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Research Article

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Spontaneous In Vitro Differentiation of Antigen-specific Lymphocytes from a Patient with Immunoglobulin M Gammopathy

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ABSTRACT Recently we have identified two monoclonal immunoglobulin M (IgM) proteins that bind Klebsiella polysaccharides. The lymphocytes of one of these patients (M.A.Y.) were available for study. A substantial proportion of the B lymphocytes isolated from this patient's peripheral blood also bound Klebsiella polysaccharides with a pattern of specificity identical to that of the monoclonal IgM, and reacted with an anti-idiotypic antiserum directed against this IgM. Stripping the surface immunoglobulin from these lymphocytes eliminated this reactivity. Although no plasma cells were detected in the freshly isolated peripheral blood lymphocytes of this patient, plasma cells binding Klebsiella polysaccharide appeared after 7 d of in vitro culture. This occurred regardless of whether the cultures were supplemented with autologous plasma, normal human plasma, or fetal calf serum. Pokeweed mitogen neither stimulated nor inhibited the in vitro differentiation of the monoclonal B lymphocytes into plasma cells. This differentiation was, however, abrogated by $F(ab')_2$ fragments of anti-human IgM and by anti-idiotypic antibodies, as well as by the Klebsiella polysaccharide with which the monoclonal IgM reacted.

INTRODUCTION

Studies of the peripheral blood lymphocytes (PBL) of patients with Waldenstrom's macroglobulinaemia (WM)[1] and the closely related disorder, chronic lymphocytic leukemia associated with the production of monoclonal immunoglobulin M (IgM) (CLL^{IgM}), have demonstrated the presence of members of the clone at different stages of maturation in vivo (1, 2). These cells at different stages of maturation can be identified morphologically; in addition, the patterns of expression of surface immunoglobulin (SIg) and of Ia antigens change with cellular maturation in a similar way to that observed in normal B lymphocytes (1, 2, 3). Although in CLL^{IgM} the majority of the members of the neoplastic clone are arrested at the B lymphocyte level, the rate of differentiation of such lymphocytes into plasma cells can be accelerated by certain signals that induce maturation of normal B lymphocytes into plasma cells, e.g., pokeweed mitogen (PWM) and allogeneic T cells (1). The similarities between normal B lymphocytes and those associated with monoclonal IgM production, the potentiality of using the IgM for preparing anti-idiotypic antibodies for in vitro studies, and the ready availability of monoclonal B lymphocytes make these disorders useful models for studying B lymphocyte differentiation.

It would be of particular interest to study the interaction of cells from such disorders with compounds specifically bound by their SIg receptors, i.e., putative antigens. Monoclonal lymphocytes from cold agglutinin disease that bind human erythrocytes have been described (4) and recently an example of CLL^{IgM} has been reported in which both the serum IgM and the B lymphocytes bound sheep erythrocytes (5). However, neither of these studies examined the effect of the sheep erythrocytes on lymphocyte differentiation, and it might be anticipated that these studies would be made difficult by the particulate nature of the ligand.

Recently, in the course of screening monoclonal IgM for reactivity with polysaccharides, we found two such proteins that bound various Klebsiella polysaccharides

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Abbreviations used in this paper: CLL^{IgM}, chronic lymphocytic leukemia associated with the production of a monoclonal IgM protein; $IgM^{M.A.Y.}$, monoclonal IgM of patient M.A.Y.; PBM, peripheral blood mononuclear cells; PBS-A, PBS containing 0.1% azide; PWM, pokeweed mitogen; SIg, surface immunoglobulin; WM, Waldenstrom's macroglobulinaemia.

(6). Cells from one of these patients were available for study. When assayed by routine markers, this patient's PBL had a normal distribution of T and B lymphocytes. Studies of the binding of Klebsiella polysaccharide K21 indicated that a high proportion of the B lymphocytes bound K21 through their SIg. Furthermore, the lymphocytes of the patient spontaneously differentiated in vitro into plasma cells with intracytoplasmic IgM capable of binding K21 polysaccharide. The addition of PWM was not required for this maturation process. The maturation was essentially abrogated by addition to the cultures of K21 polysaccharide. Anti-idiotypic or anti-IgM antibodies also prevented this spontaneous maturation.

METHODS

Case description. M.A.Y. is a 71-yr-old white male with a background of chronic peptic ulcer disease, chronic cholecystitis, cholelithiasis, and possible inactive pulmonary tuberculosis. He was hospitalized in August 1978 for an atypical pneumonia, and sputum cultures showed *Haemophilus influenzae* and *Streptococcus pneumoniae*. Serum electrophoresis demonstrated a somewhat paucidispersed monoclonal peak (~1.5 g/dl) shown to be an IgMκ previously studied (6) and it was designated IgM$^{M.A.Y.}$ The concentrations of the immunoglobulins were (in milligrams per deciliter): IgM, 1,512 (normal = 50–200); IgG, 771 (normal = 700–1,700); and IgA, 150 (normal = 100–350). The peripheral blood count was normal except for a moderate anemia that cleared after resolution of the pneumonia. Bone marrow aspiration showed an increase in lymphocytes (~20%) and the bone marrow biopsy was consistent with well-differentiated lymphocytic lymphoma. In the subsequent 36 mo, the patient has remained asymptomatic without specific therapy. There is no detectable lymphoadenopathy or hepatosplenomegaly, and the serum concentration of IgM is unchanged. At this time (January 1982) this case is classified as an asymptomatic plasma cell dyscrasia or so-called benign monoclonal gammopathy.

Preparation and culture of cells. Human peripheral blood mononuclear cells (PBM) were isolated from heparinized blood by means of discontinuous Ficoll-Hypaque gradients, and subsequently processed as described previously (2).

PWM stimulation was performed as previously described (3). Briefly, PBM isolated from blood of normal subjects or patient M.A.Y. were cultured for 7–8 d in medium RPMI 1640 supplemented with 20% fetal calf serum (Biofluids, Rockville, MD) containing 1% PWM (Gibco, Grand Island Biological Company, Grand Island, NY). Cultures in which PWM was omitted were set up in parallel. In certain experiments heat-inactivated serum from AB nontransfused males, autologous plasma, or fetal calf serum obtained from Gibco were used as serum sources.

Preparation of antibodies. Rhodamine- or fluorescein-conjugated rabbit anti-human immunoglobulin F(ab')$_2$ antibody fragments monospecific for γ, μ, α, δ, κ, and λ determinants and Ia antigens were prepared as previously described (3). Anti-idiotypic antiserum directed against IgM$^{M.A.Y.}$ was prepared following the procedure of Kunkel et al. (7). In brief, IgM$^{M.A.Y.}$ was isolated by Pevikon block electrophoresis and one rabbit was immunized with a course of four intramuscular and subcutaneous injections of 0.5–1 mg protein. 7 d after the last injection, the rabbit was bled and the antiserum was rendered idiotype-specific by solid-phase absorption with normal human serum, Cohn Fraction II, and two IgMκ and IgMλ monoclonal proteins. The anti-idiotypic specificity was confirmed by hemagglutination and hemagglutination inhibition.

Rabbit antisera specific for K21, K33, and K7 Klebsiella polysaccharides were kindly provided by Dr. M. Heidelberger (New York University College of Medicine, New York).

Membrane immunofluorescence. Lymphocyte-surface immunoglobulin was demonstrated by direct immunofluorescence. F(ab')$_2$ reagents or goat anti-immunoglobulins were used to avoid cytophilic uptake of reagents and the PBM incubated in media containing fetal calf serum to eliminate uptake of serum immunoglobulin by B cells and monocytes through the Fc receptor (8). Cells were routinely suspended and washed in ice-cold phosphate-buffered saline (PBS), pH 7.4, containing 0.1% sodium azide (PBS-A) and incubated with the antibodies at 0°C to prevent capping and minimize patching. In those experiments in which we desired the SIg to be endocytosed, we employed PBS at room temperature without azide and the cells were incubated with antibodies at 37°C for 1–2 h.

Expression of idiotypic determinants was also assayed by direct immunofluorescence using conjugated F(ab')$_2$ fragments prepared from the IgG fraction of the rabbit anti-idiotypic serum. The failure of the anti-idiotypic antibodies to react with more than 0.2% of the lymphocytes of 10 normal subjects provided evidence for its idiotypic specificity at the level of sensitivity of surface immunofluorescence.

Binding of Klebsiella polysaccharides to the surface of viable cells was assayed by a three-step indirect immunofluorescence procedure (K21 sandwich). 5×10^5 viable PBM were suspended in 50 μl of iced PBS-A, and 50 μl of the polysaccharide to be tested (at 25 μg/ml in PBS) was added. After 30 min at 0°C, the cells were washed three times in iced PBS-A and resuspended in 50 μl iced PBS-A. 50 μl of the anti-Klebsiella antiserum at a 1:50 dilution was added to the cells and the mixture was placed at 0°C for 30 min. The washings and resuspensions were repeated three times and 50 μl of a fluorescein-conjugated IgG fraction of goat anti-rabbit Ig (1 mg/ml; fluorescein to protein ratio of 2.5) was added. After 30 min at 0°C and three washes, the cell suspension was mounted on slides under cover slips that were sealed with nail polish and viewed immediately. The limited amount of rabbit anti-K21 antiserum available precluded preparation of the F(ab')$_2$ fragment of these antibodies. However, the use of a 1:50 dilution of the antiserum, which was freed of aggregates by ultracentrifugation before use, minimized binding of the rabbit immunoglobulins to the Fc receptors of PBM.

The specificity of the K21 "sandwich" was assessed by omitting the K21 polysaccharide or the rabbit anti-K21 serum, or employing rabbit anti-K7 serum as the second step. Another control was the use of a sandwich in which the first two reagents were K7, a polysaccharide nonreactive with IgM$^{M.A.Y.}$, by immunoprecipitation analysis at pH 7 and a rabbit antiserum directed against K7. Also, there was no staining with the K21 sandwich of the PBM from normal subjects or from patients with monoclonal IgM disease whose protein did not react with K21. Because these controls were all negative, it appears that Fc uptake of the rabbit anti-K21 antiserum was negligible. Double staining was performed with the K21 sandwich with fluorescein-conjugated goat anti-rabbit antibodies followed by staining with rhodamine-conjugated goat antisera directed against human immunoglobulin isotypes; these goat antisera were previously absorbed with rabbit immunoglobulins.

Cytoplasmic immunofluorescence. Intracytoplasmic immunoglobulins were studied on cells flattened in a cytocentrifuge (Shandon Southern Instruments, Inc., Sewickley, PA) and then fixed in cold 95% ethanol. The fixed cells were stained for intracytoplasmic immunoglobulin and for K21 binding activity using standard techniques (3). Direct immunofluorescence was employed for idiotypic, and SIg determinants and the sandwich technique for Klebsiella polysaccharide, binding.

Microscopy. Immunofluorescent slide preparations were examined by a Leitz Dialux microscope (E. Leitz, Inc., Rockleigh, NJ) equipped with alternating phase optics, incident fluorescent illumination, and filter systems appropriate for alternate selective visualization of fluorescein or rhodamine.

E-rosette formation. E-rosette-forming cells were enumerated according to Hoffman and Kunkel (9) by using *Vibrio cholerae* neuraminidase (Sigma Chemical Co., St. Louis, Mo.) treated sheep erythrocytes at 4°C.

RESULTS

Characterization of freshly isolated PBM of patient M.A.Y. When studied on several occasions, 50–70% of the PBM of patient M.A.Y. were T cells as determined by E-rosette formation, whereas between 5 and 7% were B cells as identified by SIg determination. Similar values for B cells were obtained with anti-IgM reagents. Thus, by conventional markers the PBL of this patient appeared normal. However, when studied with the K21 sandwich technique on five separate occasions (Table I), from 0.8 to 5.4% of the PBL were specifically stained. The number of K21-positive cells did not significantly change after overnight incubation of the cells under conditions that lead to loss of passively bound immunoglobulin and monocyte depletion.

When studied by double staining techniques, all K21-binding cells were SIgM-positive and about two-thirds expressed SIgD, indicating that these were B lymphocytes at different stages of differentiation that included $SIgM^+D^+$ and $SIgM^+D^-$ cells. However, the

TABLE I
SIg Expression and Klebsiella K21 Polysaccharide Binding by PBM of Patient M.A.Y.

Date	Cells expressing			Cells binding	
	SIg	SIgM	SIgD	K21	K7
	%			%	
1/80	4.8	—	—	0.8	<0.2
3/80	5.2	—	—	2.1	<0.2
7/80	6.7	—	—	2.2	<0.2
9/80	6.5	6.5	3.5	4.6	<0.2
10/81	5.0	4.8	3.1	5.4	<0.2
Normal (n = 10)	2–13	3–12	1–7	<0.2	<0.2
IgM gammopathy (n = 4)	4–18	6–19	1–12	<0.2	<0.2

SIg expression was assessed by immunofluorescence with directly conjugated $F(ab')_2$ fragments of rabbit IgG antibodies or directly conjugated IgG goat antibodies. Klebsiella polysaccharide binding was assessed by a three-step sandwich technique as described in Methods.

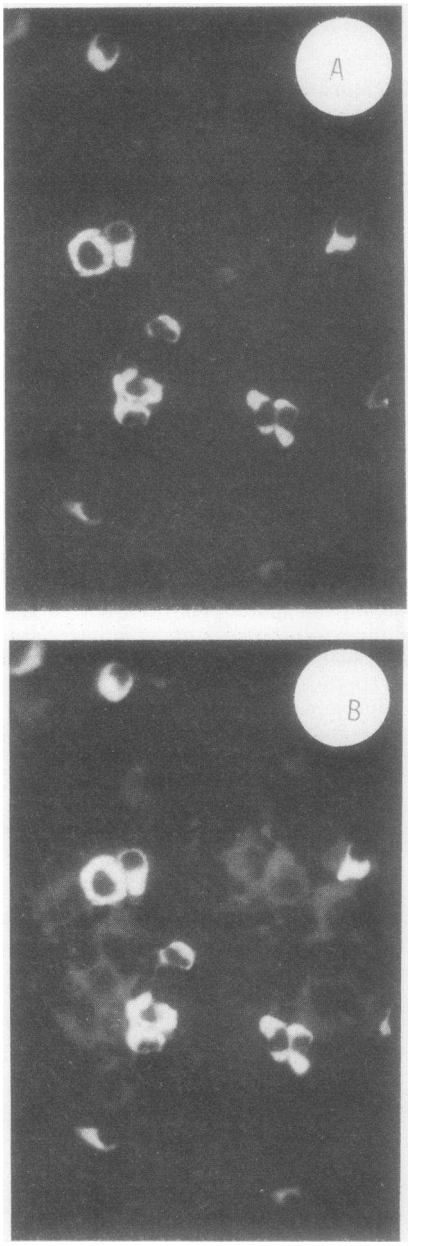

FIGURE 1 Spontaneously appearing plasma cells in a 7-d culture of M.A.Y. PBL without addition of mitogens. Intracytoplasmic double staining with rhodamine-conjugated goat anti-human immunoglobulin and K21 sandwich (with fluorescein-conjugated goat anti-rabbit Ig, see Methods). a. Illumination for rhodamine. All immunoglobulin-containing cells are shown. b. The same field with illumination for fluorescein. It appears that all immunoglobulin-containing cells are positive for K21 binding. The background shows the other cells in the culture outlined by faint autofluorescence.

presence of the clone in the peripheral blood was apparently limited to the B lymphocyte stage because no plasma cells could be demonstrated using anti-Ig reagents or the K21 sandwich on fixed cells to detect intracytoplasmic immunoglobulins.

When PBM from M.A.Y. were first reacted with goat anti-human $F(ab')_2$ and incubated at 37°C for 1 h, most of the SIg was capped and internalized; such cells failed to bind K21. A similar number of cells were detected with the anti-idiotypic antibodies directed against $IgM^{M.A.Y.}$ and with the K21 sandwich. Double staining experiments indicated that the idiotypic determinants were limited to B cells. Less than 0.2% of B lymphocytes from the four subjects with IgM-producing plasma cell dyscrasias or from more than 10 normal subjects reacted with the anti-idiotypic antibodies.

During the course of observation (January 1980–October 1981), the K21 binding cells in the peripheral blood of patient M.A.Y. have increased from 0.8 to 5.4%, corresponding to 16% and virtually all of the B lymphocytes, respectively.

Spontaneous maturation of PBM of patient M.A.Y. into plasma cells. Although no plasma cells could be demonstrated in the freshly isolated PBL of patient M.A.Y., after 7–8 d of culture in RPMI 1640 supplemented with 20% fetal calf serum, ~2–4% of the remaining cells were plasma cells as detected by intracellular immunofluorescence by using anti-immunoglobulin reagents and the K21 sandwich technique. Because cell recovery was 60–80%, this number of plasma cells corresponds to from 30 to 60% of the number of K21-binding small lymphocytes present at the initiation of culture and is too great to be explained by selective survival of plasma cells. Indeed, we did not detect any plasma cells in the samples examined before the initiation of the cultures. These cells had the appearance of mature plasma cells (Fig. 1) rather than of plasmablasts as often seen in PWM-stimulated cultures. In four experiments, at least 98% of the spontaneously arising plasma cells contained IgMκ (staining brightly for intracellular heavy and light chains), reacted with anti-$IgM^{M.A.Y.}$ idiotypic antibodies, and were K21-positive when studied with the K21 sandwich technique. They did not bind K7 or react with other components of the K21 sandwich when the polysaccharide was omitted (Table II). These results indicate that these were members of the clone producing $IgM^{M.A.Y.}$.

As seen in Table II, essentially identical results were obtained when the PBM were cultured in RPMI supplemented with 20% fetal calf serum from two different sources, with 20% autologous plasma and with 20% pooled AB sera. Adding $IgM^{M.A.Y.}$ to a final con-

TABLE II

Spontaneous Appearance of K21-binding Plasma Cells after In Vitro Culture of PBM of Patient M.A.Y.

Date	Plasma cells* in cultures supplemented with			
	20% fetal calf serum	20% pooled AB serum	20% autologous plasma	20% fetal calf serum and 2.5 mg/ml of $IgM^{M.A.Y.}$
	%			
4/80	4* (>99)‡	—	—	—
7/80	3.8 (>99)	2.2 (>99)	—	—
9/80	3.3 (>99)	5 (>99)	4 (>99)	3.3 (>99)
Normal (n = 4)	<0.2	<0.2	—	—
IgM gammopathy (n = 4)	<0.2	<0.2	—	—

* Percent plasma cells, i.e., percent cultured cells shown to contain intracellular immunoglobulin.

‡ Number in parentheses represents percent of plasma cells binding K21. These cells react both with anti-immunoglobulin and the K21 sandwich.

10^7 cells were cultured in 10 ml RPMI 1640 supplemented with glutamine, antibiotics, and indicated sera. After 7–8 d the cells were harvested (recovery was 60–80% of original number of cultured cells), cytospun, and fixed for intracellular staining. The preparations were then stained for intracellular immunoglobulin with rhodamine-conjugated goat anti-human $F(ab')_2$ and for Klebsiella binding with the three-step sandwich as in Methods and examined for both rhodamine and fluorescein fluorescence by using appropriate filters. As in Table I, omission of either of the first two steps led to a decrease in K21-positive cells to <0.2%. No staining was seen in the cultured cells of patient M.A.Y. or the other subjects with a K7 sandwich. 500 cells were counted.

centration of 2.5 mg/ml did not affect the maturation process. No plasma cells were seen in cultures assayed on day 4.

No spontaneous maturation of PBM to plasma cells was observed with four normal subjects and four patients with monoclonal IgM gammopathies, as assayed by intracellular immunofluorescence with anti-Ig reagents.

Effect of anti-Ig reagents and Klebsiella polysaccharides on the spontaneous differentiation of PBM of patient M.A.Y. Because polymeric ligands and anti-Ig reagents have been shown to affect differentiation of B cells in a variety of systems (5, 10–13), it was decided to test the effects of anti-IgM, anti-idiotype, and K21 on the PBL of patient M.A.Y. As shown in Table III, K21 polysaccharide dramatically decreased the degree of spontaneous maturation when employed at final concentrations of 2 and 0.2 μg/ml. At similar concentrations K7 had a much less marked effect.

$F(ab')_2$ fragments of IgG fractions prepared from anti-idiotypic antiserum and antiserum directed against IgM also decreased spontaneous maturation (Table IV). Because these were not affinity-purified, no conclusions can be drawn as to their relative potencies. Neither the Klebsiella polysaccharides nor the antibodies affected cell yield or viability.

Effect of PWM on the maturation of PBM of patient M.A.Y. Because certain leukemic cells have been shown to respond to PWM stimulation (in the presence of T cells) by an increased degree of differentiation into plasma cells (1, 5), the effect of PWM on the PBM of patient M.A.Y. was examined. As shown in Table V, there was only a slight increase in the number of plasma cells generated in the PWM-stimulated, as compared with unstimulated, cultures. In the unstimulated culture, at least 98% of the plasma cells bound K21, whereas in the PWM-stimulated cultures, 80% of the plasma cells bound K21. The 20% non-K21-binding cells included all the IgG and λ-containing plasma cells.

[^{3}H] Thymidine incorporation in the PWM-stimulated cultures was 7,000 cpm/10^5 cells in contrast to 2,200 in the cultures without mitogen and ~25,000 in PWM-stimulated cultures of normal subjects. Thus, the effect of PWM on the PBM cells of patient M.A.Y. is to induce a subnormal proliferative response and a minor degree of differentiation of cells other than those belonging to the clone producing $IgM^{M.A.Y.}$. There is apparently no effect on the cells of the anti-K21 clone, because the absolute number of K21-binding plasma cells was similar (~2.5×10^4) in PWM-treated and untreated cultures.

Induction of a B lymphoblastoid cell line from patient M.A.Y. B cell lines were established on three separate occasions from the PBL of M.A.Y. On each occasion, from 2 to 4% of the lymphoblastoid cells

TABLE IV

$F(ab')_2$ Fragments of Anti-IgM and Anti-idiotypic Antibodies Abrogate the Spontaneous Maturation of Lymphocytes Belonging to the K21-Binding Clone

$F(ab')_2$ concentration	Plasma cells* in cultures with $F(ab')_2$ fragments of		
	Normal rabbit IgG	Anti-idiotypic antibodies	Anti-IgM antibodies
mg/ml		%	
0.0	NT	3.8	3.8
0.05	NT	1.1	0.1
0.20	3.0	0.4	0.1
0.5	4.1	0.1	0.1

* Percent plasma cells, i.e., percent cultured cells shown to contain intracellular immunoglobulin.

NT, not tested.

Cells were cultured as in Table II except for indicated additions and plasma cells identified by intracellular staining with anti-$F(ab')_2$ antibodies. Equivalent cells recovery was obtained in all cultures.

TABLE III

Effect of Klebsiella Polysaccharides on Spontaneous Appearance of K21-Binding Plasma Cells

Date	Plasma cells* binding K21 in cultures containing								
	No polysaccharides	K21 (μg/ml)					K7 (μg/ml)			
		2	0.2	0.02	0.002	0.0002	2	0.2	0.02	0.0002
		%								
7/80	3.8* (98)‡	0.3 (100)	0.6 (100)	—	—	—	1.3 (100)	2.0	—	—
9/80	3.3 (99)	0.2 (50)	0.3 (86)	2.0 (100)	1.8 (92)	2.3 (98)	1.8 (98)	1.4	3.4 (88)	(100)

* Percent plasma cells, i.e., percent cultured cells shown to contain intracellular immunoglobulin.

‡ Number in parentheses represents percent of plasma cells binding K21. These cells react both with anti-immunoglobulin and the K21 sandwich.

Cells were cultured and assayed by direct immunofluorescence and sandwich techniques for intracellular staining as in Table II except for the indicated additions. At the time of harvesting, cell numbers and viability were not different in the cultures containing, and those without Klebsiella polysaccharides.

TABLE V

Effect of PWM on the Appearance of K21 and Non-K21-Binding Plasma Cells of Patient M.A.Y.

Pokeweed mitogen concentration	Percent plasma* cells	Plasma cells:‡ Binding K21	Containing IgM	Containing IgG
%§				
0	3.6	(98)	(99)	(0.5)
1	4.0	(75)	(80)	(6.0)

Cells were cultured and processed as described in Table II except that the PWM was added as indicated. Percent plasma cells containing indicated immunoglobulin or binding K21 was determined by appropriate double staining techniques.

* Percent plasma cells, i.e., percent cultured cells shown to contain intracellular immunoglobulin.

‡ Figures in parentheses indicate that among 100 plasma cells, identified as such by intracellular immunoglobulin, the stated number binds K21 or contains IgM or IgG.

§ % refers to lyophilized PWM reconstituted as recommended by Gibco Laboratories.

showed binding of K21 either by surface or intracytoplasmic immunofluorescence. Attempts to isolate these cells by cloning were unsuccessful; more than 50 clones were isolated, all K21-negative. Although the original lines have been carried in culture for over 1 yr, the K21-binding cells in them became progressively more rare until they eventually disappeared.

DISCUSSION

In the present studies, a major proportion of peripheral blood B lymphocytes isolated from the blood of a patient with a monoclonal IgM with antibody activity against Klebsiella K21 polysaccharide were shown to bind the polysaccharide as well as the cross-reacting polysaccharide K33 (data not shown). Both these polysaccharides give identical precipitin curves with $IgM^{M.A.Y.}$, although K21 contains 4,6 pyruvated-D-galactose and K33, 3,4 pyruvated-D-galactose (6, 14). No lymphocyte binding of Klebsiella polysaccharide K7, which is unreactive with $IgM^{M.A.Y.}$ as studied by precipitin analysis at pH 7, was observed. The parallels between the precipitin reaction of the K polysaccharides and their ability to bind to the lymphocytes, the lymphocyte expression of the idiotypic determinants of $IgM^{M.A.Y.}$, and the blocking of the binding of K21 to lymphocytes by anti-human $F(ab')_2$ antibodies indicate that the lymphocytes were binding K21 (and K33) through their SIg receptors. The binding was not modified by overnight incubation at 37°C, showing that it was not due to passive uptake of serum IgM. Lymphocytes binding K21 were present at two stages of differentiation, with two-thirds being $SIgM^+D^+$ and one-third being $SIgM^+D^-$, a situation similar to that found in some WM and CLL^{IgM} (1, 2).

No plasma cells could be demonstrated in the peripheral blood by using anti-Ig reagents including anti-idiotype antibodies or the K21 sandwich. However, K21 binding plasma cells appeared after 7–8 d of in vitro culture of PBM in the absence of a mitogen. Virtually all the spontaneously appearing plasma cells bound K21, reacted with the anti-idiotypic antisera, and contained IgMκ. The failure to detect K21-binding plasma cells containing IgG indicates that the IgM-IgG "switch" (15) did not take place and is consistent with the previously reported observation (6) that the anti-K21 activity is confined to the IgM of the patient's serum. The K21 plasma cells had a morphology typical of mature plasma cells (Fig. 1) rather than of plasmablasts, which are seen in mitogen-induced maturation.

This spontaneous in vitro maturation of lymphocytes into plasma cells (as assessed by intracellular immunofluorescent staining) is distinctly unusual. It was not observed in studies of four normal subjects or of four subjects with monoclonal IgM proteins and has not been described with WM or CLL^{IgM}. Although rare cells with intracytoplasmic immunoglobulins may be present in PBM isolated from patients with these lymphoproliferative disorders, they do not increase, but rather tend to disappear if the PBM cells are cultivated in vitro without an additional stimulus such as allogeneic T cells or PWM (1).

The absence of plasma cells in the freshly isolated PBM does not appear to result from the presence in the blood of suppressor cells or factors, because the spontaneous in vitro maturation also occurred when the PBM were cultivated in autologous plasma and did not require the removal of T cells or monocytes. This observation, together with the general finding that in WM plasma cells are more readily demonstrable in lymphoid tissue or marrow than in blood (2, 3), suggests that in patient M.A.Y. any plasma cell precursors maturing in the blood may rapidly home to these tissues. However, because no plasma cells appeared in vitro before 4 d of culture, the presence of labile and/or concentration-dependent suppressor factors cannot be totally excluded. At any rate, the in vitro maturation resembles the in vivo situation in which maturation takes place, as evidenced by the presence of a significant quantity of the monoclonal IgM in the patient's serum.

PWM, a potent T cell-dependent inducer of B cell maturation (16), has been shown to induce differentiation into plasma cells of the monoclonal B cells found in some but not all cases of CLL^{IgM} (1, 5). We have recently obtained similar results in two cases of mixed cryoglobulinemia (17), where the rheumatoid

factor was a monoclonal IgM expressing the cross-reacting idiotype. These patients' PBL contained a small number of B lymphocytes expressing SIg with the cross-reactive idiotype; more than one-half of the plasma cells induced by PWM stimulation contained the cross-reactive idiotype and were therefore members of the malignant clone. In patient M.A.Y., however, PWM had no effect on the K21-binding clone, neither stimulating nor inhibiting its maturation, although it did lead to the induction of a small number of plasma cells belonging to the residual normal clones. Similar effects—preferential stimulation of normal clones and failure of monoclonal B cells to mature in response to PWM, as assayed using the plaquing technique—have recently been reported in other cases of WM (18). The insensitivity of monoclonal populations to PWM has also been demonstrated in certain patients with CLL^{IgM} and in a patient with hypogammaglobulinaemia, associated with production of a monoclonal IgG in whom idiotype-positive B cells could be demonstrated in the peripheral blood (19). Thus, the capacity of B cells to respond to PWM with maturation to plasma cells appears to be a property of individual clones.

The maturation of the K21-binding clone found in M.A.Y. was blocked by addition of $F(ab')_2$ fragments of rabbit antibodies directed against either human IgM or idiotypic determinants of $IgM^{M.A.Y.}$. This suppression is similar to the effects of anti-IgM and anti-idiotypic antibodies, respectively, on the PWM-induced maturation of human tonsillar lymphocytes (13) and B cells from a patient with CLL^{IgM} (5). Anti-idiotypic antibodies have also been shown to block spontaneous IgM secretion in this case of CLL^{IgM} and spontaneous IgG secretion by the monoclonal B cells in the hypogammaglobulinemic patient referred to above (18), whereas in our studies, the anti-IgM antibodies appeared to be more effective than the anti-idiotypic antibodies. Studies with affinity-purified antibodies will be required to determine whether there are quantitative differences between the effects of these two types of anti-SIg antibodies.

Another ligand was studied that was unique for the cells of M.A.Y., namely, the K21 polysaccharide. The K21 polysaccharide also abrogated the spontaneous maturation. This is in agreement with studies of mouse cells, where both inactivation of B cell precursors and blockade of secretion of immunoglobulin by antibody-secreting cells have been reported to result from polymeric antigens (10–12) including hapten-coupled pneumococcal polysaccharide (12). Although similar studies with human cells have not been reported, it has been shown that tetanus toxoid, considered a soluble T cell-dependent antigen, can suppress or enhance the terminal stages of B cell differentiation depending on the stage of maturation of the B cell (20). K7, which is unreactive with $IgM^{M.A.Y.}$ by quantitative precipitin analysis at pH 7 and by surface staining of the monoclonal lymphocytes and intracellular staining of the spontaneously appearing plasma cells, also inhibited the maturation of PBM of patient M.A.Y. to some extent. This might reflect a weak cross reaction at the level of the membrane receptors, because some reactivity of K7 with $IgM^{M.A.Y.}$ has recently been detected when quantitative precipitin curves were performed at pH 4 (14).

In conclusion, patient M.A.Y.'s PBL contains a monoclonal population of antigen-binding lymphocytes belonging to the clone producing $IgM^{M.A.Y.}$. The monoclonal B cells are unaffected by PWM but are capable of spontaneously differentiating into plasma cells. In this regard they differ from B lymphocytes found in normal subjects as well as those found in monoclonal lymphoproliferative disorders in which spontaneous differentiation of lymphocytes has not been demonstrated, even in those cases in which small numbers of plasma cells are found in the blood.

This case is of interest because it indicates that the screening of monoclonal proteins for antibody activity may yield cases from which antigen-binding B lymphocytes may be isolated for further studies. This allows direct identification of members of the clone in the peripheral blood and studies of the effects of antigen on their maturation. The study of the maturation potential of the monoclonal B cells in vitro, with or without the addition of antigens or mitogens, will contribute to our understanding of normal B cell subpopulations and, conversely, to the classification of plasma cell dyscrasias.

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