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Idiotypic Studies on Myeloma B Cells*

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Abstract—*In the blood of myelomatous patients, B lymphocytes-bearing receptors with idiotypic specificities similar to those of the secreted myeloma protein were found both in untreated and treated patients. This monoclonal pool represents 5–10% of peripheral blood lymphocytes. Resynthesis experiments have been performed to prove that these idiotypic receptors were actually synthesized by the cells bearing them. The partial resynthesis observed could suggest that some lymphocytes bearing* I^+ *receptors belong to a population of immature B lymphocytes unable to resynthesize their receptors after capping and endocytosis. When premyelomatous* I^+ *B cells were incubated with anti-idiotypic antibodies covalently coupled to adriamycin, the resynthesis of receptors recognized by these antibodies, was blocked.*

INTRODUCTION

PLASMA cell myeloma, Waldenström macroglobulinemia and chronic lymphatic leukemia are the result of a neoplastic transformation of bone marrow derived lymphocytes [1–2], occuring at different stages of maturation. The clinical and biological characteristics of human myeloma led to the conclusion that this disease is due to a malignant transformation of a single clone of the most differentiated B cells, the immunoglobulin producing cell [3–5]. However, kinetic studies *in vitro* and *in vivo* have shown that:

(1) the myeloma population can be divided in a proliferating and a non-proliferating compartment [6];

(2) the proliferating compartment does not possess the characteristics of stem cells [6];

(3) the passage from the proliferating compartment to the non-dividing pool is accompanied by a decrease in actinomycin binding capacity, which can be considered as an index of genetic activity [7].

All these data suggest that the non-dividing cells do not re-enter into the cell cycle, in contrast with acute leukemias [8, 9]. These studies and the fact that plasma cells derive from B lymphocytes bearing surface immunoglobulins [10–12] have led to the hypothesis of a "premyelomatous" compartment made up of B lymphocytes whose surface immunoglobulins have the same variable region as the secreted myeloma proteins [13–16]. Such an hypothesis can be tested by using anti-idiotypic sera which recognize antigenic determinants of the variable regions of immunoglobulins. Using such antisera, we have shown the existence of a B cell myeloma compartment, whose surface immunoglobulins are bearing idiotypic specificities, similar or identical to those of the secreted myeloma protein. We have demonstrated that these surface immunoglobulins are indeed synthesized by the cells bearing them. Treatment of these B cells with anti-idiotypic antibodies, covalently linked to adriamycin, prevented the re-expression of face immunoglobulins.

The present chemotherapy of myeloma is discussed in the light of these findings.

MATERIALS AND METHODS

Patients

Six patients with well established diagnosis of multiple myeloma, 5 IgGK and 1 IgGs were studied. Diagnosis was based on bone

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marrow infiltration by plasma cells, presence of serum or urinary M component, reduction in the serum level of normal Ig and osteolytic lesions.

Four patients (men from 50 to 80 yr DeB, Ma, Ro, Mas) were studied after the onset of chemotherapy only (Alkeran + prednisone essentially). These four patients were considered in remission or partial remission on the basis of the following criteria: less than 2% of myeloma cells in the bone marrow (at the time of diagnosis, the values ranged between 15 and 45%), reduction of more than 50% of monoclonal serum protein, increase in polyclonal circulating IgG, regression of osteolytic lesions. Patients Wa and DeS were studied before and after the onset of chemotherapy.

Controls

The specificity of anti-idiotypic sera was checked using blood cells from 20 healthy donors (from 23 to 86 yr old) and occasionally on cells from patients with chronic lymphatic leukemia, benign monoclonal gammopathy and myeloma.

Preparation of anti-idiotypic antisera

Monoclonal IgGs were purified by elution on DEAE Whatman cellulose columns, with phosphate buffers gradients 0.015–0.3 M pH 8.

Purity and homogeneity of these IgGs were tested by isoelectric focusing on polyacrylamide gels containing ampholines ranging from pH 5 to 9.5.

Rabbits were immunized according to the following scheme: three intramuscular injections with 3 mg of purified myeloma protein emulsified in complete Freund's adjuvant at 3–4 days intervals, then intravenous injections of the purified protein (2 mg) precipitated with 10% potassium alum (ALK $(SO_4)_2 \cdot 12\,H_2O$) twice a week. The appearance of an anti-idiotypic activity was followed by immunodiffusions in agar containing 3% polyethylene glycol (PEG).

Rabbit antisera were made specific for the immunizing protein by repeated passages on a column of Sepharose 4 B coupled to pooled normal and monoclonal human immunoglobulins.

Specificity was tested by immunodiffusion in agar containing 3% PEG and membrane immunofluorescence on blood cells from the homologous patients and controls.

Membrane immunofluorescence staining

Heparinized blood was mixed with plasmagel (2v: 1v) and red blood cells allowed to sediment for 30 min. The supernatant was then removed, white blood cells washed three times in PBS and adjusted to a cellular concentration of 2.10^7 cells/ml. Lymphocytes bearing surface Ig were detected by direct staining with sheep anti-Hu IgA + IgM + IgG coupled to Rhodamine isothiocyanate (RITC, Cappel).

Cells were incubated for 30 min at 4°C with the fluorescent antiserum and then washed twice with cold PBS-NaN3 10^{-2} M. Idiotype positive lymphocytes were identified by indirect staining: cells were first incubated 30 min at 4°C or 20°C with anti-idiotype (Fab') 2 pepsic fragments (to avoid binding to the Fc receptors of B cells), washed with PBS NaN3 10^{-2} M and incubated another 30 min at 4°C or 20°C with a sheep antirabbit IgG + IgA + IgM antiserum coupled to RITC (Cappel). The fluorescent staining of the cells was examined with a Leitz Orthoplan microscope equipped with an Osram Hg 100 W mercury vapor lamp and an Opak Fluor Vertical illuminator. Controls omitting the first step were always made. Percentages of fluorescent cells were estimated on a number of viable cells ranging from 800 to 2000 lymphocytes. These were identified by staining the cells in suspension with Acridine Orange 10^{-5} M for 1 min. All the leukocytes are stained in orange (cytoplasm) and green (nucleus) except the small lymphocytes which had only a green nucleus.

Capping endocytosis and resynthesis of receptors in culture [17]

10^7 cells/ml M 199 Hepes were incubated with 50 µl anti-Hu Ig antiserum for 1 hr at 37°C. After one washing in M 199, cells were resuspended in M 199 Hepes, supplemented with 10% FCS, antibiotics and allowed to stand at 37°C for 2 hr, before testing the removal of the receptors by immunofluorescence staining as described above. Resynthesis of Ig receptors was tested, after a culture of 24 hr at 37°C, in the same way.

Coupling of antibodies to adriamycin [18]

Periodate oxidation of the drug (40 mg/ml) was performed by mixing adriamycin with a slight molar excess of 0.1 M $NaIO_4$ and incubating at room temperature for 1 hr in the dark. Excess periodate is consumed with 0.05 M glycerol. Oxidized drug is mixed with antibodies (20–25 mg/ml) in potassium carbonate buffer pH 9.5, and incubated another hour at room temperature. $NaBH_4$ was then

added at a final concentration of 0.3 mg/ml and the reaction proceeded for 2 hr at 37°C. Free drug is eliminated by filtration on a Biogel P 100 column.

EXPERIMENTAL RESULTS

A. *Specificity of the anti-idiotypic antisera*

The specificity of the anti-idiotypic antisera was tested before and after passages on immunoadsorbant, by immunodiffusion in agar containing PEG 3% against homologous and heterologous myeloma proteins and a pool of human normal Ig (see Materials and Methods).

Figure 1 shows the pattern obtained for one anti-idiotypic antiserum. After repeated adsorptions, the specific antisera reacted only with the immunizing protein.

The idiotypic specificity was also tested at the cellular level by membrane immunofluorescence staining of the lymphocytes, from the homologous patient and from different controls.

Table 1 shows that, for 20 control healthy donors, an average of 0.4% of the blood lymphocytes (cross) reacted with the different anti-idiotype antisera. These results are similar to those described by Wernet *et al.* [19].

B. *Immunofluorescence studies*

(1) Table 1 shows the percentages of peripheral blood lymphocytes bearing Ig receptors (Ig^+) and those characterized by idiotypic specificities similar to those of the monoclonal pathological protein (I^+). The proportion of monoclonal and polyclonal lymphocytes depends on the individual and represents from 50 to 100% of the lymphocytes, depending on the stage of the disease (expanding phase, remission, relapse).

Are the Ig receptors synthesized by the I^+ cells? The biosynthetic capacities of the lymphocytes were tested by the resynthesis method in culture. These experiments are based on the fact that receptors, cross-linked by an appropriate multivalent ligand, migrate to one pole of the cell and form a cap which is then endocytosed. In the case of normal mature B lymphocytes, endocytosis is followed by the reappearance of surface immunoglobulins. This resynthesis can be blocked by inhibition of protein synthesis. Table 2 shows that after stripping of receptors by endocytosis with an anti-Ig antiserum, 70 to 100% of the I^+ cells resynthesized surface immunoglobulins characterized by the idiotypic determinants of the circulating protein, after 24 hr of culture.

Resynthesis of polyclonal Ig receptors is of the same range. These results are similar to those described by Mellstedt [20, 21].

Table 1. *Percentages of lymphocytes bearing Ig receptors*

		Peripheral blood		Bone marrow	
		Ig^+	I^+	Ig^+	I^+
Wa	before treatment	21.0%	18.0%		
	1 yr after treatment	20.2%	7.4%		
Roc	first trial after treatment	17.5%	11.5%		
Ma	first trial after treatment	6.7%	6.6%	5.8%	6.4%
	13 months later	7.2%	6.4%		
	3 months later	8.0%	5.2%		
	15 months later	6.0%	5.1%		
DeS	first trial after treatment	7.8%			
	17 months later	7.3%	7.3%		
DeB	first trial after treatment	7.0%	4.7%		
Mas	first trial after treatment	7.0%	5.7%	4.1%	
	2 months later	9.0%	4.4%		
Controls: 20 healthy individuals		17.2%	0.4%	mean for the different anti-I	

However, in his experiments, removal of surface immunoglobulins was done by trypsinization.

(2) *Specific immunotherapy*

This part of our results shows the presence, in the myeloma patients, of a pool of circulating B lymphocytes, bearing Ig receptors characterized by idiotypic determinants, similar or crossreactive with those of the monoclonal secreted protein. Hoping to block selectively this idiotype positive lymphocytic compartment, we have studied the effect of anti-

Table 2. Test of the biosynthetic capacities of the lymphocytes bearing Ig receptors (by the method of capping-endocytosis and resynthesis in culture)

		Before capping		After capping-endocytosis		Resynthesis after 24 hr of culture	
		Ig^+	I^+	Ig^+	I^+	Ig^+	I^+
Wa	1 yr after treatment	20.2%	7.4%	5.1%	1.4%	11.8%	6.0%
Ma	first trial after treatment	6.7%	6.6%	—	3.6%	6.2%	3.6%
	13 months later	7.2%	6.4%	0.5%	3.5%	5.5%	—
	3 months later	8.0%	5.2%	2.7%	2.0%	8.7%	4.3%
DeS	first trial after treatment	7.3%	7.3%	2.0%	3.3%	—	4.4%
Mas	first trial after treatment	7.0%	5.7%	1.8%	1.3%	6.6%	5.4%
	2 months later	9.0%	4.4%	3.1%	0.0%	6.7%	4.0%

Ig receptors are removed by incubation with anti-Hu Ig

idiotypic antibodies on neoplastic lymphocytes *in vitro*. These antibodies had been previously coupled to a cytotoxic drug, adriamycin [18]. The efficiency and specificity of the anti-idiotypic antibodies coupled to adriamycin were evaluated by testing the resynthesis of receptors (I^+) of peripheral blood lymphocytes of a myeloma patient (Ma), after capping by homologous anti-I antibodies and endocytosis of the cross-linked receptors. Table 3 shows the results obtained for one typical experiment and gives the percentages of cells bearing polyclonal and monoclonal receptors after capping with anti-idiotypic antibodies coupled to adriamycin (AD) and not coupled (T), and the subsequent resynthesis of the Ig^+ and idiotype$^+$ receptors obtained in both cases.

Table 3 shows that only a part of the Ig^+ receptors is removed by capping with the anti-idiotype. The percentage obtained after capping shows that the coupling procedure does not seem to modify the binding capacity of the antibodies [18]. Only in the case of capping with anti-idiotype-adriamycin, an important inhibition of the resynthesis of I^+ receptors is observed after 24 hr of culture.

No inhibition is observed after capping with anti-I mixed, but not coupled, to adriamycin in the same concentration as above (not shown here).

DISCUSSION

The presence in the blood of myelomatous patients of a B lymphocytic population, bearing receptors with idiotypic specificities similar to those of the secreted myeloma protein, has recently been reported by several authors [20].

We have confirmed and extended these findings in treated myeloma patients using fluorescent pepsic fragments of anti-idiotypic antibodies, in order to avoid cytophilic binding of immunoglobulins to the Fc receptors of B cells. In treated patients, this monoclonal pool represents 5–10% of the peripheral blood lymphocytes. The proportion of this I^+ population depends mainly on the stage of the disease (expanding phase, remission, relapse). A certain restoration of the polyclonality of the Ig receptors can be observed during remission (patients Wa, Ma, DeB and Mas) [22].

Table 3. Physiological action of an anti-I coupled to adriamycin on peripheral blood lymphocytes of patient Ma (one typical experiment)

	Ig^+	I^+		
Before capping and endocytosis	9.7%	5.2%		
	T		AD	
After capping and endocytosis with an anti-I coupled to AD (AD) and not coupled (T)	Ig^+	I^+	Ig^+	I^+
	6.3%	1.3%	5.9%	1.9%
Resynthesis after 24 hr of culture	9.0%	4.5%	6.2%	0.6%

(The percentages refer to 100% of viable cells)

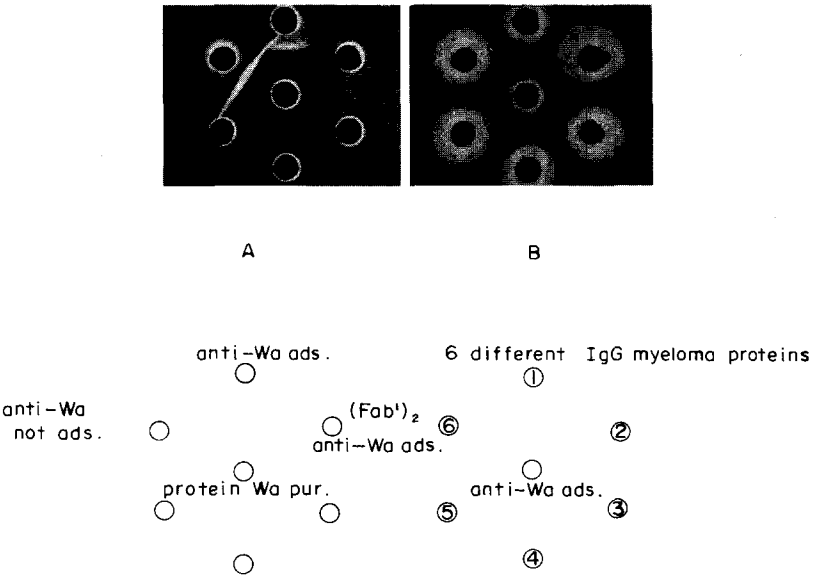

Fig. 1. Immunodiffusions in agar containing 3% PEG showing: A. the reactivity of an anti-idiotypic antiserum (anti-Wa) before and after 4 adsorptions, with the homologous protein. B. the absence of reactivity with different heterologous IgG myeloma proteins.

Resynthesis experiments have been performed to prove that these idiotypic receptors were not simply adsorbed on the cell membrane, but were actually synthesized by the cells bearing them. After capping and endocytosis of immunoglobulin receptors promoted by anti-Ig antisera which recognize only B cells, the majority of these lymphocytes (70–100%) were able to resynthesize their receptors after 24 hr of culture. Endocytosis of idiotypes is only partial. This could suggest the occurrence of T lymphocytes bearing the same idiotypic specificities. Such cells have indeed been found by Preud'Homme *et al.* [23].

The incomplete resynthesis could be due to overestimation of the number of I^+ lymphocytes before stripping of the receptors, due to passive adsorption of the myeloma protein on the lymphocyte's membrane [24]. More interestingly, this partial resynthesis could suggest that some lymphocytes bearing idiotypic specificities of the myeloma protein belong to a population of immature B lymphocytes. Indeed, Bruyns *et al.* [25] have shown that foetal and neonatal lymphocytes do not re-express their receptors after capping and endocytosis induced by an anti-Ig serum. This phenomenon also holds true for mouse bone marrow B lymphocytes which are believed to be precursors of the mature B lymphocytes of the spleen and lymph nodes [26]. The possible occurrence of such immature B lymphocytes deserves further experiments, since it could suggest that the neoplastic transformation does not occur at the level of the fully mature plasma cells, but of the early precursors of secreting cells. In myeloma patients, there could be some idiotypic recruitment of newly born lymphocytes. Such a possibility of idiotypic recruitment fits very well with the new network concepts of the immune system [27, 28].

In fact, the immunodeficiency observed in myeloma patients could be the result of reduced polyclonality of the lymphocyte's receptors, whether the neoplastic clone is becoming dominant, or the neoplastic cells "recruit" normal ones.

Salmon's data [29, 30] on the kinetics of multiple myeloma show that chemotherapy (mostly alkylating agents) can reduce from 10 to 100 times the number of the myeloma secreting cells (from 10^{12} myeloma cells at diagnosis, to 10^{11} to 10^{10} after treatment) and allows clinical improvement. On the other hand, we have shown that during remission, the monoclonal lymphocytic pool represents at least from 5 to 10% of the peripheral blood lymphocytic population. Considering that there are 10^{12} lymphocytes in the whole human body, 5–10% of this population represent 5×10^{10}–10^{11} lymphocytes at least, without considering a possible preferential localization in some tissues (spleen, bone marrow, lymph nodes...). This means that, at any time, the size of the monoclonal B lymphocytic pool is of the same order of magnitude as the myeloma secreting cell pool.

The reported observation of a patient (Ma) who stayed in remission for two years, with a high percentage of monoclonal B lymphocytes despite the absence of chemotherapy and the data from literature showing that there are no differences in survival between continuously treated and untreated myeloma patients in remission [31–36], lends support to the view that differentiation of monoclonal B lymphocytes into secreting cells is not a common event, at least during remission. On the other hand, these observations justified the evaluation of other treatment modalities during remission.

Bearing this in mind, we have studied the effect of specific anti-idiotypic antibodies coupled to a cytotoxic drug (adriamycin) on pre-myelomatous B cells *in vitro*.

The method of capping, endocytosis of the Ig receptors and their resynthesis in culture, is very useful to test the actual biosynthetic capacities of cells and could perhaps allow the demonstration of an eventual blockade of the genetic activity.* Sela *et al.* [18, 38] have in fact shown a marked inhibition of Uridine ^3H incorporation into RNA, in cultures of tumor cells treated by specific antibodies coupled to adriamycin or daunomycin. The results reported here are very suggestive because they show a very selective action of the anti-idiotypic antibodies coupled to adriamycin on the idiotypic population, leaving the polyclonal Ig population untouched. Other experiments are needed to study the fate of the cells which are unable to resynthesize their receptors after treatment with anti-idiotypic sera coupled to adriamycin.

Unpublished experiments from this laboratory have shown that it is possible to depress significantly a primary immune response by using specific antibodies coupled to adriamycin. We can therefore presume that such a type of treatment might be very effective at the level of virgin B lymphocytes.

*The drug used can inhibit the template activity of DNA by intercalating between the base pairs [37].

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REFERENCES

1. A. Aisenberg and K. J. Bloch, Immunoglobulins in the surface of neoplastic lymphocytes. *New Engl. J. Med.* **287,** 282 (1972).
2. J. L. Preud'Homme and M. Seligmann, Surface-bound immunoglobulins as a cell marker in human lymphoproliferative diseases. *Blood* **40,** 772 (1972).
3. J. G. Waldenström, Benigne und maligne monoklonale hypergammaglobulinemie (Gammapathie). *Schweiz. med. Wschr.* **100,** 290 (1970).
4. E. F. Ossermann, Multiple myeloma and related plasma cell dyscrasias. In *Immunological Diseases.* (Edited by M. Santer) Little and Brown, Boston (1971).
5. H. A. Azar, Plasma cell myelomatosis and other monoclonal gammopathies. *Pathol. Ann.* **7,** 1 (1972).
6. A. Pileri and R. P. Tarocco, *In vivo* kinetic studies in human myeloma. *Haematologica* **59,** 10 (1974).
7. J. Brachet and N. Hulin, Binding of tritiated actinomycin and cell differentiation. *Nature (Lond.)* **222,** 481 (1969).
8. V. Gabutti, A. Pileri, R. P. Tarocco, F. Gavosto and E. H. Cooper, Proliferative potential of out-of-cycle leukaemia cells. *Nature (Lond.)* **224,** 375 (1969).
9. F. Gavosto and A. Pileri, Cell cycle of cancer cells in man. In *The Cell Cycle and Cancer.* (Edited by R. Baserga) p. 99. Dekker, New York (1971).
10. W. E. Paul, Functional specificity of antigen-binding receptors of lymphocytes. *Transpl. Rev.* **5,** 130 (1970).
11. E. E. Sercarz, A. R. Williamson and C. F. Fox, *The immune system, Genes, Receptors, Signals.* Academic Press, New York. (1974).
12. W. H. Kreth and L. A. Herzenberg, Fluorescence-activated cell sorting of human T and B lymphocytes. I. Direct evidence that lymphocytes with a high density of membrane-bound immunoglobulins are precursors of plasmocytes. *Cell Immunol.***12,** 396 (1974).
13. M. Seligmann, J. L. Preud'Homme and J. C. Brouet, B and T cell markers in human proliferative blood diseases and primary immunodeficiencies, with specific references to membrane-bound immunoglobulins. *Transpl. Rev.* **16,** 85 (1973).
14. F. D. Lindström, W. R. Hardy, B. J. Eberle and R. C. Williams, Multiple myeloma and benign monoclonal gammopathy: differentiation by immunofluorescence of lymphocytes. *Ann. int. Med.* **78,** 837 (1973).
15. H. Mellstedt, S. Hammarström and G. Holm, Monoclonal lymphocyte population in human plasma cell myeloma. *Clin. exp. Immunol.* **17,** 371 (1974).
16. N. I. Abdou and N. L. Abdou, The monoclonal nature of lymphocytes in multiple myeloma. Effects of therapy. *Ann int. Med.* **83,** 42 (1975).
17. F. Loor, L. Forni and B. Pernis, The dynamic state of the lymphocyte membrane: Factors affecting the distribution and turnover of surface Ig. *Europ. J. Immunol.* **2,** 203 (1972).
18. E. Hurwitz, R. Levy, R. Maron, M. Wilchek, R. Arnon and M. Sela, The covalent binding of daunomycin and adriamycin to antibodies, with retention of both drug and antibody activities. *Cancer Res.* **35,** 1175 (1975).
19. P. Wernet, T. Feizi and H. G. Kunkel, Idiotypic determinants of immunoglobulin M detected on the surface of human lymphocytes by cytotoxicity assays. *J. exp. Med.* **136,** 650 (1972).
20. G. Holm, H. Mellstedt, D. Pettersson and P. Biberfeld, Idiotypic immunoglobulin structures on blood lymphocytes in human plasma cell myeloma. *Immunological Rev.* **34,** 139 (1977).
21. H. Mellstedt, S. Hammarström and G. Holm, Monoclonal lymphocyte population in human cell myeloma. *Clin. exp. Immunol.* **17,** 371 (1974).
22. H. Mellstedt, D. Pettersson and G. Holm, Monoclonal B lymphocytes in peripheral blood of patients with plasma cell myeloma. *Scand. J. Haematol.* **16,** 112 (1976).

23. J. L. PREUD'HOMME, M. KLEIN, S. LABAUME and M. SELIGMANN, Idiotype-bearing and antigen-binding receptors produced by blood T lymphocytes in a case of human myeloma. *Europ. J. Immunol.* **7,** 840 (1977).
24. P. I. LOBO, F. B. WESTERVELT and D. A. HORWITZ, Identification of two populations of immunoglobulin-bearing lymphocytes in man. *J. Immunol.* **114,** 116 (1975).
25. C. BRUYNS, G. URBAIN-VANSANTEN, C. PLANARD, C. DE VOS-CLOETENS and J. URBAIN, Ontogeny of mouse B lymphocytes and inactivation by antigen of early B lymphocytes. *Proc. nat. Acad. Sci. (Wash.)* **73,** 2462 (1976).
26. M. C. RAFF, J. J. T. OWEN, M. D. COOPER, A. R. LAWTON, M. MEGSON and W. E. GATHINGS, Differences in susceptibility of mature and immature mouse B lymphocytes to anti-immunoglobulin induced suppression *in vitro:* possible implications for B cell tolerance to self. *J. exp. Med.* **142,** 1052 (1975).
27. J. URBAIN, Idiotypes, expression of antibody diversity and network concepts. *Ann. Immunol.* (Inst. Pasteur), **127,** C, 357 (1976).
28. N. K. JERNE, *The Immune System. A Web of V Domains.* Harvey Lectures Ser. Academic Press, New York (1976).
29. P. W. SULLIVAN and S. E. SALMON, Kinetics of growth and regression in IgG multiple myeloma. *J. clin. Invest.* **41,** 1697 (1972).
30. S. E. SALMON and B. G. M. DURIE, Application of kinetics to chemotherapy for multiple myeloma. In *Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells.* Williams and Wilkins, Baltimore (to be published).
31. R. ALEXANIAN, S. BALCERZAK, A. HAUT, J. HEWITT and E. GEHAN, (for the South West Oncology Group), Remission maintenance therapy for multiple myeloma. *Arch. int. Med.* **135,** 147 (1975).
32. D. E. BERGSAGEL, Plasma cell myeloma: prognostic factors and criteria of response to therapy. In *Cancer Therapy: Prognostic Factors and Criteria of Response.* (Edited by M. J. Staquet) p. 73. Raven Press, New York (1975).
33. R. A. KYLE, Therapy and prognosis of multiple myeloma. In *Topics in Hematology.* Proceedings of the 16th International Congress of Hematology, Kyoto (Edited by S. Beno, F. Takakai and S. Irino) *Excerpta Medica,* p. 81. Amsterdam (1977).
34. R. ALEXANIAN, S. SALMON, J. BONNET, E. GEHAN, A. HAUT and J. WEICK, Chemotherapy for multiple myeloma. In *Recent Advances in Cancer Treatment.* (Edited by H. J. Tagnon and M. J. Staquet) p. 19. Raven Press, New York (1977).
35. D. E. BERGSAGEL, The treatment of plasma cell myeloma. *Brit. J. Haemat.* **33,** 443 (1976).
36. D. E. BERGSAGEL and W. PRUZANSKI, Treatment of plasma cell myeloma with cytotoxic agents. *Arch. int. Med.* **135,** 172 (1975).
37. W. J. PIGRAM, W. FULLER and L. D. HAMILTON, Stereochemistry of intercalation: interaction of daunomycin with DNA. *Nature New Biol.* **235,** 19 (1972).
38. R. LEVY, E. HURWITZ, R. MARON, R. ARNON and M. SELA, The specific cytotoxic effects of daunomycin conjugated to antitumor antibodies. *Cancer Res.* **35,** 1182 (1975).