A Monoclonal IgM With Antibodylike Specificity for Phospholipids in a Patient With Lymphoma


Both murine and human myeloma proteins have been shown to have antibodylike activity for serum lipoproteins. We studied a 58-yr-old woman (M.L.) with a lymphoma and a VDRL titer of 1:4096 and a nonreactive FTA-ABS. Serum electrophoresis revealed an M-component which was shown to be IgM-kappa by immunoelectrophoresis and 2-mercaptoethanol treatment of the serum reduced the VDRL titer to 1:64. Immunolectrophoresis (IEP) of the purified IgM against sonicates of the specific components of the VDRL antigen (cardiolipin, lecithin, cholesterol) produced a precipitin line with both cardiolipin and lecithin. All IgM in the serum could be removed by absorption with cardiolipin, and elution experiments demonstrated the specificity of this reaction. A quantitative precipitin test demonstrated that at the point of equivalence, 0.009 mg of antigen (cardiolipin) reacted with 0.4 mg of IgM (antibody). Additional studies showed that purified IgM (M.L.) gave a positive reaction with nine of 15 phospholipids tested. Similar studies on sera from 50 normal individuals, ten patient’s sera without M-components giving false positive VDRL reactions, and serum from one patient with a congenital absence of IgA and IgG and increased IgM showed no evidence of such a precipitin reaction with any phospholipid tested. Thus, it appeared that the IgM-kappa monoclonal protein behaved like an antibody directed against specific phospholipid antigenic components. The role of lipid antigens in the initiation of such neoplastic and immunologic aberrations should be further investigated and monoclonal proteins screened more extensively against such antigens.

The study of monoclonal immunoglobulins associated with neoplastic processes has contributed significantly to our understanding of the structure and function of immunoglobulins.\(^1\) It has been proposed that such proteins arise from the activation of a single clone of cells, which then proliferates and produces a homogeneous molecular species of immunoglobulin.\(^2\) Although it has been speculated that the activation of a particular clone of immunoblasts may be a response to a particular antigen, the identification of such antigens has

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been infrequent. Plasma cell tumors induced by foreign materials in mice attest to the capacity of chronic immunologic stimulation to produce uncontrolled clones of immunoblasts with concomitant production of myeloma proteins. Moreover, a number of these murine proteins have been shown to have antibody specificity for bacterial polysaccharides of polyNitrophenyls.

Though a wide spectrum of antibodylike activities among human monoclonal immunoglobulins has been reported, the frequency of this occurrence is unclear, and some survey reports have failed to find any such activities. Furthermore, the relevance of the antibodylike activities to the disease process has been questioned because of the unusual nature of some of the antigens studied. Serum lipoproteins have been among the activities reported from both murine and human myeloma proteins. However, this study presents the first report of a patient with a lymphoma and associated IgM M-component possessing antibodylike specificity for certain phospholipids. This activity against membrane components in this patient's clinical setting suggests that antibodylike activity against certain antigens may be of more than trivial importance and that such antigens may play a role in the evolution of the neoplastic process and the production of the monoclonal immunoglobulin.

CASE REPORT

The patient (M.L.), a white female, was found to have chronic lymphocytic leukemia and Coomb's positive hemolytic anemia in 1963 at the age of 50. Treatment with chlorambucil and prednisone controlled the disease until February 1971, when massive splenomegaly recurred. Anemia and leukopenia were noted, and the patient became refractory to chemotherapy. On March 25, 1971, a Rapid Plasma Reagin test (RPR), performed as a routine screening procedure for syphilis during the patient's hospitalization, was negative. On March 29, 1971, a splenectomy was performed, and a 2200-g spleen was removed, and a histologic diagnosis of lymphoblastic lymphosarcoma was made. The patient was discharged on the sixth postoperative day with a normal hemogram. Three weeks after splenectomy, the patient was found to have a low-grade fever and progressive dependent edema.

The patient's third and final hospitalization was on June 1, 1971 for evaluation of progressive diarrhea, 10-lb weight loss, fever, and poor oral intake. Physical examination showed a cachectic 58-yr-old white female with massive ascites, right pleural effusion, hepatomegaly, and marked pitting edema of the lower extremities. Serum studies showed evidence of severe malabsorption with a serum albumin of 1.7 g/100 ml, cholesterol of 76 mg/100 ml, serum carotene of 20 mg/100 ml, and prothrombin time of 26.4 sec/10.0 sec. Radiographic studies of the small bowel showed changes consistent with malabsorption. A routine RPR during this admission revealed a titer of greater than 1:256, and VDRL was 1:4096. Serological studies are summarized in Table I. A serum electrophoresis at this time revealed a large band of anomalous protein in the "slow-beta" region characterized further below.

Despite intensive support, the patient's condition, marked by persistent fever, diarrhea, progressive pleural effusion, and ascites, deteriorated, and she expired on June 17, 1971. An autopsy showed Hodgkin's disease of the mixed cellularity type involving cervical, mediastinal, and abdominal lymph nodes. Tumor had replaced at least 40%, 50%, of the liver (weight, 2280 g). Examination of the small bowel showed lymphatic obstruction by lymphoma cells, and focal accumulations of lymphoma cells were also seen in multiple organs. There was no histologic evidence of syphilis.

MATERIALS AND METHODS

Serum electrophoresis on cellulose acetate was performed at pH 8.6 in veronal buffer. Microimmunoelectrophoresis (IEP) was performed in 1.5%, noble agar at pH 8.6 in veronal buffer by the method of Scheidegger. Ouchterlony diffusion analysis was done in a standard manner.
Table 1. Serological Studies on Patient M.L.

<table>
<thead>
<tr>
<th>Test</th>
<th>Date</th>
<th>3-25-71</th>
<th>6-1-71</th>
<th>6-10-71</th>
<th>6-15-71</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPR</td>
<td>Nonreactive</td>
<td>&gt;1:256</td>
<td>&gt;1:1024</td>
<td>&gt;1:1024</td>
<td></td>
</tr>
<tr>
<td>VDRL</td>
<td>—</td>
<td>1:4096</td>
<td>1:4096</td>
<td>1:4096</td>
<td></td>
</tr>
<tr>
<td>FTA-ABS</td>
<td>—</td>
<td>Nonreactive</td>
<td>Nonreactive</td>
<td>Nonreactive</td>
<td></td>
</tr>
<tr>
<td>Direct Coombs</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Anticomplement</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Cold agglutinins</td>
<td>Negative</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Febrile agglutinins</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Nonreactive</td>
<td>Nonreactive</td>
</tr>
<tr>
<td>Ig A</td>
<td>(60–330 mg/100 ml)*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>29 mg/100 ml</td>
</tr>
<tr>
<td>Ig G</td>
<td>(570–1900 mg/100 ml)*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>155 mg/100 ml</td>
</tr>
<tr>
<td>Ig D</td>
<td>0–6 mg/100 ml)*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>160 mg/100 ml</td>
</tr>
<tr>
<td>Ig M</td>
<td>(45–145 mg/100 ml)*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2000 mg/100 ml</td>
</tr>
</tbody>
</table>

*Normal range.

Immunoglobulins were identified and quantitated using Hyland antisera and quantitative plates and by a single immunodiffusion technique using Oudin tubes. Purification of the pathologic IgM was accomplished by gel filtration on Sephadex G-200 of whole serum with phosphate-buffered saline as the eluent, followed by zone electrophoresis in starch of the void volume peak. This final IgM preparation was shown to be pure by IEP using antihuman serum. The effect of 2-mercaptoethanol on the anti-VDRL activity of the serum was studied.

Antibody specificity of the monoclonal protein for the antigen in the Venereal Disease Research Laboratory test for syphilis (VDRL) was studied by specific elution of monoclonal protein bound to the antigen as follows: Equal amounts of the patient’s serum and saline were added to tubes containing concentrated VDRL antigen obtained from Sylvana Laboratories (The Sylvana Company, Millburn, N.J. 07041). Following incubation at 4°C for 30 min, the tubes were centrifuged and the precipitates washed three times with 0.9%, saline, combined, then suspended 3 M NaCl (2 ml) and ether (10 ml) and agitated vigorously. After settling, the clear aqueous layer was removed, dialyzed overnight against 0.9% saline, and analyzed by VDRL titration, IEP, and Ouchterlony diffusion. For controls, the same procedure was followed with three other IgM-kappa monoclonal proteins and with an IgG and an IgA myeloma protein.

Immunoelectrophoresis was used to demonstrate the precipitin reaction between the patient’s serum and monoclonal protein and various phospholipids. The patient’s serum was placed in the well of a microimmunoelectrophoresis plate and electrophoresed. The various phospholipid suspensions were then allowed to diffuse from the trough in 1% agar. Adequate phospholipid antigen diffusion in this system was demonstrated by following the diffusion of 14C-labeled lecithin.

Beef heart and bacterial cardiolipin, bis-phosphatidic acid, phosphatidyl glycerol, lecithin (phosphatidyl choline), lysolecithin, phosphatidyl serine, pig liver phosphatidyl ethanolamine, L-3-phosphatidyl ethanolamine, phosphatidic acid (dioleoyl and dipalmitoyl), and sphingomyelin were obtained from Serdary Research Laboratories, London, Ontario, Canada. Suspensions of phospholipids were prepared by sonication of the phospholipid in water.

Absorption experiments were carried out using an aqueous suspension of cardiolipin and serial twofold dilutions in normal saline of the patient’s serum beginning with a dilution of 1:5. The precipitates formed were centrifuged to 10,000 rpm for 30 min, and the supernatants were removed. The supernatants were tested for the presence of antigen and antibody by means of double diffusion in agar gel against undiluted patient’s serum (for the detection of antigen) and cardiolipin suspension (for the detection of antibody).

Quantitative precipitin tests were carried out by the method of Kabat, using a 1:25 dilution...
in saline of the patient’s serum and a cardiolipin suspension prepared as described above, except that the cardiolipin was reconstituted to ten times its original volume with water. The suspension was then clarified by centrifugation for 18 hr at 10,000 rpm in the cold. The actual cardiolipin content of the clarified suspension was 0.092 mg/ml, as determined by the method of Chalvardjian and Rudnicki.17 Similar studies were performed using lecithin as the antigen.

RESULTS

Electrophoresis of serum M.L. on cellulose acetate strips on June 1, 1971 revealed a large anomalous “slow-beta” migrating protein component (Fig. 1). No prior serum electrophoresis was available for comparison. IEP of the serum demonstrated an IgM-kappa component (Fig. 2). VDRL reactions at that time were positive at 1:4096 dilution, whereas the Rapid Plasma Reagin test had been nonreactive 3 mo earlier. The Fluorescent Treponemal Antibody Absorption test (FTA-ABS) was consistently nonreactive. Incubation of the serum with 2-mercaptoethanol reduced the RPR titer from 1:1024 to 1:64. IEP of the patient’s serum against sonicates of the specific components of the VDRL antigen (cardiolipin, lecithin, and cholesterol) produced a precipitin line with both cardiolipin and lecithin, but not cholesterol (Fig. 2). The precipitin line followed the same pattern as that of the anomalous IgM-kappa on IEP.
When the immunoglobulin components of the patient’s serum were separated on a Sephadex G-200 column (Fig. 3), a large initial peak consisting of IgM and other macromolecular proteins was seen, while the area containing IgG was quite small and did not demonstrate the peak normally seen. VDRL titers of the fractions can be seen to follow the first peak exactly, and the only immunoglobulin detectable by Ouchterlony diffusion in this first peak was the IgM-kappa monoclonal protein. Though there was a small amount of spread of the IgM monoclonal protein into the trough area where IgG of both light chain types could be found, there was insignificant anticardiolipin activity in this area.

When either the patient’s whole serum or protein obtained from peak 1 of the G-200 column was incubated with VDRL antigen and subjected to elution with 3 M NaCl and ether, only IgM-kappa with the monoclonal bowed configuration on IEP could be eluted from the washed antigen-antibody precipitate. The eluate was free of all other proteins by IEP analysis against antihuman serum. This eluted material was found to contain the anti-VDRL activity upon retesting with standard methods. The same procedure, performed with three other IgM-kappa proteins, an IgA, and an IgG myeloma protein, resulted in the failure of any of these proteins to bind the VDRL antigen, as shown by the absence of detectable eluted immunoglobulin or anti-VDRL activity. Thus, the patient’s IgM-kappa appears to possess specific antibody-like activity against the VDRL antigen, presumably the cardiolipin and possibly the lecithin components, judging from the precipitin information. In addition, the absorption experiments showed that the removal of antibody to cardiolipin by absorption of serum M.L. with an aqueous cardiolipin suspension resulted in the disappearance of IgM from all serum dilutions tested. With an IgM concentration of 5.6 mg/ml, all detectable IgM could be removed by absorption with cardiolipin suspensions.

The quantitative precipitin test demonstrated that, at the point of equivalence, 0.009 mg of antigen (cardiolipin) reacted with 0.4 mg of IgM (M.L.). This would indicate that 20 molecules of antigen (1.1 × 10^3 mol wt) were reacting with each molecule of IgM (1 × 10^6 mol wt). Theoretically, one would expect ten molecules of antigen to react with one molecule of IgM (with ten potential receptor sites). However, since the phospholipid is present in small micelle
form, each antigenic site on a micelle could carry with it several molecules of phospholipid.

Precipitin tests using purified IgM (M.L.) and lecithin resulted in precipitation of an IgM-lecithin complex (Fig. 4). It can be seen that with greater amounts of lecithin there were associated smaller concentrations of IgM remaining in the supernatant of each tube tested. This strongly suggests that lecithin could also react with the monoclonal protein. The demonstration that all antibody activity could be removed with cardiolipin alone, however, suggests that the reaction with lecithin is on the basis of the reactivity of a single antibody species against structural components shared by the two phospholipids. Because of the possibility that the antibody activity of the pathological IgM could also be directed against an antigen similar in molecular structure to cardiolipin or lecithin, other phospholipids were used for IEP precipitin analysis (Fig. 2).

It was found that purified IgM (M.L.) gave a positive precipitin reaction to nine of 15 phospholipids tested (Table 2). Similar studies on sera from 50 normal individuals, ten patients having false positive VDRL reactions without anomalous protein components, and one patient (J.G.) with a congenital absence of IgG and IgA but elevated level of IgM showed no evidence of an IEP reaction with any phospholipid tested.

DISCUSSION

A number of investigations of both animal and human myeloma proteins have provided evidence to establish the fact that antibody activity can occur among monoclonal proteins. Furthermore, animal studies have demonstrated that a true antibody response to well-defined antigens can in fact be "monoclonal" in certain instances. To date, however, only a small proportion of monoclonal proteins from patients with multiple myeloma or macroglobulin-
Table 2. Reaction of Specific Phospholipids With M. L Serum

<table>
<thead>
<tr>
<th>Phospholipid Tested</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiolipin—beef heart</td>
<td>+</td>
</tr>
<tr>
<td>Cardiolipin—bacterial</td>
<td>+</td>
</tr>
<tr>
<td>Bisphosphatidic acid</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidic acid—dipalmitoyl</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidic acid—dioleoyl</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidyl glycerol</td>
<td>-</td>
</tr>
<tr>
<td>Phosphatidyl glycerol—dioleoyl</td>
<td>-</td>
</tr>
<tr>
<td>Lecithin</td>
<td>+</td>
</tr>
<tr>
<td>Lysolecithin</td>
<td>-</td>
</tr>
<tr>
<td>Phosphatidyl serine</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>+</td>
</tr>
<tr>
<td>L-3-phosphatidyl ethanolamine</td>
<td>-</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>+</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>-</td>
</tr>
</tbody>
</table>

emia have shown such activity, and the relationship of the antibody activity to the associated disease process remains unclear.\(^7,20\)

Though little information is available concerning activity against phospholipids, a large body of information has accumulated regarding the antibody activity possessed by the monoclonal proteins produced by BALB/c mice with plasma cell tumors. One can induce the formation of plasma cell tumors in mice of this strain by implanting mineral oils of various types or plastic materials intraperitoneally. The tumors can then be transplanted and will produce monoclonal immunoglobulins during many generations.\(^4\) Schubert et al.\(^21\) have demonstrated that about 10% of the anomalous immunoglobulins produced by such myeloma tumors will precipitate with nitrophenyl derivatives or nucleic acid bases. Eisen et al.\(^22\) screened 116 mouse myeloma proteins for activity and found seven with antibody activity against dinitrophenyl (DNP) and/or tri-nitrophenyl. Because of the high percentage of IgA mouse myelomas and the suspicion that they arise from plasma cells of the gastrointestinal system, Potter\(^7\) screened a large number of these proteins for precipitin activity against antigenic components of enteric flora. Among these proteins, several were found with precipitin activity against various lipopolysaccharide antigens of assorted bacterial cell walls and membranes, leading to the suggestion that such antigens were part of the stimulus for plasma cell reaction and subsequent tumor formation. Leon and Young\(^8\) studied six murine IgA myeloma proteins that precipitated the C polysaccharide of \textit{Pneumococcus} and found that all recognized the phosphoryl choline determinant of the antigen.

Attempts to uncover antibody activity among human monoclonal immunoglobulins by screening against various antigens have produced conflicting results. Although some studies have shown precipitating activity for various haptons and bacterial antigens,\(^1,20\) the incidence was about 1% in one study,\(^23\) and another group was unable to demonstrate activity by fluorescent quenching or double diffusion in agar after screening 275 myeloma sera against 20 antigens.\(^7\) None of the antigens used in these studies were phospholipids. Much of the conflict may depend upon different methodologies and the selection of
screening antigens. However, the study of antigens more obviously detectable in clinical situations may provide some clues to the stimulus for uncontrolled cell growth.

At least eight cases of monoclonal proteins (all IgG) with activity against streptolysin O antigens have been described. Several myeloma proteins have been described which possess antilipoprotein activity. Specific activity was demonstrated by extracting lipoprotein–immunoglobulin complexes and then removing the anomalous immunoglobulin in a manner similar to ours described above. Beaumont and Lemort have reported one case in which hyperlipoproteinemias apparently resulted from the activity of an IgA protein against heparin. An IgG myeloma protein with Donath-Landsteiner hemolysin activity and several IgG components, which were cryoglobulins and appeared to be antibodies against IgG, have been reported.

A substantial number of monoclonal immunoglobulins with antibody activity have been described among the Waldenstrom’s macroglobulins. The most frequent activity has been anti-IgG. This “rheumatoid factor activity” has been demonstrated in more than 20 cases and seems to represent antibody activity directed against the Fc fragment of IgG. Warner et al. have recently reported a Waldenstrom’s macroglobulin that reacted specifically with immunoglobulin (IgM or IgG) only when it was in combination with antigen, thus appearing to be a true anti-antibody. In chronic cold agglutinin disease, an antibody is produced which is monoclonal, almost always IgM-kappa, and which reacts with the I antigen of red blood cells in the cold, producing agglutination and hemolysis. There is rarely, however, any abnormality visible on serum electrophoresis or routine IEP, since the protein is present in low concentrations.

The agglutinating reactivity of a macroglobulin with stored nonviable red cells and with an isologous tissue extract suggests the possibility of autogenous membrane antigens acting as a stimulus for an anomalous (monoclonal) antibody response.

The particular membrane components involved in the reactivity of our patient’s protein were phospholipids, and the reactivity was initially manifest as a false positive VDRL. The VDRL reagent consists of cardiolipin, lecithin, and cholesterol in globular suspension. Cardiolipin, a mammalian mitochondrial membrane phospholipid, consists of two molecules of phosphatidic acid esterified to the two primary alcoholic groups of a glycerol molecule. Thus, it is a polyglycerol phospholipid and closely related to other phospholipid components of mammalian cell membranes, such as phosphatidyl ethanolamine, against which our patient’s protein also reacted.

Such reactions (positive VDRL in the absence of syphilis, demonstrable Treponema pallidum immobilization, or reactive FTA-ABS) have frequently been reported among patients with various collagen diseases, sometimes preceding other manifestations of the disease. However, the titer has usually been very low. In the majority of the cases studied, the antibody has resided in the IgM fraction, but anomalous immunoglobulin components have not been observed. The reported occurrence of biologic false positive reactions in lymphomas is very rare. In one instance, a patient with lymphosarcoma was reported to have a biological false positive reaction with a VDRL titer of 1:256,
but there was no evidence of any other protein abnormality, and the antibody was not further characterized.\textsuperscript{36} Among sera from patients with Waldenstrom’s macroglobulinemia, a few have been reported to have anti-Wasserman activity.\textsuperscript{35} In only two cases has such activity been shown to reside in an anomalous immunoglobulin. In one report, Gisler and Pillot\textsuperscript{37} described a patient with Waldenstrom’s macroglobulinemia whose anomalous protein formed a mixed cryoprecipitate with IgG. The IgM–IgG complex possessed anticardiolipin activity, but neither of the components alone could react with the antigen.

Killander et al.\textsuperscript{38} reported a monoclonal macroglobulin in a patient with a high-titer Wasserman reaction. This protein appeared to complex specifically with lecithin in a complement fixation assay but to have low reactivity toward other VDRL components. Pruzanski et al.\textsuperscript{39} described a patient with malabsorption who had a mononuclear infiltration of the bone marrow composed primarily of small lymphocytes. Silica gel chromatography showed both sphingomyelin and lecithin in the precipitated macroglobulin. Jejunal biopsies showed deposition of IgM of the lambda type intimately associated with phospholipid in the lamina propria.

Our patient represents the first in whom antibodylike activity against specific phospholipid antigens has been shown to reside in a monoclonal IgM. We have shown conclusively that the VDRL activity of the serum resides in the IgM-kappa monoclonal protein and that the activity is directed against specific phospholipid antigenic components. Though we could not demonstrate that the anomalous component in the serum appeared at the same time as the VDRL activity, since no prior SEP was available, the fact that all IgM could be absorbed from the serum with the VDRL antigen makes this most likely. This antibody has both flocculating and precipitating activity, and although we were unable to test for localization of activity in the Fab portion of the IgM molecule, the precipitin characteristics and specificity strongly suggest true antibody activity. Furthermore, it is of interest that the antibody cross-reacted with phospholipids found in mammalian mitochondrial and plasma membranes.

The occurrence of a monoclonal IgM with antibodylike activity for phospholipids in a patient with a history of an autoantibody (causing Coomb’s positive hemolytic anemia) and a lymphoproliferative disorder raises several interesting speculations. It is certainly true that in the normal state, and probably to a greater degree in lymphomas, there is a significant degree of cell destruction and release of membrane components. Lee\textsuperscript{40} has shown a consistent increase in the serum phosphatidyl choline to sphingolipid ratio in patients with cancer, and he has also demonstrated an increase in serum phosphatidyl ethanolamine and phosphatidyl serine levels in patients with reticulum cell sarcoma. Phospholipids are important structural constituents of cell membranes\textsuperscript{41} and particularly play an important role in maintaining the integrity of red cells.\textsuperscript{42} In addition, recent studies by Green\textsuperscript{43} have shown that red cell Rh antigenicity depends on erythrocyte lipids. It was shown that lecithin (phosphatidyl choline) and phosphatidyl ethanolamine were most active in restoring antigenicity to lipid-depleted red cell membranes. Cardiolipin and phosphatidyl serine were also active.
If such phospholipid membrane components were present in, or available to, a cell of lymphoid origin at a time when a malignant transformation was taking place, it is possible that specific antibody formation could be induced in that clone of cells, resulting in a monoclonal antibody. In our patient, such an event might have occurred during the transition to a more aggressive phase of the disease, perhaps as the result of a second mutation. Furthermore, persistent antigenic stimulation, such as that provided by increased cell breakdown, has been suggested as a mechanism for the induction of neoplastic processes. This has often been postulated for the mineral oil-induced myelomas in BALB/c mice, and there have been reports of the appearance of monoclonal proteins after a period of chronic immunologic stimulation in the Aleutian disease of mink. Osserman and Takatsuki have speculated that protracted reticuloendothelial stimulation, such as that occurring in chronic cholecystitis, may result in myelomatosis in man, and Schwartz has suggested that a chronic immune response, such as an autoimmune process, accompanied by impaired feedback control, may result in derepression of a viogene, followed by viral replication and neoplastic transformation. The recent demonstration of an IgG myeloma protein with antihorse γ2 macroglobulin activity in a patient having received horse antitetanus serum a number of years previously suggests the possibility of exogenous antigens acting in a similar manner. The increased incidence of neoplasms, especially lymphomas, in kidney graft recipients and immunologically deficient patients supports this hypothesis.

The antibodylike activity of this monoclonal IgM for various phospholipids suggests that these lipids may have functioned as persistent autogenous antigens with the eventual production of a malignant clone of immunoblasts. The previously suggested relationship between lipid antigens and disorders involving monoclonal immunoglobulin production is further emphasized by this study. The possibility that membrane antigens play a role in the initiation of such neoplastic and immunologic aberrations, as suggested by our findings, deserves further investigation, including more extensive screening of monoclonal proteins against such antigens.

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