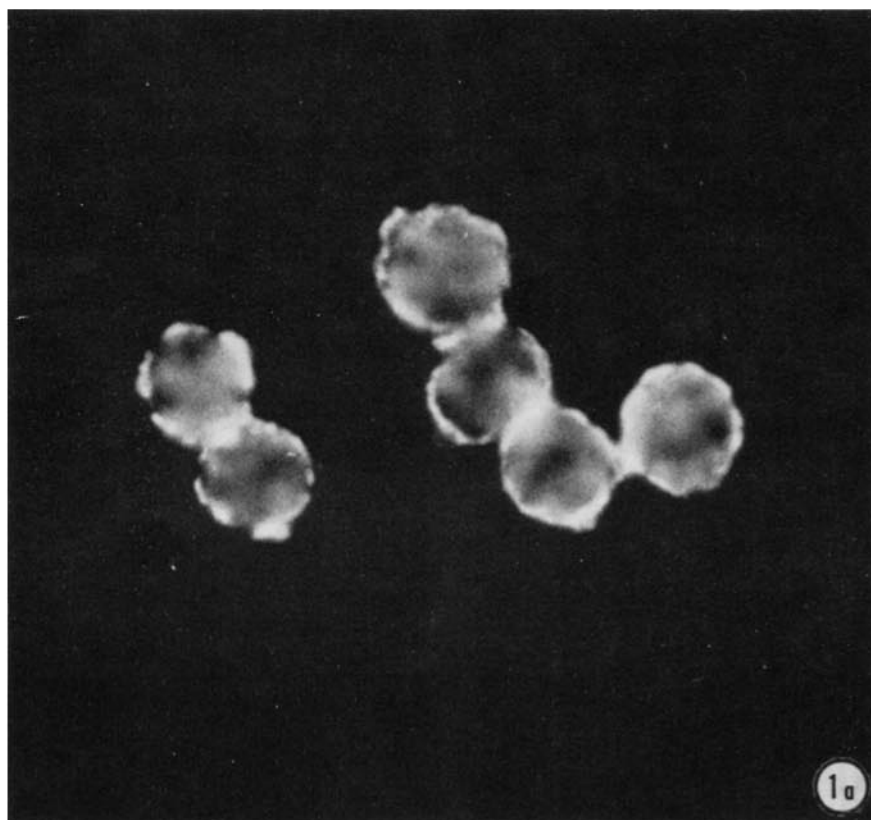
tensity of fluorescence diminished with dilution of the anti-idiotype serum, but some staining was still seen even at 1:1,280 dilution (Table I). To confirm the specificity of this staining reaction, inhibition studies were performed using purified monoclonal IgM from lymphoma 141 and from a BALB/c plasmacytoma (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

Titer of anti-141	Indirect immunofluorescence*	Specific cytotoxicity‡		
		Percent	Percent after inhibition by:	
			141 IgM	104E IgM
80	+3§	92		
160	+3	92	4	90
320	+3	92	5	93
640	+2	91	0	
1,280	+1	80		
2,560	0			

* After staining with fluorescein-conjugated, goat antiserum to rabbit IgG, fluorescence was graded on a scale of 0 to +3.
‡ Specific release of ^{51}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.

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 1.5×10^7 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

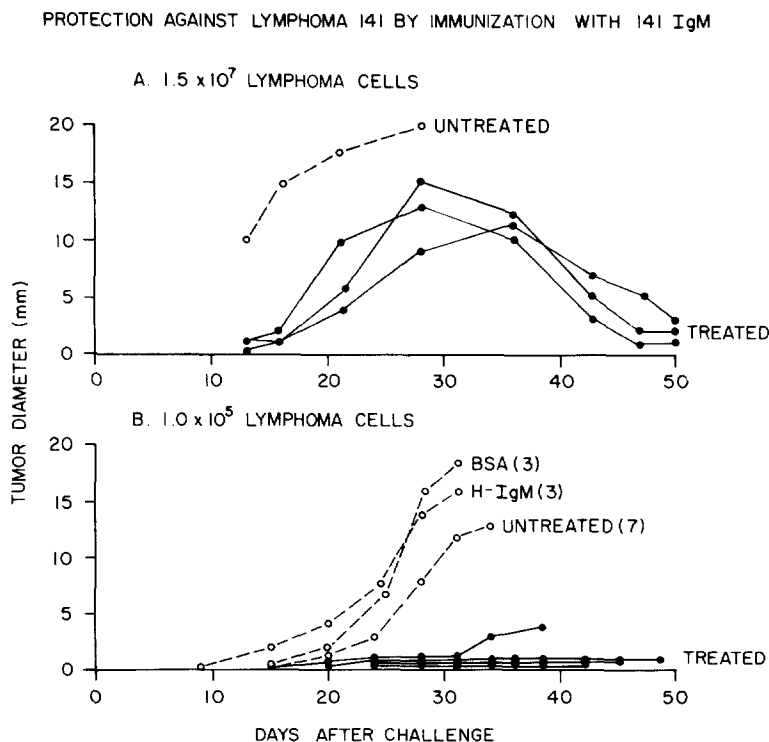PROTECTION AGAINST LYMPHOMA 141 BY IMMUNIZATION WITH 141 IgM

Fig. 2. Tumor diameter as a function of days after challenge with lymphoma 141 cells in unimmunized (untreated) and immunized (treated) mice. The challenge inoculum was either 1.5×10^7 cells (A) or 1.0×10^5 cells (B). Three mice were immunized with bovine serum albumin (BSA) and three with a human monoclonal IgM (H-IgM).

below). 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 II

Cellular Immunity Against Lymphoma 141 by Spleen Cells from Protected Mice

Mouse no.	Specific cytotoxicity*		
	Percent	Percent after inhibition by:	
		141 IgM	104E IgM
477	88		
505	9		
501	27	5	23
536	26	0	23
537	45		

* Specific release of ^{51}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, presum-
ably 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 [³H]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

*Specificity of the In Vitro DNA Synthetic Response to IgM 141 by Spleen Cells from
Protected Mice*

Protocol*				Incorporation of [³H]TdR (cpm ± SE) after:			
Exp.	Concentration of IgM	Wash	Mouse	No IgM	141 IgM		104E IgM
	µg/ml						
I	5	+	Control	3,877 ± 518	3,804 ± 254		3,509 ± 800
	5	+	501	6,189 ± 38	10,899 ± 336		8,499 ± 875
						p < 0.2	
	50	+	Control	3,877 ± 518	2,221 ± 190		3,501 ± 123
	50	+	501	6,189 ± 38	10,187 ± 645		7,206 ± 222
						p < 0.001	
II	50	+	Control	1,958 ± 22	3,180 ± 127		2,505 ± 98
	50	+	536	3,806 ± 121	2,605 ± 84		2,659 ± 284
	83	–	536	4,902 —	12,264 —		3,917 —
III	100	–	Control	5,401 ± 1,084	5,414 ± 844		6,736 ± 707
	100	–	535	537 ± 45	1,306 ± 173		459 ± 6
						p < 0.001	

* 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 idiotype 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 antireceptor (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 F_1 (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-idiotypic 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.

References

1. Burnet, F. M., and M. C. Holmes. 1965. The natural history of the NZB/NZW F_1 hybrid mouse: a laboratory model of systemic lupus. *Australas Ann. Med.* 14:185.
2. Aarons, L. 1964. Renal immunofluorescence in NZB/NZW MICE. *Nature (Lond.).* 203:1080.
3. Lambert, P. H., and F. J. Dixon. 1968. Pathogenesis of the glomerulonephritis of NZB/W mice. *J. Exp. Med.* 127:507.
4. Talal, N., and A. D. Steinberg. 1974. The pathogenesis of autoimmunity in New Zealand Black Mice. *Curr. Top. Microbiol. Immunol.* 64:79.
5. Talal, N. 1970. Immunologic and viral factors in the pathogenesis of systemic lupus erythematosus. *Arthritis. Rheum.* 13:887.
6. Barthold, D. R., S. Kysela, and A. D. Steinberg. 1974. Decline in suppressor T cell function with age in female NZB mice. *J. Immunol.* 112:9.
7. Allison, A. C., A. M. Denman, and R. D. Barnes. 1971. Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet.* 1:135.
8. Bach, J. F., M. Dardenne, and J. C. Salomon. 1973. Studies of thymus products. IV. Absence of serum thymic activity in adult NZB and (NZB × NZW) F_1 mice. *Clin. Exp. Immunol.* 14:247.
9. Talal, N., M. Dauphinee, R. Pillarisetty, and R. Goldblum. 1974. Effect of thymosin on thymocyte proliferation and autoimmunity in NZB mice. *Ann. N. Y. Acad. Sci.* In press.
10. Sugai, S., R. Pillarisetty, and N. Talal. 1973. Monoclonal macroglobulinemia in NZB/NZW F_1 mice. *J. Exp. Med.* 138:989.
11. Cosenza, H., and H. Kohler. 1972. Specific suppression of the antibody response by antibodies to receptors. *Proc. Natl. Acad. Sci. U. S. A.* 69:2701.
12. Rowley, D. A., F. W. Fitch, E. P. Stuart, H. Kohler, and H. Cosenza. 1973. Specific suppression of immune responses. Antibody directed against either antigen or

receptors for antigen can suppress immunity specifically. *Science (Wash. D. C.)*
181:1133.

13. Lynch, R. G., R. J. Graff, S. Sirishinha, E. S. Simms, and H. N. Eisen. 1972.
Myeloma proteins as tumor-specific transplantation antigens. *Proc. Natl. Acad. Sci.
U. S. A.* **59:**1540.

14. Hannenstad, K., M. S. Kao, and H. M. Eisen. 1972. Cell-bound myeloma proteins on
the surface of myeloma cells. Potential targets for the immune system. *Proc. Natl.
Acad. Sci. U. S. A.* **69:**2295.

15. Potter, M. 1972. Immunoglobulin-producing tumors and myeloma proteins of mice.
Physiol. Rev. **52:**631.

16. Greenspan, J. S., G. A. Gutman, N. Talal, I. L. Weissman, and S. Sugai. 1974.
Thymus-antigen and immunoglobulin positive cells in lymph nodes, thymus and
malignant lymphomas of NZB/NZW mice. *Clin. Immunol. Immunopathol.* **3:**32.

17. Canty, T. G., and J. R. Wunderlich. 1970. Quantitative *in vitro* assay of cytotoxic
cellular immunity. *J. Natl. Cancer Inst.* **45:**761.

18. Hartzman, R. J., M. L. Bach, F. H. Bach, G. B. Thurman, and K. W. Sell. 1972.
Precipitation of radioactively labeled samples: a semi-automatic multiple-sample
harvester. *Cell. Immunol.* **4:**182.

19. Attias, M. R., R. A. Sylvester, and N. Talal. 1973. Filter radioassay for antibodies to
reovirus RNA in systemic lupus erythematosus. *Arthiritis. Rheum.* **16:**714.

20. Bozicevich, J., J. J. Bunim, J. Freund, and S. B. Ward. 1958. Technique of bentonite
flocculation test for rheumatiod arthritis. *Proc. Soc. Exp. Biol. Med.* **97:**180.

21. Shirai, T., and R. C. Mellors. 1971. Natural thymocytotoxic autoantibody and
reactive antigen in New Zealand black and other mice. *Proc. Natl. Acad. Sci. U. S. A.*
68:1412.

22. Seligman, M., and J. C. Brouet. 1973. Antibody activity of human myeloma globulins.
Semin. Hematol. **10:**163.

23. 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-γ-globulin
activity. *J. Exp. Med.* **137:**331.

24. Williams, R. C., Jr., J. R. DeBoard, O. J. Mellbye, R. P. Messner, and F. D.
Lindstrom. 1973. Studies of T and B lymphocytes in patients with connective tissue
diseases. *J. Clin. Invest.* **52:**283.

25. Preud'homme, J. L., and M. Seligman. 1972. Surface bound immunoglobulins as a
cell marker in human lymphoproliferative diseases. *Blood J. Hematol.* **49:**777.

26. Wernet, P., T. Feiz, and H. G. Kunkel. 1972. Idiotypic determinants of immunoglobu-
lin M detected on the surface of human lymphocytes by cytotoxicity assays. *J. Exp.
Med.* **136:**650.

27. Sirisinha, S., and H. N. Eisen. 1971. Autoimmune-like antibodies to the ligand-bind-
ing sites of myeloma proteins. *Proc. Natl. Acad. Sci. U. S. A.* **68:**3130.

28. Rollinghoff, M., and H. Wagner. 1973. *In vitro* induction of tumor specific immunity:
requirements for T lymphocytes and tumor growth inhibition *in vivo*. *Eur. J.
Immunol.* **3:**471.

29. Herzenberg, L. A., E. L. Chan, M. M. Ravitch, R. J. Riblet, and L. A. Herzenberg.
1973. Active suppression of immunoglobulin allotype synthesis. *J. Exp. Med.*
137:1311.

30. Jerne, N. K. 1974. Clonal selection in a lymphocyte network. *In* Cellular Selection
and Regulation in the Immune Response. G. M. Edelman, editor. Raven Press,
New York, 39.