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Differential Immunocytological Expression of a Paraprotein-associated Idiotype is Possibly Related to Variant Immunoglobulin Molecules in Plasmacytoma Cells

OTTO WETTER,*† DIETER BRANDHORST*† and WINRICH BREIPOHL‡

Innere Universitätsklinik (Tumorforschung), West German Tumor Center and ‡Department of Anatomy, University of Queensland, St. Lucia, Australia

Abstract—*An anti-idiotype antibody (anti-Id Ab) was made by immunization of a rabbit with the IgG-2/λ paraprotein from a plasmacytoma patient (Sa). The antibody was directed against determinants of heavy (H) and light (L) chains on the $F(ab)_2$ piece of the paraprotein. Using this antibody an unusual pattern of cytoplasmic fluorescence was seen with bone marrow cells of the patient. One population (type I) showed reactivity in association with γ and λ chains, the other (type II) with the anti-Id Ab only. So, by immunological methods, biclonality could be demonstrated using the anti-Id Ab but not with anti-isotype antibodies. L chains of mol. wt* 23.5 *and* 17.0 kd *were observed. The H and L chains of small molecular weight were not detected in the patient's serum nor in the culture supernatant. Thus, deleted and intracellularly degraded H and L chains may cause the unusual staining pattern of the type II cells.*

INTRODUCTION

THE CONCEPT of the clonal nature of the antibody response suggests that anti-Id Ab are most reliable reagents for the detection of malignant cells derived from antibody producing cells. Using anti-Id Ab it has been shown for the first time that plasmacytoma patients harbor cells in the peripheral blood expressing the paraprotein idiotype on their outer surface membrane [1]. Also by the use of such antibodies it was possible to trace back the origin of plasma cell malignancy to the pre-B cell stage in two cases [2].

In the following we report on our experience with an anti-Id Ab which was applied in an immunofluorescence procedure to plasmacytoma cells of a patient with an IgG-2/λ paraprotein. A peculiar staining pattern was observed in a morphologically distinct population of plasmcytoid cells using this antibody. With commercial antibodies against H and L chain isotypes these cells were negative. From an analysis of the cell sap proteins after biosynthetic labelling we conclude that deleted H chains were present intracellularly and cause the unusual pattern of immunofluorescence. We also found a L chain fragment of 17 kd. It is suggested, that the use of anti-Id and anti-isotype Ab for immunoprecipitation of cell sap proteins in parallel experiments, followed by SDS-PAGE is an optimal screening procedure for variant Ig molecules. Of course, the precondition of this approach is that the anti-Id Ab is directed against determinants on H and L chains.

MATERIALS AND METHODS

The patient studied here is a 48 year old female referred to as Sa in the following. The diagnosis of plasmacytoma was verified by an abnormal increase of plasma cells in the bone marrow, atypical cells and a monoclonal serum protein of IgG-2/λ type at a concentration of 4.6 g%. The patient experienced a period of 12 years with stable disease without cytostatic or radiotherapeutic treatment until a pathological fracture of the left humerus and severe anemia indicated the progression of disease. Our study was performed on bone marrow cells obtained during this phase prior to the application of cytostatic agents. The patient died 15 months after the

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Correspondence to: Prof. Dr. O. Wetter, Innere Universitätsklinik (Tumorforschung), Hufelandstraße 55, D-4300 Essen 1, F.R.G.
Abbreviations: kd, kilodalton; CLL chronic lymphatic leukemia; MAL, mercaptoethanol.

initiation of cytostatic treatment with signs of tumor progression. Indications for the presence of amyloid light chain disease or monoclonal Ig deposition disease were not given.

Preparation of cells

Bone marrow cells were obtained by aspiration from the iliacal crest at three occasions during a 6 month period. Heparin (Novo Ind., Mainz) was used as anticoagulant at a dose of 5000 I.U. per ml aspirate. Mononuclear cells were isolated using Ficoll-Isopaque (Lymphoprep, Nyegaard & Co., Oslo) according to Bøyum [3] with minor variations. For conventional staining according to Wright the cells were brought onto slides by cytocentrifugation.

Morphometric analysis

Measurements of the nuclear and the cytoplasmic areas of the cells were performed with the digiplan MOP-A 03 instrument (Kontron, Eching) in connection with a Dialux light microscope (Leitz, Wetzlar). One hundred cells were measured in each category (see later) and the nucleo-cytoplasmic ratio was determined. Cells with two or more nuclei were excluded. Morphometry of cells after immunofluorescence staining was performed on photographic reproductions.

Antisera (As) and immunofluorescence analyses

Intracellular Ig was detected by a direct technique using polyclonal FITC-conjugated antibodies (Meloy, Springfield) and by an indirect method with monoclonal Ab against μ, δ, γ, κ, λ isotypes (BRL) and against α chains (Becton-Dickinson). The second Ab was a FITC-conjugated anti-mouse Ab from the goat (Tago Inc.). For the detection of surface Ig a polyvalent As against μ, γ and α chains (Meloy) in a direct procedure was used. The anti-Id Ab was used in an indirect assay with a FITC-conjugated anti-rabbit As from the goat (Medac, Hamburg) as the second antibody. Cells of patient Sa and of a healthy donor were used for comparison. An Orthoplan microscope (Leitz, Wetzlar) equipped with a Philips CS 100 W mercury lamp and an Opak Fluor vertical illuminator was used. Photographs were taken on Agfachrome 50L Professional and 3M ASA 1000 film.

Production and testing of the anti-Id Ab

The idiospecific antibody was produced in the rabbit following the procedure given by Bernier [4]. Absorption and testing of the IgG fraction of the antiserum was performed as described previously [5]. The Sa paraprotein was resistant to digestion with pepsin as was expected from a G2 protein. So, papain (Sigma, Deisenhofen) was used for proteolysis following the procedure of Gergely *et al.* [6]. The digests were separated on a Sephacryl S 200 column with PBS-Cystein. The $F(ab)_2$ and the Fc fractions were purified on protein A Sepharose. They showed mol. wt 108 kd and 25.3 kd, respectively, by SDS-PAGE. Electrophoresis of these fractions was done in Paragon SPE II gel (Beckman Instr.), followed by precipitation with affinity-purified anti-$F(ab)_2$ and anti-F_C antibodies from the rabbit (Jackson Lab.) for control of the digestion and separation procedures. H and L chains of the Sa paraprotein were separated by SDS-PAGE according to Laemmli [7]. Immunoblotting was done after electrophoretical transfer to a nitrocellulose membrane (Hybond, Amersham).

In the patient's serum the detection of Id^+ molecules was performed after SDS-PAGE (Pharmacia PAA 4/30 gradient gel) and electroblotting (Immobilon PDF, Millipore). The transfer membrane was developed with the anti-Id Ab in combination with a HRP detection kit (Biorad).

Cell culture for internal labelling and analysis of radiolabelled protein

Freshly prepared cells (20×10^6) from the patient's bone marrow and from a healthy donor were incubated with 30 μCi [^{14}C]leucine for 2 h at 37°C. The supernatant was collected and dialyzed against PBS for 48 h at 4°C with two changes. The cells were washed five times in saline. To the pellet 250 μl lysis buffer (0.145 M NaCl, 0.0015 M $MgCl_2$, 0.01 M Tris–HCl, 0.1% SDS, 0.5% NP 40 and 1% Triton X 100, pH 7.2) was added and the mixture was held on ice with occasional rocking followed by centrifugation for 30 min at 10^5 ***g*** at 4° C. To the lysate and to the supernatant the anti-Id Ab (50 μl) was added and the mixture was incubated for 2 h at 4°C. After another 2 h incubation at 4°C with 100 μl packed protein A Sepharose, five washings in PBS were performed. The Sepharose-bound protein was put into 200 μl of SDS-PAGE sample buffer according to Weber and Osborn [8] and boiled for 5 min. After centrifugation at 1200 ***g*** for 5 min SDS-PAGE was carried out according to Shapiro *et al.* [9] in rod gels (11×0.5 cm) of 4.5% PAA in the absence and presence of 1% MAL. Electrophoresis was done at 2.2 mA/gel for 17 h. Marker proteins were bought from Pharmacia, Uppsala. After processing in Amplify solution (Amersham) for fluorography the gels were dried and exposed to Kodak XAR film at −70°C.

Electron microscopy

Electron microscopy was performed as described previously [10]. Briefly, cells from the bone marrow were prepared by density gradient centrifugation and fixed in 3.2% glutyaraldehyde and 2.6% for-

maldehyde in cacodylate buffer and embedded in Epon 812.

RESULTS

Anti-Id As and Ab

The result of an analysis of the anti-Id As in an Ouchterlony agar diffusion plate is shown in Fig. 1. Four IgG/λ paraproteins (wells 2–5) and normal human gamma globulin (well 6) were compared with the patient's serum (well 1). A precipitation line was observed in well 1 only. The same result was obtained with the IgG fraction of the anti-Id As (not shown). All further experiments were performed with this fraction called anti-Id Ab. In the hemagglutination inhibition assay inhibition was observed down to 62 μg of the Sa protein, but not with normal gamma globulin up to 5 mg. So, idiospecificity of our antibody still is given in the presence of non-specific Ig up to 80 times the amount of idiotypic protein.

Figure 2 shows the result of electrophoresis in paragon SPE II gel of $F(ab)_2$ and F_C of the Sa paraprotein and the immunoprecipitation with specific antibodies including the anti-Id Ab. It appears that the $F(ab)_2$, but not the F_C, is precipitated by the anti-Id Ab. Some contamination of the $F(ab)_2$ with F_C is visible (lane 5). In order to clarify the contribution of the paraprotein H and L chains to the idiotope the chains were separated by SDS-PAGE and immunoblotted using the anti-Id Ab as developer. Figure 3 shows that H chains (mol. wt 50.5 kd) as well as L chains (mol. wt 26.0 kd) bind to the anti-Id Ab as the paraprotein did (not shown). By immunoblotting after SDS-PAGE of cryopreserved serum at serial dilutions precipitation with the anti-Id Ab of protein at a position of 159 kd was observed but not at 49.1 or 17.0 kd. So, only the complete idiotype bearing IgG molecules and no fragments were detected in the serum.

Idiotypic cytoplasmic determinants

Two easily distinguishable patterns of immunofluorescence were observed using the anti-Id Ab with the patient's cells (Fig. 4a,b,c). The cell shown in Fig. 4a represents plasma cells with strong fluorescence evenly distributed over the cytoplasm. The nuclei of these cells are relatively small and eccentrically located. Cells of this type are called type I throughout this text. The other cell type (type II) is shown in Fig. 4b and c. These cells show discrete spots of fluorescence located invariably in a paranuclear position. This is illustrated in Fig. 4b where two cells are photographed with their greatest circumference in focus. One is tempted to align the fluorescence with a zone marking the Golgi apparatus. In Fig. 4c the speckled character of the fluorescence is evident. A distinctive feature of the two cell types besides this pattern of fluorescence is their different nucleo-cytoplasmic ratio (see later).

Table 1. Results of morphometry and immunofluorescence analyses of plasmacytoma cells from the bone marrow of patient Sa

	Type I	Type II
Nucleo-cytoplasmic ratio*	0.21	0.52
Surface Ig (μ, γ, α)	Negative	Negative
Surface Id	Negative	Negative
Cytoplasmic Ig†	$\gamma^+ \lambda^+$	Negative
Cytoplasmic Id (cId)	Positive	Positive
cId, mode of expression	Homogeneous	Restricted to the peri-Golgi site

*From measurements on photographic reproductions of cId positive cells.
†Conventional (polyclonal) antibodies.

Table 2. Results of a representative experiment with bone marrow cells from patient Sa with the idiospecific antibody and monoclonal antibodies against isotypic determinants. Numbers are percentages of all mononucleated cells

	Total	Type I	Type II
Number of plasmacytoma cells (Wright staining)	28	19	9
Number of positive cells			
Anti-Id	22	15	7
Anti-γ chain	25	25	0
Anti-λ chain	17	17	0
Anti-δ chain	2	2	0
Anti-μ chain	0	0	0
Anti-α chain	1	1	0
Anti-κ chain	2	2	0

Isotypic cytoplasmic and surface determinants

Cells of type I and type II were distinguished easily by their morphology. Only cells of the type I were stained when conventional or monoclonal antibodies against γ and λ chains were used for immunofluorescence. Type I cells showed the color evenly distributed over the cytoplasm as was also the case with anti-Id Ab, but not as intensively as with the latter. These results, including those of tests for surface Ig and surface Id, are summarized in Table 1. The results of a representative experiment with monoclonal anti-isotype Ab and our anti-Id Ab are shown in Table 2. Cells not listed in the table were erythroid and myeloid precursor cells and rare granulocytes. The few cells stained by anti-δ, anti-α and anti-κ chains are probably normal plasmacytes.

Morphology of cells of type I and II

The relationship between the two cell types (I and II) was not recognized initially when smears of

Table 3. Morphometric data of two types of plasmacytoma cells (type I, II) from the bone marrow of patient Sa after cytocentrifugation and conventional staining

	Nuclear area (mean ± s.d.) (μm^2)	Cytoplasmic area (mean ± s.d.) (μm^2)	Nucleo-cytoplasmic ratio
Type I	58.9 ± 15.4	240.4 ± 67.7	0.24
Type II	126.2 ± 24.2	211.2 ± 45.4	0.59

Table 4. Results of fluorography of internally labelled protein after SDS-PAGE in the presence of MAL. Proteins were precipitated by the anti-Id Ab from culture supernatants and cell sap from bone marrow cells of patient Sa. The molecular weights of H and L chains of Sa paraprotein and normal IgG are also given. Numbers are kd

Supernatant	Cell sap	Paraprotein	Normal IgG
67.5	68.5	66.4	69.6
	49.1		
23.0	23.5	24.8	24.8
	17.0		

the patient's bone marrow cells were examined routinely. Figure 5a shows the two cell types which occur also in binucleated (Fig. 5b) and occasionally in trinucleated forms. The cells of type I show the appearance of mature plasma cells, those of type II show a relatively great nucleus with a nucleolus, deep blue cytoplasm and a paranuclear bright zone. Table 3 shows the morphometric data of the two types.

Electrophoresis in SDS-PAGE of radiolabelled protein

Molecules precipitated from the cell sap by the anti-Id Ab were separated and fluorographed (Fig. 6). In the absence of MAL four bands of 151, 123, 100 and 37.2 kd were observed (Fig. 6a). In the presence of MAL bands of 68.5, 49.1, 23.5 and 17.0 kd were observed (Fig. 6b). The component of 49.1 kd seems to originate from the 123 kd component as judged by the intensity of the radiolabel. It appears that in addition to ordinary H and L chains small chains are present (H_F, L_F). The supernatant of the cultured cells showed two bands of 67 kd and 23 kd, respectively, when electrophoresed in the presence of MAL. H and L chains of small molecular weight were not detected in the supernatant and, therefore, were probably degraded intracellularly. In the cell sap molecules resulting from a partial assembly block (H_F.L), a dimer of H_F and a heterodimer of L chains and L_F chains were found in addition to ordinary H_2L_2 molecules. The results together with the molecular weights of the H and L chains of the paraprotein and of normal IgG are summarized in Table 4. We also performed a control experiment with normal bone marrow cells for biosynthetic labelling. With our anti-Id Ab no radiolabelled molecules were found after immunoprecipitation in the sap of these cells.

Electron microscopy

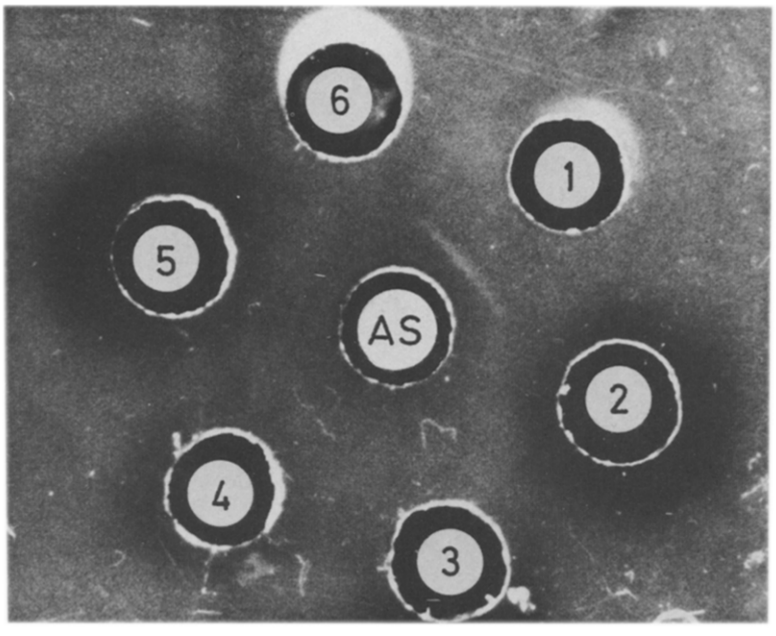
Two types of secretory cells were observed in preparations of the patient's bone marrow. One type (Fig. 7a) shows the features of mature plasma cells with an eccentrically situated nucleus, a pronounced Golgi apparatus and richly developed RER. The other cell type (Fig. 7b) had a more immature appearance with the RER dispersed in the form of shorter strands within the cytoplasm. The tubules of the RER contain a smaller amount of electron dense material when compared to the cell in Fig. 7a.

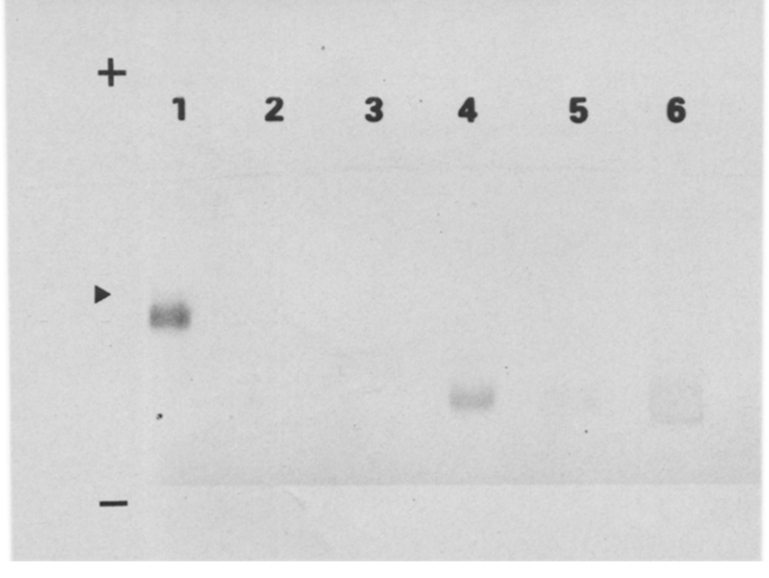
DISCUSSION

Heterogeneous tumor cells and their products

The application of our anti-Id Ab in the immunofluorescence procedure revealed two patterns of cytoplasmic expression of the idiotype. These two populations of tumor cells behaved as stable variants as the same proportion of each type of cell, called type I and II, was observed at several occasions in the patient's bone marrow. The malignant character of both cell types is evident; they are present in osteolytic lesions and they occur in binucleated and multinucleated forms. By these criteria they differ from idiotypic presursor cells which were observed also in the bone marrow of plasmacytoma patients but did not behave as malignant cells [2]. As the cells of type II not only by their immunofluorescence staining pattern but also by their morphometric criteria could be distinguished clearly from type I (Table 3), it was possible to ascertain if such cells were stained by anti-isotype antibodies also. Using polyclonal and monoclonal antibodies against H and L chain isotypes we could not detect any reactivity with the cells of type II. Only ϵ chains were not tested. In this situation with cells stained by idiospecific Ab but not by isospecific Ab it was interesting to analyze the cell sap proteins after immunoprecipitation with the anti-Id Ab. The occurence of 'small chain' variants has been shown in the case of MPC-11 tumors [11]. These small chains no longer reacted with antibodies against the isotypic determinants but still reacted with antibodies against the MPC-11 idiotype [11]. The intracellular presence of such molecules, therefore, would explain the differential immunofluorescence reaction pattern observed with type II cells of our case also.

A biosynthetic study of immunoprecipitable cytoplasmic proteins was performed in a case of Bence-Jones plasmacytoma of type κ [12]. In this study





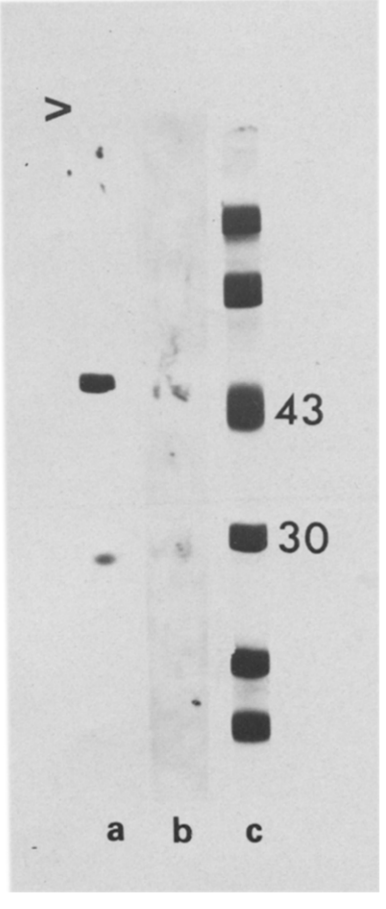


Fig. 1. *Double immunodiffusion in agarose with the anti-Id Ab against Sa protein (center well) and four individual IgG/γ paraproteins (well* 2–5) *pooled normal IgG (well* 6) *and Sa serum (well* 1). *A precipitation line is visible in well* 1 *only.*

Fig. 2. *Electrophoresis in agarose of* F_C (*lanes* 1,2,3) *and* $F(ab)_2$ (*lanes* 4,5,6) *of Sa paraprotein with consecutive immunoprecipitation by anti-*F_C *antibody (lanes* 1,5), *anti-*$F(ab)_2$ *antibody (lanes* 2,4) *and anti-Id Ab (lanes* 3,6). *The cuneiform sign indicates the start position.*

Fig. 3. *SDS-PAGE of H chains (mol. wt* 50.5 kd) *and L chains (mol. wt* 26.0 kd) *of the Sa paraprotein (lane a), immunoblot from lane a developed by the anti-Id Ab (lane b) marker proteins (lane c). Numbers are the molecular weights of two of the marker proteins, the arrow indicates the start position.*

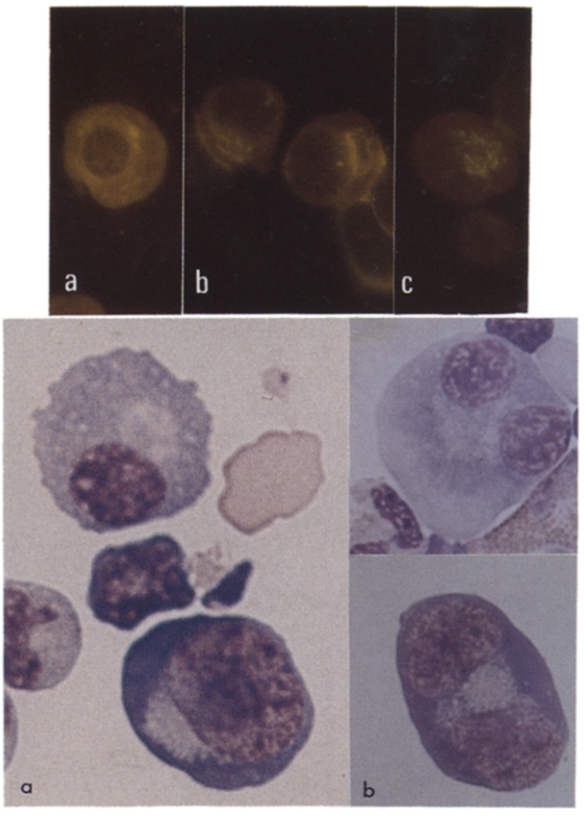


Fig. 4. Typical plasmacytoma cell (type I) from patient Sa stained by the anti-Id Ab in an indirect fluorescence procedure (a) and cells showing spots of fluorescence photographed at different positions of focus (b,c). These cells are called type II cells. Note the different nucleo-cytoplasmic ratio of the two types of cells (× 500).

Fig. 5. Cells of type I (upper part) and type II (lower part) in mononucleated form (a) and binucleated form (b) after Wright staining. Note the difference in the nucleo-cytoplasmic ratio between type I and II which also is found with the binucleated cells. Nucleoli were present in type II only (× 1050).

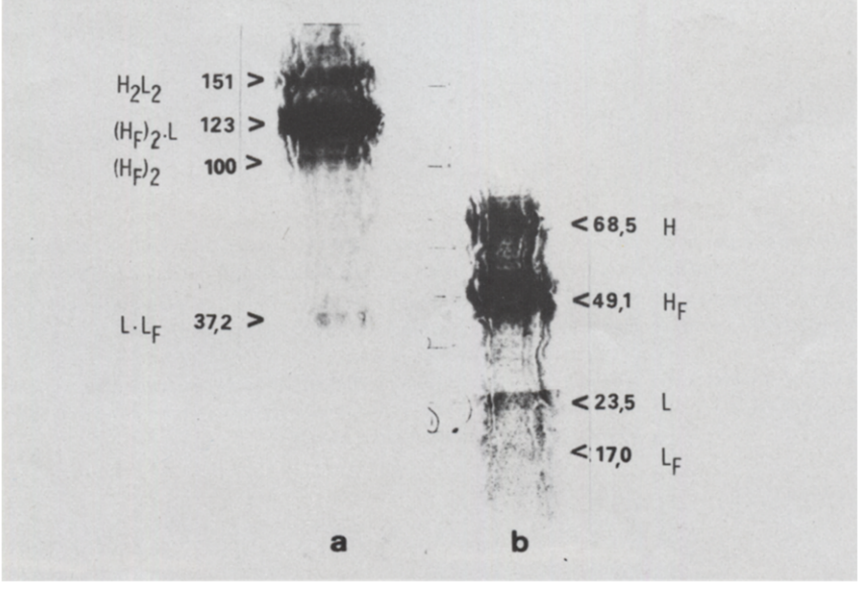


Fig. 6. Fluorography of biosynthetically labelled cell sap proteins of Sa bone marrow cells after precipitation with anti-Id Ab and electrophoresis in SDS-PAGE in the absence (lane a) and the presence (lane b) of MAL. Assumed molecular structures and molecular weights in kd *are given.*

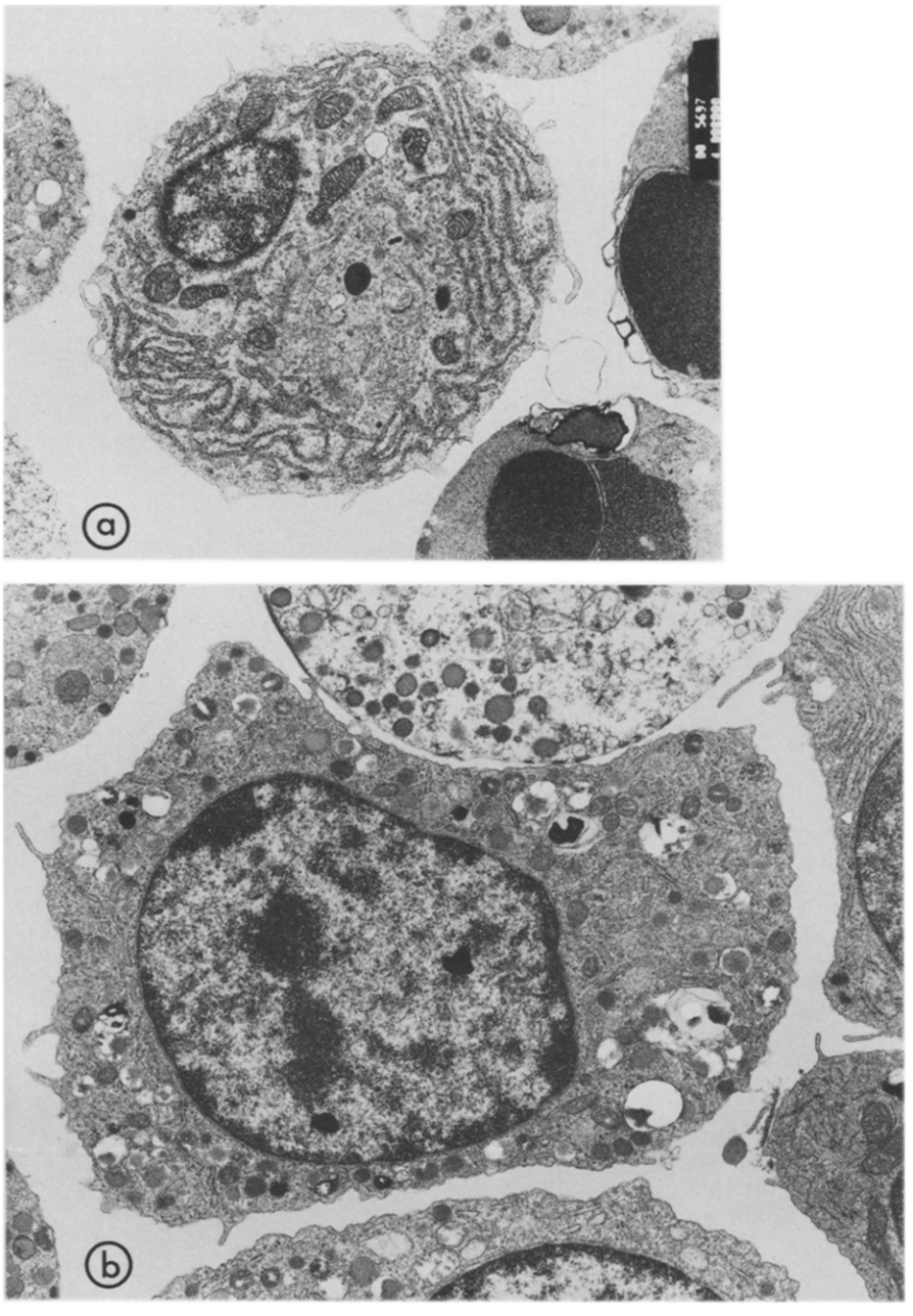


Fig. 7. (*a*) *Electron micrograph of a cell from the patient's bone marrow with the appearance of a mature plasma cell. This type of cell probably corresponds to type* 1 *of Figs.* 4 *and* 5. *In the lower part* (*b*) *a cell with scarce RER and various types of vesicles is shown which probably corresponds to type II* (× 8300, *both micrographs*).

abnormally short H chains were observed which assembled with a partial block with normally sized L chains, a finding similar to our case showing a species of 123 kd compatible with a $(H_F)_2$.L molecule (Fig. 6). The intracellular presence of abnormally short L chains of a molecular weight in the range 12–15 kd accompanied by a monomer-fragment heterodimer in a plasmacytoma with a λ chain paraprotein has been reported [13]. This observation also closely resembles some of the features of our case with a L chain fragment of 17 kd and a $L.L_F$ heterodimer. To our knowledge the simultaneous occurence of fragments of H and L chains in a plasmacytoma patient has not been reported before.

With respect to the technical procedure of immunoprecipitation and the following isolation of the precipitated material it can be noted that most workers including the cited authors [12, 13] use anti-isotype antibodies without or with subsequent binding to protein A beads. It is possible that the procedure applied by us, namely the use of anti-Id Ab in a sandwich technique followed by exposure to protein A beads, is very useful to obtain representative biosynthetic profiles as both parts of the Ig molecule—the v region (via idiotype) and the c region (via protein A affinity) are covered. Of course, a precondition of this approach is that an anti-Id Ab is available that binds to epitopes on both the H chain and the L chain. The applicability of the above procedure is limited to paraproteins of the IgG class because of the exclusive affinity of protein A to these molecules. In order to include IgA and IgM also in this screening procedures the combination of anti-Id Ab and anti-isotype Ab together and separately in aliquot samples followed by protein A exposure and subsequent analysis in SDS-PAGE probably is the most adequate approach.

As we did not perform cold amino acid chase experiments we do not know if the small chains in our case result from *de novo* synthesis or from intracellular degradation. Most if not all of the heavy chain proteins show internal deletions with defects in the v_H and the c_H1 domain. Many observations indicate that deleted chains are very susceptible to proteolytic cleavage ([14] for review). Synthesis of deleted chains and rapid intracellular degradation to immunologically non-reactive material certainly may explain some of the puzzling discrepancies between biosynthetic and immunocytologic observations on these patients as discussed by the above-mentioned author [14]. In our case, however, an additional hypothesis would be needed to explain why amino acid sequences with idiotypic determinants are preserved while isotypic determinants are lost. Binding of v region sequences to an antigen might provide such an element of preservation but in our case seems very unlikely.

The idiotope detected by the anti-Id Ab

In the present case it has been shown that $F(ab)_2$, H and L chains of the Sa paraprotein bind to anti-Id Ab. The fact that H and L chains after separation still bind the antibody indicates that it is directed mainly against determinants which are relatively conformation-independent. With respect to the molecular weight of the H chains of the Sa paraprotein the difference between the apparent molecular weight obtained at different PAA gel concentrations is noteworthy. The molecular weight obtained using the Laemmli buffer system with 12.5% PAA is 50.5 kd and in the Weber and Osborne buffer system with 10% PAA is 66.5 kd. The values obtained for L chains are 26.0 and 24.8 kd, respectively. It appears, that the H chains behave anomalously and that the molecular weights derived from their R_f values are unreliable.

Our failure to detect isotypic determinants in association with idiotypic determinants by the immunofluorescence procedure with type II cells may have other causes than that of a 'defective' molecule favored by us. For example, it is possible that the anti-isotype antibodies used for the indirect fluorescence procedure or the second FITC-conjugated antibody did not bind because of steric hindrance. With respect to this possibility, it would have been interesting to look for isotypic determinants of the biosynthetically labelled proteins after immunoblotting from SDS-PAGE. Unfortunately, the patient was no longer available to us when we became aware of the presence of intracellular Ig chain fragments. Another possibility would be the presence of an Ig molecule belonging to an hitherto unknown class or of an IgE protein. Both possibilities to us seem very remote. Finally, the presence of an epitope of non-Ig nature, which cross-reacts with the Sa idiotype by virtue of internal imaging, has to be considered. Such a putative cross-reacting epitope then would qualify as a tumor-specific antigen as it could not be detected in bone marrow cells from a normal individual.

Relationship between type I and II cells

The finding in patient Sa of two morphologically distinct types of cells expressing the same idiotype suggests a common origin of these cells. It is possible that the two types were created by the same transforming event at one time. During the observation period of 6 months the proportion (2:1) of type I and II, respectively, remained stable. It is possible that our observation of a striking difference of the labelling intensity associated with H chains and H_F chains is relevant to the discussion of the relationship between the two types. One explanation for this difference would be that the cells of type I in addition to complete IgG-2/λ molecules produce H_F molecules. On the basis of similar rates of synthesis for H and H_F chains by both cell types

and the 2:1 proportion mentioned above three times as much H_F chains as H chains are calculated. Indeed, about this proportion of H_F to H chains was obtained from the densitometric evaluation of the fluorogram. It then seems reasonable to assume that the cells of type II originate from type I by a second event causing the loss of ordinary H and L chains. It is accordance with this speculation that with mouse plasmacytomas it has been proposed that in the absence of L chains H chains—probably in order to get rid of their toxicity—have to be mutated if the cell is to express them [15]. In our case this would mean that after acquisition of a H chain mutant of 49.1 kd from subsequent variants loosing the expression of L chains only those with defective but not those with complete H chains would survive. Unfortunately, our attempt to separate the cells of type I and II failed. So, we were not able to prove or disprove the presence of H_F chains in type I cells. The phenomenon of a striking difference of radiolabelled protein was not observed in the case of L and L_F chains (Fig. 6). It is possible, therefore, that a third population of plasmacytoma cells is present producing L chains and L_F chains exclusively and that this population escaped the detection by the immunofluorescence procedure due to a small number of cells.

Morphological considerations

A comparison between the two cell types shown in the electron micrograph (Fig. 7) reveals significant differences in their size, their nucleo-cytoplasmic ratio and the amount of RER. As judged by these criteria the cells shown in Fig. 7a and b correspond to the cells of type I and II, respectively (Figs. 4 and 5). By the relatively great size of the nucleus, the presence of a nucleolus and the strong basophilic cytoplasm the cells of type II are clearly 'immature' when compared to type I and show marked signs of asynchrony [16]. Asynchrony to a certain degree is shown also by the cells of type I as evident from the occurence of mitoses in cells with a well-developed Golgi apparatus. In a systematic study of possible correlations between synthesis/secretion of Ig and the ultrastructure of CLL cells it was shown that secreting cells had a well-developed Golgi apparatus, various types of vesicles and strands of the RER whereas non-secreting cells did not possess these elements in a significant amount [17]. These non-secreting cells had μ chains which were degraded intracellularly.

Several attempts have been made to establish a relationship between morphology and clinical findings of prognostic relevance ([10] for literature). The observations made in the present case with two populations of tumor cells at different maturation stages, which numerically remained stable over a period of 6 months of rapid deterioration of the patient's condition, may show the limits of such attempts. Cellular metabolic conditions related to the proliferative capacity of plasma cells as measured after exposure to lectins [18] or anti-Id antibodies [5] may provide better criteria with respect to tumor progression and prognosis. An interesting question is, if the presence of variant Ig molecules and/or the process of intracellular degradation is accompanied by the appearance of certain types of cytoplasmic vesicles as is suggested by the findings in cases with μ chain disease [19, 20]. As it is known that an intracellular excess of H chains might be toxic [21] such vesicles may indicate damage of cell structures initiated by metabolic alterations. It should be considered also that these vesicles may constitute precursor elements of vacuoles which may be observed in plasma cells of patients with a variety of diagnoses as CLL, malign lymphoma and μ chain disease by light microscopy [14]. With plasmacytoma cells from a patient with an IgG/κ paraprotein [5] and with cells from the peripheral blood secreting γ chains (unpublished results) such vesicles were not observed. In the present case numerous vesicles, some of which were filled with a dense material and others with a fuzzy material, were observed with a preferential localization near the Golgi zone (Fig. 7b). These latter vesicles possibly correspond to the deposits of fluorescence stain shown in Fig. 4b and c.

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