Immunology 1985 **55** 45

Multiple myeloma with monoclonal IgG and IgD of lambda type exhibiting, under treatment, a shift from mainly IgG to mainly IgD

TERESA GALLART,* J. BLADÉ,† J. MARTÍNEZ-QUESADA,* J. SIERRA,† C. ROZMAN† & J. VIVES*
**Immunology Laboratory, and †Postgraduate School of Haematology Farreras-Valenti, Hospital Clinic i Provincial, University of Barcelona, Barcelona, Spain*

Accepted for publication 16 November 1984

Summary. A patient with multiple myeloma (MM), who initially presented with a predominant IgGλ and a minor IgDλ paraprotein pattern, is described. After chemotherapy, levels of the IgDλ protein increased and the IgGλ levels decreased. The following results were obtained when serum IgD was predominant. In the bone marrow, there were three plasma cell populations: a major one containing only δ chains, a minor one containing only γ chains, and another minor one containing both δ and γ chains. All these plasma cell populations contained λ chains. Stimulation of circulating mononuclear cells with pokeweed mitogen (PWM) achieved differentiation of circulating B lymphocytes into plasma cells: 30% with only cytoplasmic $\delta\lambda$ chains and 10% with only cytoplasmic $\gamma\lambda$ chains. These IgG-containing plasma cells showed cytoplasmic reactivity with rabbit antiserum raised against monoclonal IgD which was shown to contain specificities recognizing both δ chains and idiotypic determinants present in both serum IgDλ and IgGλ. Circulating B lymphocytes were 'monoclonal': almost all expressed surface $\delta\lambda$ chains, and a small proportion of them expressed both $\delta\gamma$ and λ chains. High levels of IgD were detected in the supernatants of all cultures, but high concentrations of IgG were only detected in those from PWM-stimulated cultures with very low levels of IgM and IgA. These findings suggest that plasma cells producing either IgD or IgG were derived from a common B-cell clone. Double paraproteinaemia exhibiting a shift in immunoglobulin production from IgG to IgD has not been previously described.

Correspondence: Dr Teresa Gallart, Servicio de Inmunologia, Hospital Clinic i Provincial, Villarroel 170, 08036 Barcelona, Spain.

INTRODUCTION

Double paraproteinaemia (DP) or double monoclonal gammopathy (DMG) is a rare event, since it only occurs in 1–1·5% of all monoclonal gammopathy, the more frequent isotype associations being IgG-IgA and IgM-IgG, often with the same type of light chain (Bouvet *et al.*, 1975; Kyle, Robinson & Katzmann, 1981).

There have been only a few reports where immunofluorescence studies and analysis of idiotypic determinants and/or N-terminal sequences have been performed, but the majority of these results suggest that plasma cells producing each of the isotypes had arisen from a common B-cell clone, and, in such cases, the monoclonal immunoglobulins shared the same light chain type (Yagi & Pressman, 1974; Todel, Franklin & Rudders, 1974; Hopper, Haren & Kmiecik, 1979; Fair, Schaffer & Krueger, 1976). This is probably the most

common cellular basis of DP (Bouvet *et al.*, 1975; van Camp *et al.*, 1978). Such studies have provided experimental data to support a mechanism of intraclonal sequential isotype switching. They have also given rise to the 'two genes, one polypeptide chain' model for the genetic control of immunoglobulin synthesis (Fudenberg *et al.*, 1971; Fair & Krueger, 1978).

Very few reports of multiple myeloma (MM) with double monoclonal IgD and IgG exist and, to our knowledge, the primary situation was always a patient producing IgD with later development of monoclonal IgG (Fahey *et al.*, 1968; Gore, Riches & Kohn, 1979; Oxelius, 1971), and analysis of idiotypic determinants and/or immunofluorescence studies was not carried out. Such studies could be of interest in providing information with regard to the well-known paradox of IgD biology: the rare frequency of normal IgD-secreting plasma cells and, hence, the very low concentration of IgD in normal sera, in spite of the fact that it is expressed as surface IgD (sIgD) on the majority of B lymphocytes, together with surface IgM (sIgM).

In this report, we describe a patient with multiple myeloma who initially exhibited a predominant monoclonal IgGλ with a minor IgDλ. Under chemotherapy, the latter increased, reaching a high concentration, whereas the IgGλ decreased until undetectable. The results of several investigations suggest that the populations of plasma cells producing the IgD of IgG were derived from a common B-cell clone.

Case report

MM was diagnosed in a 57-year-old man in February 1981. Bone marrow aspirate disclosed 90% of atypical plasma cells. Serum electrophoresis and immunoelectrophoresis revealed two M-components, a major one in the slow gamma region (IgGλ) and a minor one in the rapid gamma region (IgDλ). Free λ-chain excretion, assessed by electrophoresis and immunoelectrophoresis of concentrated urine, was minimal at that time (Figs 1 & 2). A skeletal X-ray survey showed generalized osteoporosis and lytic lesions in the skull.

Treatment with intermittent courses of vincristine, cyclophosphamide, melphalan and prednisone (VCMP) at 4-week intervals was started in March 1981. During this treatment, no improvement was attained, and both λ light-chains proteinuria and the serum IgDλ component increased, whereas the serum

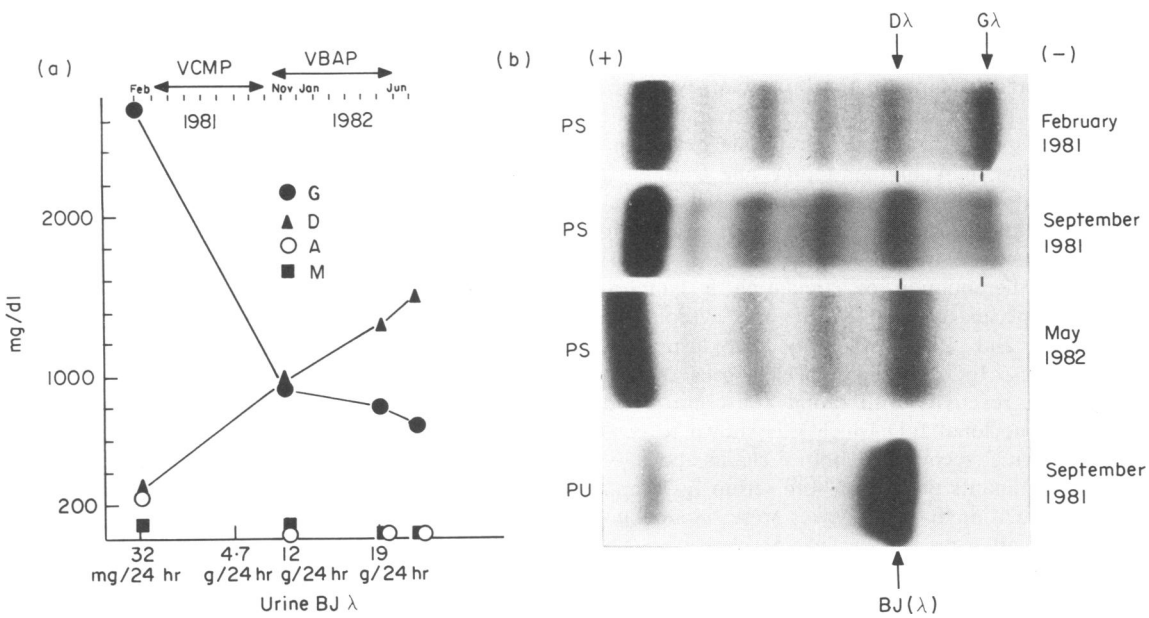

Figure 1. (a) Levels of serum IgD (D), IgG (G), IgA (A), IgM (M), and urine λ chains excreted during the evolution of the disease. The periods of chemotherapy with VCMP (vincristine, cyclophosphamide, melphalan and prednisone) and with VBAP (vincristine, BCNU, doxorubicin and prednisone) are indicated. (b) Electrophoresis of patient's serum (PS) at various times of the disease, and electrophoresis of patient's urine (PU).

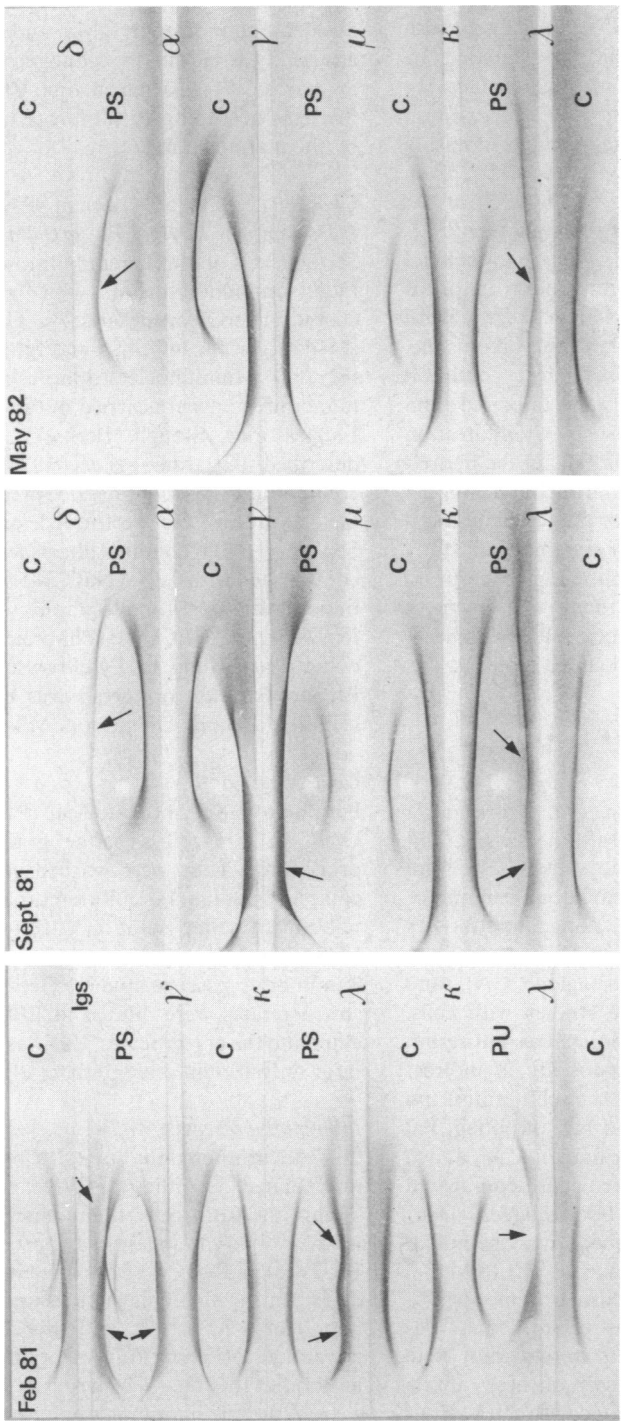

Figure 2. Immunoelectrophoresis of the patient's serum (PS) and urine (PU) at various times of the disease (same dates of electrophoresis as in Fig. 1): February 1981, when IgG was predominant; September 1981, when IgD was predominant but IgG was still clearly detectable; May 1982, when IgD was even more increased and monoclonal IgG was not perceptible by immunoelectrophoresis (C, normal serum; δ γ μ α κ λ, anti-δ, anti-γ, anti-α, anti-μ, anti-κ, anti-λ antisera; Igs, anti-IgG-IgA-IgM antiserum containing antibodies for heavy and light chains).

IgGλ component decreased (Figs 1 & 2). After seven courses of chemotherapy with VCMP, treatment with vincristine, BCNU, doxorubicin, and prednisone (VBAP) at 30-week intervals was instituted in October 1981. After nine such courses, the patient's condition deteriorated progressively, severe anaemia requiring packed red cells transfusions appeared, and λ chain proteinuria continued increasing to 19·1 g/24 hr. At that time (May 1982), the serum IgDλ component was predominant (1300 mg/100 dl), while monoclonal IgGλ was undetectable by immunoelectrophoresis (Figs 1 & 2). The patient was then treated with intermittent courses of melphalan and prednisone. After two cycles, the patient's clinical condition worsened, his renal function became impaired, and pancytopenia due to bone plasma cell infiltration developed, with 5–10% of plasma cells in the peripheral blood. The patient died of bronchopneumonia after a surgical procedure for a traumatic subdural haematoma in August 1982. A postmortem study showed right lower lobe bronchopneumonia due to fungi (*Candida* sp.), myeloma kidney, and extensive infiltration by plasma cells of bone marrow, spleen, retroperitoneal lymph nodes and choroid plexus.

MATERIAL AND METHODS

Antisera

Goat antisera specific for human μ, δ, γ, α, κ and λ chains, and for human IgG-IgA-IgM (heavy and light chains), purchased from Kallestad, Austin, TX, were used for immunoelectrophoresis and double immunodiffusion studies. F(ab')$_2$ fragments of goat antibodies FITC or rhodamine-conjugated, specific to human μ, δ, γ, α, κ and λ chains (Tago, Burlingame, CA), were utilized for immunofluorescence studies with cells. Goat anti-rabbit IgG FITC-conjugated antiserum (Tago) was used as a second antibody in indirect immunofluorescence studies with rabbit antiserum produced against the monoclonal IgD of patient Pal (IgD Pal). Rabbit IgG specific for human μ, δ, γ and α, chains non-conjugated and peroxidase-conjugated (POC), purchased from Dako (Copenhagen, Denmark), were utilized in a solid phase, enzyme-linked immunosorbent double-sandwich assay (ELISA).

A rabbit antiserum was obtained against monoclonal IgD (Pa). Immunization was performed as described by Kubagawa *et al.* (1979) in two adult New Zealand white males. High titre sera of these rabbits were pooled and the IgG precipitated with $(NH_4)_2SO_4$. The IgG was sequentially absorbed with normal human sera (NHS), polyclonal IgG (Kabi, Stockholm, Sweden), monoclonal IgMκ and monoclonal Ig$a\lambda$, covalently attached to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the procedures recommended by the manufacturer.

Characterization and isolation of monoclonal IgG and IgD of patient Pa(IgG Pa, IgD Pa)

Serum levels of immunoglobulins were determined by radial immunodiffusion with immunoplates purchased from Behringwerke A. G., Marburg, FRG (M-Partigen for IgG, IgA and IgM, and LC-Partigen for IgD). Immunoelectrophoresis and double immunodiffusion were carried out in 1% agarose (Miles Laboratories, Slough, Berks, U.K.) as previously described (Guardia *et al.*, 1976). Serum samples containing 0·1% sodium azide were stored at −80°. Gel filtration was performed on a 2·5 × 100 cm Sephacryl S-300 column (Pharmacia Fine Chemicals) equilibrated with 0·15 M NaCl, 0·5 M Tris-HCl, pH 8·4, buffer. Affinity chromatography was done on a protein A-Sepharose CL-4B (Pharmacia Fine Chemicals) column according to Ey, Prowse & Jenkin (1978). Preparative electrophoresis was done on blocks of cellulose acetate (Chemetron, Milan, Italy).

Cell separation

Peripheral blood mononuclear (PBM) cells were isolated on a Ficoll-Hypaque gradient by standard procedures. They were washed and resuspended in complete medium for culture purposes or in phosphate-buffered saline solution (PBS) containing 5% of FCS (Gibco, Europe) plus 0·02% of sodium azide for immunofluorescence studies. Heparinized bone marrow aspirates were diluted in PBS and, in order to eliminate the erythrocytes, this was centrifuged at 100 *g* for only 10 min on a gradient of Ficoll-Hypaque.

Immunofluorescence studies

Surface immunoglobulins (sIg) on PBM cells were investigated according to Winchester & Fu (1976). Within the PBM cells, the monocytes were identified by their ability to ingest latex particles (Winchester & Fu, 1976) and they were excluded in counting lymphocytes bearing sIg. This method implies an incubation period of 1 hr at 37°, and this step has the added advantage of permitting cytophilic or autoreactive antibodies to elute (Winchester & Fu, 1976).

Cytoplasmic immunoglobulins (cIg) on PBM cells, bone marrow cells, and PBM cells cultured for 7 days

with or without mitogen (PWM or PHA) were investigated (Janossy & Greaves, 1975). FITC or rhodamine-conjugated antisera were sequentially used for double-labelling procedures (Gathings, Lawton & Cooper, 1977). The slides were examined using a Leitz microscope equipped with a filter system permitting individual identification of the fluorescence due to FITS or rhodamine.

Immunoglobulin synthesis and plasma cell generation by PBM cells stimulated with pokeweed mitogen (PWM)
PBM cells were resuspended in complete medium: RPMI 1640 (Gibco) supplemented with 1% glutamine (Gibco), 0·5% HEPES (Flow, Irvine, Ayrshire, U.K.), penicillin-streptomicin (Flow) and 10% of a selected bath of FCS (Gibco). They were added to 96 round-bottomed microculture plates (Nunc, Roskilde, Denmark), either alone or with several doses of PWM (Flow) and PHA (Flow). Each combination was prepared in quadruplicate and each well contained $2{\cdot}5 \times 10^5$ cells in a final volume of 0·2 ml. They were cultured at 37° in a humidified atmosphere of 5% CO_2 in air, and after 7 days the supernatants of each quadruplicate were harvested, mixed and stored at −70° for the measurement of IgM, IgG, IgA and IgD by an ELISA. In order to evaluate the generation of plasma cells, the cells of each quadruplicate were collected and processed for cIg examination as indicated above.

ELISA
The coating, incubation and washing conditions, as well as the enzymatic activity determination, have already been described (Nieto *et al.*, 1984). In order to measure IgM, IgG, IgA and IgD, polystyrene microplates (Dynatech, Zurich, Switzerland) were sensitized with 100 μl/well of rabbit IgG (50 μg/ml) (Dako) specific to each one of human heavy chains μ, δ, γ and α. Samples or standard solutions of known concentrations of human IgM, IgG, IgA and IgD (Behringwerke), appropriately diluted, were added (100 μl) to each well. POC-conjugated rabbit antibodies (100 μl), appropriately diluted, namely 1/1000 for the anti-γ, and 1/400 for the anti-μ, anti-δ, and anti-α, were added to each well coated with anti-γ, anti-μ, anti-δ and anti-α chains, respectively. All tests were done in duplicate. Standard curves were constructed by plotting the optical density values against the known concentrations of each Ig. The following ranges of values were used: 3·6–50 ng/ml for the IgG, and 3·6–25 ng/ml for the IgM, IgA and IgD.

In order to evaluate whether or not the rabbit antiserum raised against the IgD (which could not be absorbed with other monoclonal IgD proteins) contained specifities to idiotypic determinants present in both IgD and IgG, an ELISA was performed following the same steps and conditions as those indicated in the ELISA for Ig measurement but, in this case, the polystyrene microplates were coated with the IgG fraction of the rabbit antiserum (50 μg/ml). Monoclonal IgG and IgD of patient Pa, as well as 'residual' polyclonal IgG of patient Pa and monoclonal IgG (of κ and λ type) isolated from other patients with MM, and standard solutions of known concentrations of human IgG, IgA, IgM and IgD (Behringwerke) were added to the wells. POC-conjugated antibodies specific for μ, δ, γ and α chains were then used to ascertain which assayed Ig was able to react with the rabbit IgG adsorbed to the microplates.

RESULTS

Immunochemical studies

Due to its rapid disappearance from the serum during treatment, an anti-idiotypic antiserum against the monoclonal IgG of patient Pa could not be obtained since serum samples were not available to isolate it in sufficient yield. A small serum sample corresponding to September 1981, when monoclonal IgG was still present (Figs 1 & 2), although in low concentration, allowed us to isolate it by preparative electrophoresis; it was kept at −80° and later used to test its reactivity with rabbit antiserum raised against the monoclonal IgD Pa by an ELISA.

On May 1982, the monoclonal IgG was not detectable by immunoelectrophoresis (Fig. 2). In order to try to concentrate it, serum samples obtained in May 1982 were also passed through a protein A-Sepharose column and, as expected, the IgD was not retained by the protein A, whereas this was the case for the majority of the IgG. Immunoelectrophoresis of this IgG showed its polyclonal character, since it reacted with both anti-κ and anti-λ chain antisera with heterogeneous precipitin arcs (Fig. 3a). This fraction was kept at −80° and later used to test its reactivity with the rabbit antiserum raised against the IgD (Pa). However, it was also observed that minute amounts of IgG were not retained by the protein A, but were eluted together with the IgD; immunoelectrophoresis revealed that it reacted with anti-λ antiserum but not with anti-κ antiserum (Fig. 3a). Since the Fc fragment

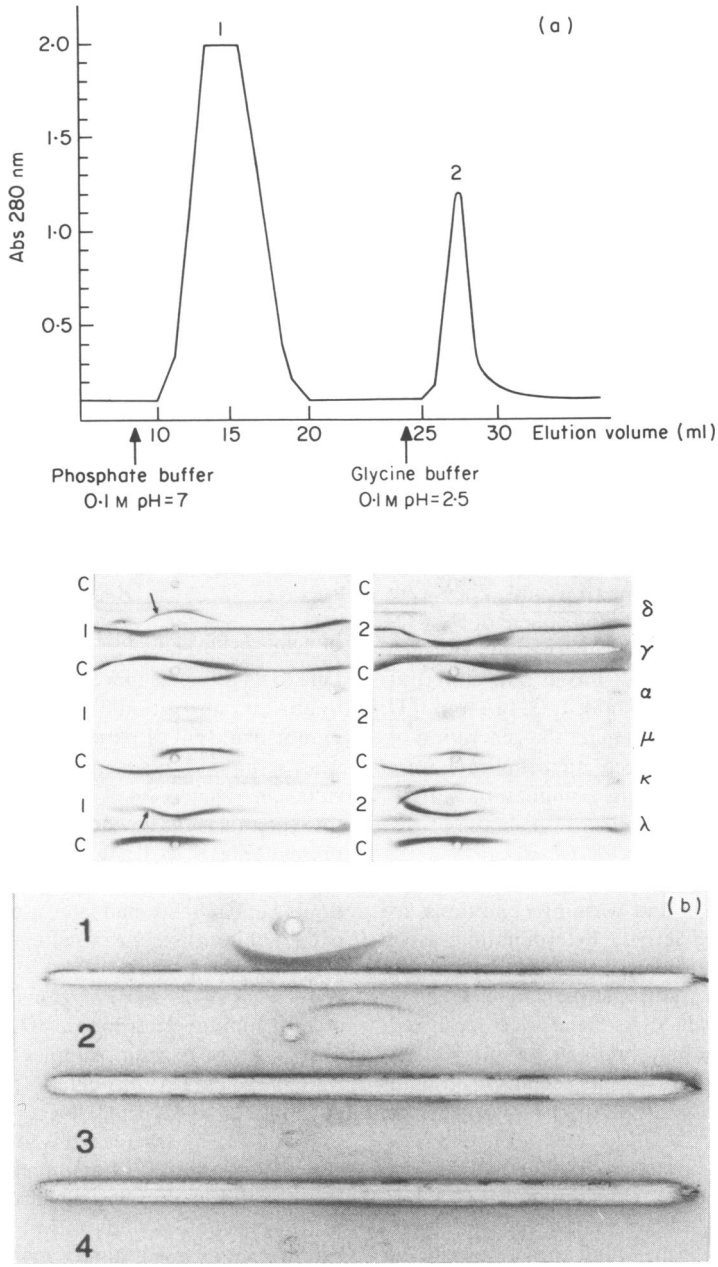

Figure 3. (a) Elution profile of patient's serum passed through a protein A-Sepharose CL-4B column. Immunoelectrophoresis of the Fractions 1 and 2 (below) showed that IgD was not retained by protein A but eluted in Fraction 1. Fraction 2 contained only IgG exhibiting a polyclonal character since it reacted with both anti-κ and anti-λ antisera with heterogeneous precipitin arcs (C, normal human serum; $\delta\ \gamma\ \alpha\ \mu\ \kappa\ \lambda$, anti-$\delta$, anti-$\gamma$, anti-$\mu$, anti-$\alpha$, anti-$\kappa$, anti-$\lambda$ antisera). (b) Immunoelectrophoresis showing that the absorbed rabbit antiserum raised against monoclonal IgD Pa (anti-δIdPa) only reacted with IgD of patient's serum (Well 1) as well with polyclonal igD contained in a standard solution (Well 2). No precipitin arcs were observed with polyclonal IgG of patient Pa (Fraction 2 from elution volume of protein A-Sepharose column) (Well 3) and with normal human serum (Well 4). (All troughs contain anti-δIdPa rabbit antiserum.)

of the human IgG3 subclass is not able to interact with the protein A, this finding suggests that the monoclonal IgG Pa was an IgG3, although it could not be confirmed with monospecific antisera due to its extremely low concentration.

Serum samples corresponding to May 1982, when IgD was predominant and IgG was undetectable by immunoelectrophoresis (Fig. 2), were used to isolate the IgD by preparative electrophoresis and utilized as the source to immunize rabbits. After proper absorption, the IgG fraction of rabbit antiserum obtained against the monoclonal IgD Pa showed, on immunoelectrophoresis, a unique precipitin arc with the whole serum of patient Pa, with isolated IgD Pa as well as with the polyclonal IgD present in standard solutions. It did not react with polyclonal IgG isolated from the serum of patient Pa by protein A-sepharose chromathography, nor with normal human serum (Fig. 3), nor with sera from other patients with MM containing monoclonal Ig, including IgGκ, IgGλ, IgMλ, nor with the urine of patients with MM containing free monoclonal Bence-Jones proteins of κ and λ type.

An ELISA performed with the microplates coated with the IgG fraction of this rabbit antiserum confirmed these results, since a strong reactivity was observed with monoclonal IgDλ Pa and with polyclonal IgD present in the standard solution (Behringwerke), but not with the polyclonal IgG of patient Pa (the fraction which was retained in the protein A-Sepharose column), nor with polyclonal IgG, IgA and IgM present in the standard solutions (Behringwerke), nor with other monoclonal IgG (of κ and λ type) from two other patients with MM. However, a strong reactivity was observed with the monoclonal IgGλ Pa (Table 1). These results suggest that, in addition to antibodies to δ chains, this antiserum also contained specificities recognizing idiotypic determinants present in both monoclonal IgD and IgG of patient Pa, and thus it will be designated as anti-δIdPa.

Immunofluorescence studies with peripheral blood mononuclear (PBM) cells and bone marrow cells

In May 1982, when serum IgD was predominant and serum IgG was undetectable, PBM cells were examined for surface and cytoplasmic immunoglobulins (sIg and cIg) (Table 2). At this stage, there were 8–10% of IgDλ-containing plasma cells. The majority of circulating B lymphocytes carried surface δ chains, but a small proportion of them carried both δ and γ chains. Cells bearing surface λ chains exceeded those with surface δ or γ chains. Cells bearing surface κ, μ or α chains were negligible or absent. Furthermore, double-labelling procedures showed that all cells with surface δ chains, and virtually all those with surface γ chains, also carried λ chains.

Bone marrow plasma cells were examined for cIg in February 1981 when serum IgD was predominant and serum IgG was still present but in very low concentrations, as well as in May 1982 when serum IgD was predominant and serum IgG was undetectable (Table 2). On both occasions, the major plasma cell population contained only δ chains. In addition to the minor

Table 1. ELISA reactivity (optical density) of IgG fraction of rabbit antiserum raised against monoclonal IgDλ (Pa) with isolated immunoglobulins of patient Pa and other polyclonal and monoclonal immunoglobulins*

Immunoglobulins added to the wells	Developing antibodies			
	POC-anti-δ†	POC-anti-γ	POC-anti-α	POC-anti-μ
Monoclonal IgDλ (Pa) May 1982	1·50	0·10	0·10	0·05
Monoclonal IgGλ (Pa) September 1981	0·10	1·30	0·10	0·10
Polyclonal IgG (Pa) May 1982	0·10	0·20	0·10	0·05
Polyclonal IgD (standard solution)	1·40	0·10	0·10	0·15
Polyclonal IgG-IgA-IgM (standard solution)	0·20	0·10	0·05	0·05
Monoclonal IgGκ (Sa)‡	0·10	0·20	0·10	0·05
Monoclonal IgGλ (Ga)‡	0·05	0·30	0·15	0·10

* In order to perform this ELISA, the microplates were coated (see Material and Methods) with the IgG fraction of rabbit antiserum which was absorbed with normal human sera, polyclonal and monoclonal IgG, IgA and IgM, but not with other monoclonal IgD.

† Peroxidase-conjugated rabbit antibodies specific for human heavy chains.

‡ Isolated monoclonal IgGκ and IgGλ from two other patients with MM (Sa and Ga, respectively).

Table 2. Cells with surface (sIg) and cytoplasmic (cIg) immunoglobulins

	sIg (%)	cIg (%)				
Ig determinants	PBM* (May 82)	PBM (May 82)	BM† (Oct 81)	BM (May 82)	BM(C1)‡	BM(C2)‡
μ	0	0	<1	<1	<1	<1
γ	4	0	7	12	80	<1
$\gamma+\lambda$	3	ND¶	6	10	ND	ND
α	0	0	<1	<1	<1	78
δ	27	8	30	42	0	0
$\delta+\lambda$	30	9	32	44	ND	ND
κ	<1	0	2	2	90	1
λ	38	10	50	88	1	85
$\delta+\gamma$	3	0	4	8	ND	ND
δIdPd§	ND	10	45	60	<0·5	<0·5
$\gamma+\delta$IdPa	ND	0	9	14	ND	ND

* PBM peripheral blood mononuclear cells.
† BM, bone marrow cells.
‡ Bone marrow cells from two other patients with MM producing IgGκ (C1) and IgAλ (C2).
§ IgG fraction of rabbit antiserum raised against monoclonal IgDλ Pa containing specificities to both δ chains and idiotypic determinants of IgDλ Pa.
¶ ND, not done.

Table 3. Generation of cells with cytoplasmic Ig (cIg) and immunoglobulin synthesis (Ig) by PBM cells stimulated with mitogens (7 days)

	Mitogens					
	None		PWM*		PHA*	
Ig determinants	cIg (%)	Ig† (ng/ml)	cIg (%)	Ig (ng/ml)	cIg (%)	Ig (ng/ml)
μ	0	2	<1	120	0	2
γ	0	44	10	>3000	0	47
$\gamma+\lambda$	ND‡	ND	9	ND	ND	ND
α	0	2	<1	220	0	3
δ	10	>2000	30	>2000	11	>2000
$\delta+\lambda$	11	ND	33	ND	10	ND
κ	0	ND	<1	ND	0	ND
λ	12	ND	41	ND	13	ND
$\delta+\gamma$	0	ND	0	ND	0	ND
δIdPa§	12	ND	44	ND	ND	ND
$\gamma+\delta$IdPa	0	ND	13	ND	ND	ND

* 1% final dilution in the cultures.
† Measured in the supernatants of cultures by an ELISA.
‡ ND, not done.
§ IgG fraction of rabbit antiserum obtained against monoclonal IgDλ Pa containing specificities to both δ chains and idiotypic determinants of IgDλ Pa.

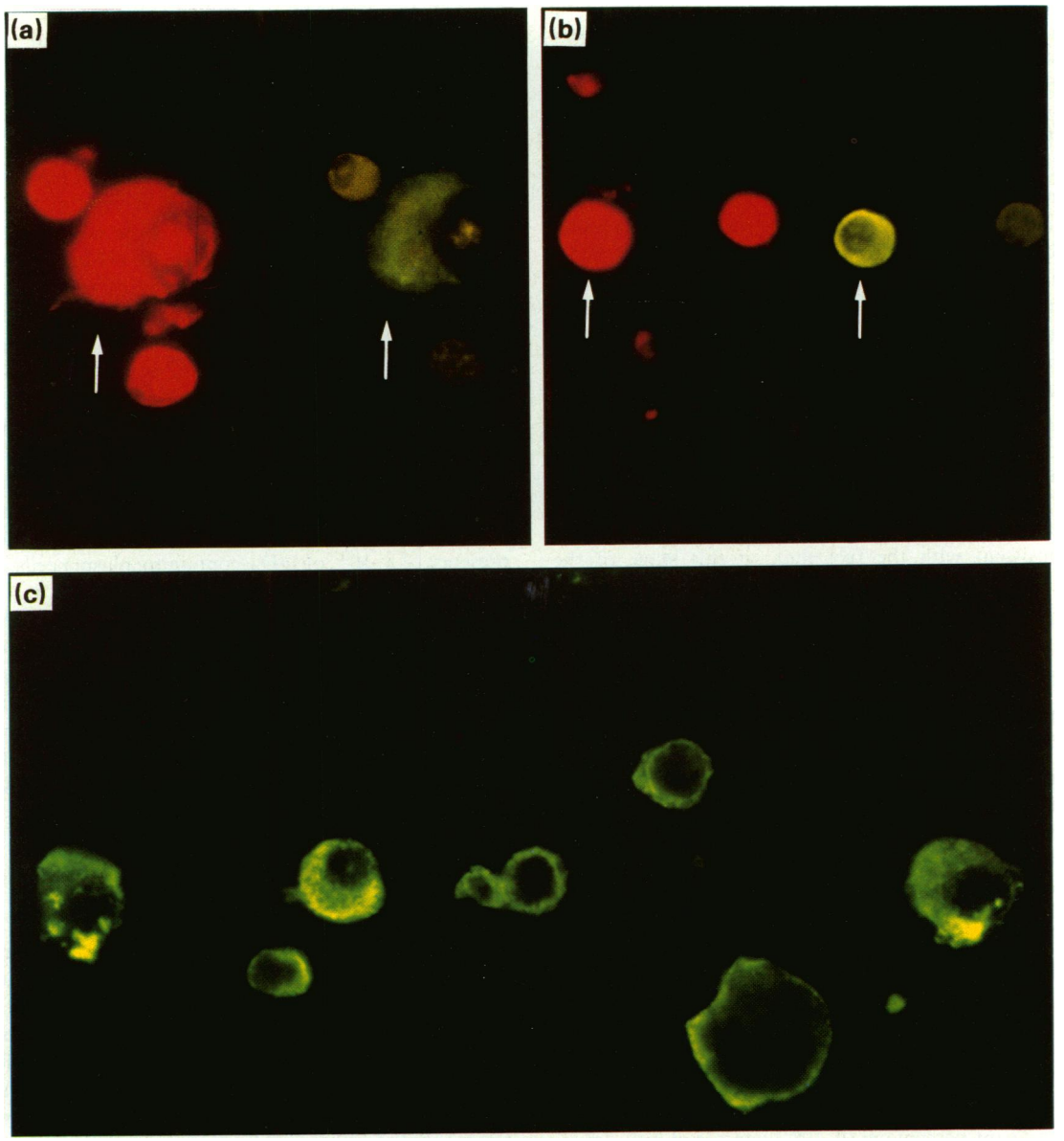

Figure 4. Bone marrow cells of patient Pa examined for cytoplasmic immunoglobulins (cIg) in May 1982 when serum monoclonal IgDλ was predominant and monoclonal IgGλ was undetectable. (a), (b), photographs of two fields sequentially stained with rhodamine-labelled anti-δ and FITC-labelled anti-γ antisera. Arrows indicate two cells clearly positive for both δ and γ chains (Table 2). (c), photographs of various fields stained with the rabbit anti-δIdPa which contained specificities to both δ chains and idiotypic determinants present in both monoclonal IgGλ and IgDλ of patient Pa.

plasma cell population containing only γ chains, there was another minor plasma cell population containing both δ and γ chains (Fig. 4). Double-labelling experiments showed that plasma cells containing either δ or γ chains also contained λ chains. Plasma cells containing cytoplasmic κ, μ or α chains were negligible or absent. The percentages of cells showing cytoplasmic reactivity with the anti-δ IdPa rabbit antiserum were 45% and 60% on October 1981 and May 1982, respectively. These figures account for the total percentages of plasma cells containing δ or γ chains on both dates (Table 2). Furthermore, double-staining procedures showed that plasma cells containing γ chains also exhibited cytoplasmic reactivity with the anti-δIdPa antiserum. This antiserum did not show reactivity with the bone marrow plasma cells containing IgGκ or IgAλ obtained from two other patients with (Table 2, C1 & C2). Plasma cells containing λ chains exceeded those containing cytoplasmic δ and/or γ chains; this finding reflected an imbalance in the production of heavy and light chains by myeloma plasma cells, and is consistent with the fact that, at this stage of the disease, a significant excretion of free λ light chains was occurring.

In vitro generation of cells with cytoplasmic Ig (cIg) and immunoglobulin synthesis by peripheral blood mononuclear (PBM) cells stimulated with mitogens

From May to June 1982, when serum monoclonal IgD was predominant and monoclonal IgG was undetectable, the PBM cells were cultured for 7 days with either complete medium alone, or with several doses of PWM (1% and 0·5% final dilution) or PHA (2% and 1% final dilution). As shown in Table 3, even in cultures without mitogen or with PHA, there were 10–12% or IgDλ-containing plasma cells as observed with fresh, non-cultured PBM cells (Table 2). A high proportion of cells with cytoplasmic immunoglobulins were observed in cultures stimulated with PWM. As indicated by double-staining experiments, the majority of them (30–32%) contained only IgDλ, but a significant percentage (9–10%) contained only IgGλ. The percentages of plasma cells containing κ, μ or α chains were negligible. As observed with the bone marrow plasma cells, the *in vitro*-generated plasma cells containing cytoplasmic λ chains exceeded those containing δ or γ chains. The percentage of plasma cells showing cytoplasmic reactivity with the anti-δIdPa antiserum was 44%, a figure which accounts for the total plasma cells containing either IgDλ or IgGλ. Contrary to that observed in the bone marrow, there were no *in vitro*-generated plasma cells containing both δ and γ chains. Thus, at least 9–10% of the *in vitro*-generated plasma cells contained only IgGλ, and double-labelling experiments disclosed that they also exhibited cytoplasmic reactivity with the anti-δIdPa antiserum (13% of plasma cells positive for both cytoplasmic γ and δIdPa determinants) (Table 3). On the other hand, when the PBM cells of three normal donors were cultured under the same conditions as the PBM cells of patient Pa (data not shown), plasma cells with cytoplasmic δ chains were never observed, a finding in agreement with those reported by other authors (Preud'homme, Brouet & Seligmann, 1977), but a mean value of 3% of plasma cells with cytoplasmic γ chains were found and, in spite of this, the rabbit anti-δIdPa antiserum did not show any reactivity.

High concentrations of IgD were found in the supernatants of all cultures, with or without mitogens (Table 3). This was an expected finding because of the presence of 8–10% of IgDλ-containing plasma cells in the peripheral blood at that time (Tables 2 & 3). However, high concentrations of IgG were only observed in the supernatants of PWM-stimulated cultures, together with very low levels of IgM and IgA (Table 3). This finding was in accordance with the high proportion of plasma cells with cytoplasmic γ chains and the absence or negligible presence of plasma cells with cytoplasmic μ and α chains generated with PWM stimulation.

DISCUSSION

In the few reports describing the simultaneous occurrence of monoclonal IgG and IgD in the serum of patients with MM (Fahey *et al.*, 1968; Gore *et al.*, 1979; Oxelius, 1971), immunofluorescence studies of bone marrow or circulating B cells with anti-isotypic or anti-idiotypic antisera, as performed in the present study, were not done. In addition, as far as we know, DP associated with a shift from predominantly IgG to IgD production has not been previously reported. The apparition of monoclonal IgD was not induced by chemotherapy since it was present as a minor component, together with the predominant IgG, before the treatment was begun.

The presence of 'monoclonal' circulating B lymphocytes in human myeloma, that is, circulating B lymphocytes bearing, on their surface, isotypic and/or

idiotypic determinants identical to those of monoclonal serum isotypes, has been reported by several groups (Preud'homme *et al.*, 1976; Holm *et al.*, 1977; Warner & Krueger, 1978; Kubagawa *et al.*, 1979; Bast *et al.*, 1982). Although they are considered to be the neoplastic progenitors of mature myeloma plasma cells, there is, at present, no direct proof of their ability to differentiate into terminal plasma cells secreting the same monoclonal isotypes present in the serum. In this study, when peripheral blood mononuclear cells were stimulated with PWM, circulating B lymphocytes were induced to differentiate into Ig-secreting plasma cells which were restricted to the synthesis of the same monoclonal isotypes (IgDλ and IgGλ) observed *in vivo*. In addition, the *in vitro*-generated plasma cells containing either cytoplasmic IgDλ alone or cytoplasmic IgGλ alone showed cytoplasmic reactivity with a rabbit antiserum raised against monoclonal IgDλ. This antiserum was shown to comprise specificities recognizing both δ chains and idiotypic determinants present in both serum monoclonal IgD and IgG. The circulating B lymphocytes were 'monoclonal', since almost all expressed surface IgDλ, but a small percentage of them also expressed both surface IgD and IgGλ. These results in the *in vitro*-generation of plasma cells paralleled those obtained with the bone marrow plasma cells at the same evolutionary stage of the disease. Within the *in vitro*-generated plasma cells, no double producers (of IgD and IgG) were observed, but they were clearly detectable in the bone marrow, although only a small percentage, together with a major plasma cell population containing only IgDλ and a minor one containing only IgGλ.

The presence of small numbers of plasma cells containing in their cytoplasm the two monoclonal isotypes present in the serum has been well documented in many cases of MM with DMG or triclonal gammopathy (van Camp *et al.*, 1978; Krueger, Fair & Kyle, 1979). In such cases, the monoclonal isotypes shared the same light chain type (van Camp *et al.*, 1978) and idiotypic determinants (Krueger *et al.*, 1979) as the patient described here. These findings have been interpreted as evidence to suggest that the major plasma cell populations producing each one of the isotypes were derived from a common single B-cell clone. Furthermore, in several mouse experimental models using the splenic focus assay, the presence of two different isotypes with the same antigen-binding specificity in the cytoplasm of at least 25% of the daughter cells derived from a single B-cell clone during their clonal expansion has been demonstrated (Gearhart, Hurwitz & Cebra, 1980; Teale, 1982). Similar observations have been made by Robertson *et al.* (1982) with mouse hybridomas secreting isotypes with anti-*p*-azophenylarsonate specificities. Equivalent findings have been found in cultures of mouse B lymphocytes stimulated with polyclonal activators in which the percentage of cells containing two isotypes

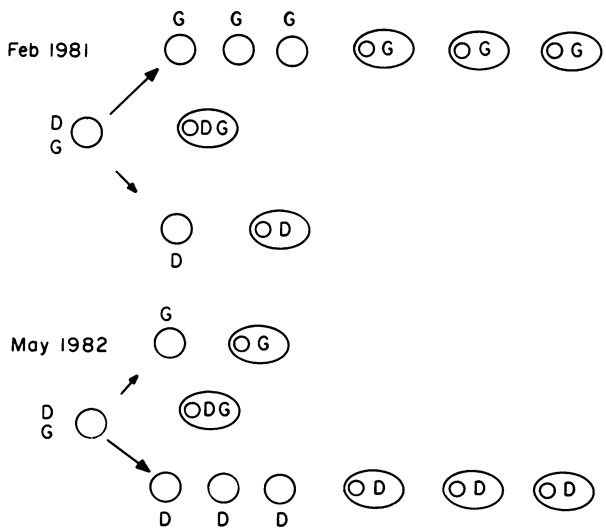

Figure 5. Scheme showing a possible interpretation of the cellular basis of double monoclonal gammopathy observed in patient Pa.

in their cytoplasm increased when cytochalasin B was added to inhibit cell division (van der Loo *et al.*, 1979; Severinson *et al.*, 1982). From these results, implications for the genetic mechanism of the intraclonal switching process have been suggested (Gearhart *et al.*, 1980; Honjo *et al.*, 1981; Serevinson *et al.*, 1982; Robertson *et al.*, 1982), in the sense that switching appears to take place during the cell cycle involving an assymetric cell division which would be consistent with the model of unequal sister chromatide exchange (Obata *et al.*, 1981). In this context, the findings reported in this study strongly suggest that the plasma cell populations present in the bone marrow producing either IgD or IgG were derived from a common precursor B-cell clone. A possible interpretation is depicted in Fig. 5.

Despite extensive studies devoted to this issue, the precise functional role of sIgD as a B-cell antigen receptor remains undefined and controversial (Vitetta, 1982). In addition, two main types of model exist for surface isotype diversification along the B-cell differentiation pathway, as far as the expression of surface IgD is concerned:

(i) the acquisition of IgD of IgD in the surface of immature B lymphocytes bearing sIgM is a necessary step for the subsequent intraclonal isotype switching to IgG or IgA (Preud'homme *et al.*, 1977; Vitetta & Uhr, 1977);

(ii) immature B cells with sIgM can directly switch to IgG (or IgA) on the B-lymphocyte surface without the previous expression of sIgD, and these cells become 'trebles' (e.g. bearing surface IgM-IgG-IgD) through the acquisition of sIgD (Parkhouse & Cooper, 1977; Cooper *et al.*, 1982).

In either case, both types of model are compatible with the existence of B lymphocytes with both surface IgD and IgG as precursors of IgG-secreting plasma cells. In fact, B lymphocytes bearing surface IgD and IgG with some containing cytoplasmic IgG have been clearly documented (Preud'homme *et al.*, 1977). The findings reported here seem to suggest that normal B lymphocytes bearing surface IgD and IgG as precursors with the potential to give rise to either IgG-secreting or IgD-secreting plasma cells (Fig. 5) could also exist, although at an extremely low frequency.

ACKNOWLEDGEMENT

This work was supported, in part, by two research grants (1982) from the Fondo de Investigaciones Sanitarias de la Seguridad Social.

REFERENCES

Bast E.J.E.G., van Camp B., Reynaert P., Wiringa G. & Ballieux R.E. (1982) Idiotypic peripheral blood lymphocytes in monoclonal gammopathy. *Clin. exp. Immunol.* **47,** 677.

Bouvet J.P., Feingold J., Oriol R. & Liacopoulos P. (1975) Statistical study on double paraproteinaemias. Evidence for a common cellular origin of both myeloma globulins. *Biomedicine*, **22,** 517.

van Camp B.G.K., Struit H.R.E., Hijmans W. & Radl J. (1978) The cellular basis of double paraproteinemia in man. *Clin. Immunol. Immunopathol.* **9,** 111.

Cooper M.D., Kuritani T., Chen C., Lehmeyer J.E. & Gathings V.E. (1982) Expression of IgD as a function of B-cell differentiation. *Ann. N.Y. Acad. Sci.* **399,** 146.

Ey P.L., Prowse S.J. & Jenkin D.R. (1978) Isolation of Pure IgG, IgG2a and IgG2b immunoglobulins from mouse using protein A-Sepharose. *Immunochemistry*, **15,** 429.

Fahey J.L., Carbone P.P., Rowe D.S. & Bachmann R. (1968) Plasma cell Myeloma with D-Myeloma protein (IgD Myeloma). *Am. J. Med.* **45,** 373.

Fair D.S. & Krueger R.G. (1978) Analysis of biclonal immunoglobulins and their contributions to understanding the development aspects of the antibody response. *Contemp. Top. Mol. Immunol.* **7,** 51.

Fair D.S., Schaffer S. & Krueger R.G. (1976) Development of monoclonal IgA and an apparent IgG in a patient with macroglobulinemia: sharing of individually specific antigenic determinants among IgM, IgA and IgG. *J. Immunol.* **117,** 944.

Fudenberg H.H., Wang A.C., Pink J.R.L. & Levin A.S. (1971) Studies of an unusual biclonal gammopathy: implications with regard to genetic control of normal immunoglobulin synthesis. *Ann. N.Y. Acad. Sci.* **190,** 501.

Gathings W.E., Lawton A.R. & Cooper M.D. (1977) Immunofluorescence studies of the development of pre-B cells, B lymphocytes and immunoglobulin isotype diversity in humans. *Eur. J. Immunol.* **7,** 804.

Gearhart P.J., Hurwitz J.L. & Cebra J.J. (1980) Successive switching of antibody isotypes expressed within the lines of a B-cell clone. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5424.

Gore M.E., Riches P.G. & Kohn J. (1979) Identification of the paraproteins and clinical significance of more than one paraprotein in serum of 56 patients. *J. clin. Pathol.* **32,** 313.

Guardia J., Rubies-Prat J., Gallart T., Moragas A., Martinez-Vazquez J.M., Bacardí R. & Vilaseca J. (1976) The evolution of alpha-heavy chains disease. *Am. J. Med.* **60,** 596.

Holm G., Mellstedt H., Petterson D. & Biberfeld P. (1977) Idiotypic immunoglobulin structures on blood lymphocytes in human plasma cell myeloma *Immunol. Rev.* **34,** 139.

Honjo T., Nakai S., Nishida Y., Kataoka T., Yamawaki-Kataoka Y., Takahashi N., Obata M., Shimizu A., Yaoita Y., Nikaido T. & Ishida N. (1981) Rearrangements of immunoglobulin genes during differentiation and evolution. *Immunol. Rev.* **59,** 33.

Hopper J.E., Haren J.M. & Kmiecik T.E. (1979) Evidence for shared idiotypy expressed by the IgM, IgG, and IgA

serum proteins of a patient with a complex multiple paraprotein disorder. *J. Immunol.* **122,** 2000.

JANOSSY G. & GREAVES M. (1975) Functional analysis of murine and human B lymphocytes subsets. *Immunol. Rev.* **24,** 176.

KRUEGER R.G., FAIR D.S. & KYLE R.A. (1979) Monoclonal IgM, IgA and IgG in the serum of a single individual: immunofluorescence identification of cells producing the immunoglobulins. *Eur. J. Immunol.* **9,** 602.

KUBAGAWA I.T., VOGLER L.B., CAPRA J.D., CONRAD M.E., LAWTON A.R. & COOPER M.D. (1979) Studies on clonal origin of multiple myeloma. *J. exp. Med.* **150,** 792.

KYLE R.A., ROBINSON R.A. & KATZMANN J.A. (1981) Clinical manifestations of biclonal gammopathy. A review of 57 of cases. *Am. J. Med.* **11,** 999.

VAN DER LOO W., GRONOWICZ E.S., STROBER S. & HERZENBERG L.A. (1979) Cell differentiation in the presence of cytochalasin B: studies on the 'switch' to IgG secretion after polyclonal B cell activation. *J. Immunol.* **122,** 1203.

NIETO A., GAYA A., JANSA M., MORENO C. & VIVES J. (1984) Direct measurement of antibody affinity distribution by hapten-inhibition enzyme immunoassay. *Molec. Immunol.* **21,** 537.

OBATA M., KATAOKA T., NAKAI S., YAMAHISHI H., TAKAHASHI H., YAMAWAKI-KATAOKA Y., NIKAIDO T., SHIMIZU A. & HONJO T. (1981) Structure of rearranged I gene and its implication to immunoglobulin class switch mechanism. *Proc. natn. Acad. Sci. U.S.A.* **78,** 2437.

OXELIUS V.A. (1971) Alternating appearance of IgD and IgG myeloma during treatment. *Scand. J. Haematol.* **8,** 439.

PARKHOUSE R.M.E. & COOPER M.D. (1977) A model for the differentiation of B lymphocytes with implications for the biological role of IgD. *Immunol. Rev.* **37,** 105.

PREUD'HOMME J.L., BROUET J.C. & SELIGMANN M. (1977) Membrane-bound IgD on human lymphoid cells, with special reference to immunodeficiency and immunoproliferative diseases. *Immunol. Rev.* **37,** 127.

PREUD'HOMME J.L., HUTEZ D., DANON F., BROUET J.C. & SELIGMANN M. (1976) Intracytoplasmic and surface-bound immunoglobulins in 'nonsecretory' and Bence-Jones Myeloma. *Clin. exp. Immunol.* **25,** 428.

ROBERTSON S.M., CLAYTON L., DEV V.G., WALL R., CAPRA J.D. & KETTMAN J.R. (1982) Antiarsonate antibody response: a model for studying antibody diversity. *Fed. Proc.* **41,** 2502.

SEVERINSON E., BERGSTEDT-LINDQUIST S., VAN DER LOOS W. & FERNANDEZ C. (1982) Characterization of the IgG response induced by polyclonal B cell activators. *Immunol. Rev.* **67,** 73.

TEALE J.M. (1982) The potential of B lymphocytes for isotype expression. *Fed. Proc.* **41,** 2497

TODEL C.W., FRANKLIN E.C. & RUDDERS R.A. (1974) Similarities of the light chains and the variable regions of the heavy chains of the IgG and IgA myeloma proteins from a single patient. *J. Immunol.* **112,** 871.

VITETTA E. (1982) Immunoglobulin receptor reevaluated. *Ann. N.Y. Acad. Sci.* **399,** 255.

VITETTA E. & UHR J. (1977) IgD and B cell differentiation. *Immunol. Rev.* **37,** 50.

WARNER T.F.C.S. & KRUEGER R.G. (1978) Circulating lymphocytes and the spread of myeloma. Review of the evidence. *Lancet*, **i,** 1174.

WINCHESTER R.J. & FU S.M. (1976) Lymphocyte surface membrane immunoglobulin. *Scand. J. Immunol.* **5** (Suppl), 9.

YAGI Y. & PRESSMAN D. (1974) Monoclonal Iga and IgM in the serum of a single patient (SC). Sharing of individually specific determinants between IgA and IgM. *J. Immunol.* **110,** 335.