PATHOLOGY OF IMMUNOGLOBULINS
SOME ASPECTS OF MONOCLONAL GAMMOPATHY

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Human immunoglobulins are a heterogeneous group of proteins closely related in biological function and highly similar in molecular structure. The immunoglobulins are synthesized in cells of the lymphoreticular system. Antigenic stimulation will produce a reaction in the lymphoreticular system in which many different cell clones are involved. This ensures a diffuse increase of immunoglobulins: a *polyclonal* reaction[1]. Malignant proliferation of a single clone will cause excessive production of a homogeneous immunoglobulin fraction: a *monoclonal* gammopathy[1].

One of the aspects of monoclonal gammopathy, viz the presence of an M-component in serum or urine, is the fact that the term "M-component" has begun to lead pretty much its own life as a result of the enormous advances in immunology and profound analytical studies of immunoglobulin structures. Today the term far exceeds its clinical significance. Thus one may find an extensive literature which discusses monoclonal gammopathy as if it were a disease. It is well-advised to bear in mind, however, that one is only dealing with a symptom.

The extent to which clinical insights have lagged behind laboratory findings can be illustrated by referring to the fact that, not too long ago, the finding of an M-component was practically tantamount to a diagnosis of multiple myeloma or Waldenström's macroglobulinaemia. But in the years between 1950 and 1960 this simple equation was no longer valid when monoclonal gammopathy was also found to occur in other syndromes[2–6].

Malignant immunoproliferative diseases

In the event of monoclonal proliferation of immunologically competent cells —immunocytes[7]—the formation of an M-component is an obvious inference. In the group of malignant immunoproliferative diseases (Table I), an M-component is always

TABLE I

MALIGNANT IMMUNOPROLIFERATIVE DISEASES

Lymphoproliferative	lymphatic leukaemia
	lympho-sarcoma
	macroglobulinaemia Waldenström
	Franklin's disease ("Heavy chain" disease)
Plasmaproliferative	multiple myeloma
	plasma cell leukaemia
	light chain disease (Bence Jones myeloma)
Reticuloproliferative	reticulo-sarcoma
	Hodgkin's disease

found in Waldenström's macroglobulinaemia and H-chain disease, and nearly always in multiple myeloma. The incidence of an M-component is low in lymphatic leukaemia, lymphosarcoma, reticulosarcoma and Hodgkin's disease.

On immunochemical analysis, the M-component in *Waldenström's macroglobulinaemia* invariably shows a structure which is characteristic of IgM.

In very many cases of *multiple myeloma*, zone-electrophoresis discloses a distinct M-component in the serum. On the basis of their immunochemical properties, these M-components can be divided into monoclonal proteins of the G-, A-, D-, or E-immunoglobulin class (G-myeloma proteins, A-myeloma proteins, etc.). Since we are dealing here with homogeneous protein fractions, the L-chains of these M-components can be either $\varkappa$ or λ.

In a number of cases, the synthesis of L- or H-chains can be out of balance in the presence of a monoclonal gammopathy. If the L-chain synthesis is excessive, the excess of free L-chains (relatively small molecules) are secreted together with the complete Ig molecule, and they can be found in the urine as so-called Bence Jones proteins. These Bence Jones proteins, therefore, are free L-chains and are either of type K or of type L. In the case of a monoclonal gammopathy these proteins are of course of the same type as the intact M-component.

In the past two years it has been established in cases of Bence Jones proteinuria that, in addition to the complete L-chain, the urine may contain fragments which correspond either with the "distinct" part (aminoacids 1–106) or with the "common" part of the Bence Jones protein (aminoacids 107–212)[8,9]. Myeloma has a much higher incidence of Bence Jones proteinuria (30–40%) than other diseases.

Another form of disbalance in H- and L-chain synthesis is found in situations in which the synthesis of H-chains is blocked. In such cases only L-chains are excreted, which are either type K or type L. This situation is known as "light chain disease" or Bence Jones myeloma. A quantitatively not so important M-component is often found in the serum in these cases, in which the urine contains a demonstrable homogeneous L-fraction of the same electrophoretic mobility and the same immunological type as that in the serum. The total serum protein is usually normal or slightly decreased, and in many cases the ESR shows only a slight increase (20–30 mm/1 h) or even is normal.

The abnormal serum component can sometimes be demonstrated only by means of agar-gel or cellulose acetate electrophoresis, and is not visible on the paper electrophoresis strip. If in examining serum from a patient suspected of multiple myeloma, a relatively low ESR, a normal or even slightly decreased total serum protein concentration and hypogammaglobulinaemia on the paper electropherogram are found, the possibility of Bence Jones myeloma must be considered: the urine must then be carefully examined for Bence Jones proteins. Our experience is that this is best done by concentrating fresh urine (containing some ε-aminocaproic acid against proteolysis) 20 times (by ultrafiltration, for example) and then submitting it to agar-gel and immunoelectrophoresis. Detection of Bence Jones proteins in *serum* is feasible with the aid of immunoelectrophoresis, using antisera specific for isolated L-chains. These antisera can be obtained by immunisation with purified Bence Jones proteins or light chains and absorbing the antisera with intact IgG molecules[10].

There is yet another form of monoclonal gammopathy, in which the synthesis of L-chains is blocked. In these cases however, complete H-chains are *not* secreted,

most probably because L-chains are required to release H-chains from the ribosome in the cell. In this so-called "heavy chain disease" the serum and urine are found to contain a homogeneous protein component with a MW of some 50,000, which consists of the Fc fragment of IgG which in addition incorporates a small peptide fraction of the N-terminal part of the γ-chain[11]. It seems, therefore, as if deletion in the gene has occurred as regards the information for synthesis of the larger part of the Fd fragment. Heavy chain disease (or preferably: Franklin's disease after its discoverer, or Fc disease) is a rare condition. The total number of cases known does not exceed eight, all derived from H-chain of IgG.

Ultracentrifugal analyses

Ultracentrifugation of serum in cases of monoclonal gammopathy discloses some characteristic features. In Waldenström's macroglobulinaemia the gammopathy of the IgM type is reflected in the ultracentrifuge diagram as a high 19 S peak.

In cases of myeloma one may find an increased 7 S or G peak (in G, A, or D-myeloma). In case of A-myeloma very often a 10 S fraction with small 13 S and 15 S fractions in addition, are found. Exhaustive studies have been devoted to these A-myelomas of the polymeric type, which showed that the M fraction often contained 7 S monomers besides the 10 S, 13 S and 15 S components.

The polymer formation in the monoclonal IgA in a number of patients with A-myeloma is quite intriguing and still awaits an adequate explanation. In earlier studies[12] differences in fingerprints (peptide maps) of 7 S monomers and corresponding 10 S dimers were found, which might suggest that no simple polymerization was involved. Immunochemical analyses, however, failed to demonstrate a difference between the 7 S IgA monomer and the 10 S IgA dimer in serum. The polymerization of 10 S myeloma IgA takes place via the Fc-fragment as could be demonstrated by peptic degradation of the 10 S IgA molecule[13].

It must finally be mentioned that IgA frequently enters protein associations (usually via -S-S-bonds) with other proteins such as albumin, haptoglobin and α_{2M}-globulin[14]. This is not a specific property of A-myeloma proteins, for it can be demonstrated that an albumin-IgA association is also present in commercial γ-globulin preparations[15].

Biclonal gammopathies

So far *monoclonal gammopathies* have been discussed in which (as the word "monoclonal" suggests) only a single M-component was found in the serum, leaving aside the cases in which an excess of L-chain was produced so that a second band was clearly visible in the electrophoretic pattern. These L-chains, it should be borne in mind, originate from the same cell clone, and the presence of the two bands in the electropherogram must therefore not be interpreted as suggestive of biclonal gammopathy.

But some cases are known in which the serum protein disturbances can be ascribed to a biclonal gammopathy with some justification. Several laboratories have reported the finding, in patients with multiple myeloma, of two M-components which had a different H- or L-chain. We had occasion to study 10 such cases. The distribution found is summarized in Table II.

In a number of combinations the L-chains are of the same immunological type,

TABLE II

CLASSIFICATION AND TYPING OF M-COMPONENTS IN BICLONAL GAMMOPATHIES

M-component I		*M-Component II*		*Frequency*
Class	*Type*	*Class*	*Type*	
IgG	K	Bence Jones	L	1 ×
IgG	K	IgA	K	2 ×
IgA	K	IgM	K	2 ×
IgG	K	IgM	K	1 ×
IgG	K	IgG	L	1 ×
IgG	L	IgA	L	1 ×
IgM	K	IgM	L	1 ×
IgG	L	IgA	L	1 ×

but this does not necessarily mean that they are identical. In this context we must mention studies made by Prendergast and Kunkel[16], who investigated the M-components in patients with an IgG and IgA biclonal gammopathy, with L-chains of type K. Although they were of the same immunological type, the isolated L-chains of the monoclonal IgG and IgA component differed in two patients on electrophoresis in acid-urea starch gel. No difference was demonstrable in the third patient. A "triclonal" gammopathy has been described by Jensen *et al.*[17]. The authors found in an apparently healthy man aged 41 three M-components of the IgG, IgA and IgM class.

The most striking pattern of an oligoclonal gammopathy was observed in the case studied by Stoop *et al.*[18], who found four abnormal fractions. Three of these were IgG, and one was not classifiable. It was established that two monoclonal G-components had L-chains of different type. Because the $\varkappa$ and λ chains are produced in different cells under physiological conditions, at least a biclonal gammopathy would seem to have existed in this case.

"Non-myeloma" gammopathies

Straightforward forms of immunoproliferative disease have been discussed so far. In a great many cases, however, a so-called "non-myeloma" monoclonal gammopathy prevails in the absence of any findings suggestive of Waldenström's macroglobulinaemia. It is difficult to classify these cases, and they must be discussed therefore in more or less random order.

There are patients, for example, with monoclonal gammopathy in whom the M-component shows antibody activity, e.g. M-components of the IgM class with cold agglutinin activity. The occurrence of monoclonal protein components with a specific antibody activity is of great fundamental importance. In the context it should be pointed out that Waldenström in particular emphasized long ago that these monoclonal globulin components might well be antibodies originating from a cell which has undergone malignant proliferation.

A group of diseases, however, which may be associated with a monoclonal gammopathy often lack any clues to a malignant immunoproliferative basis. Three categories will be discussed separately.

The first is the category of chronic infections, in which a polyclonal gammopathy usually exists. In these cases a monoclonal gammopathy can develop in the course of a few years. Osserman[19] in particular has pointed out this possibility, which is based on clinical experience and is supported by the results of animal experiments

by Potter's group[20]. A suggestive case is that of a 2-year-old child in whom a polyclonal reaction turned into a monoclonal gammopathy with Bence Jones proteinuria[21].

The second category includes the skin diseases with a monoclonal gammopathy. A high incidence of M-components of the IgA class is found in pyoderma gangraenosum[22]. These monoclonal components are not important from a quantitative point of view. In lichen myxoedematosus, a monoclonal M-component of the IgG class is found, with a light chain of the L-type[23].

The third category is that of the so-called essential benign monoclonal gammopathies—a term introduced by Waldenström and his group. An investigation of a population of normal individuals aged over 70 disclosed 3% monoclonal gammopathies (ref. 24). The relative amount of the abnormal protein component in the serum is mostly less than 2 g/100 ml, and remains constant in time.

Genetic aspects

One of the problems which arise in the study of gammopathies concerns the genetic aspect. There are reports of chromosomal abnormalities in monoclonal gammopathy; their incidence is highest in macroglobulinaemia, in which the type of abnormality is relatively constant.

A study of monozygotic twins, one of whom suffered from Waldenström's macroglobulinaemia with chromosomal abnormalities while the other was entirely unaffected and showed no chromosomal abnormalities, would seem to suggest that a direct pathogenetic correlation exists between the occurrence of the chromosomal aberration and the monoclonal gammopathy[25]. Korngold[26] described two sisters who developed multiple myeloma, one shortly after the other. One had L-chain disease (type L) and the other suffered from A-myeloma type L.

Exhaustive family studies have been carried out, especially by Seligmann[27] and by Kalff and Hijmans[28]. They have demonstrated that monoclonal gammopathies have a familial aspect, which is most clear in cases of macroglobulinaemia.

Cellular aspects

What are the repercussions of a monoclonal gammopathy at the cellular level? The normal human adult bone marrow contains about 8% lymphocytes. The frequencies of cells producing IgG, IgA or IgM are 50%, 35% and 15%. The $\varkappa/\lambda$ ratio of these cells can be estimated as 3:1[29]. A 3:2 ratio is found in the lymph glands and spleen[30]. This is reflected in the quantitative distribution of serum immunoglobulins.

In the case of a monoclonal gammopathy the predominance of demonstrable cells contain the monoclonal protein, that is: we find a predominance of cells with an immunoglobulin of a given class and type.

One can determine the frequency distribution of a large number of monoclonal proteins as to class and type. This has been done by a number of investigators, and Mul[31] has recently incorporated the data from the literature in his own large material.

The statistical analysis of typing data warrants a number of conclusions[31]. The hypothesis that M-components are random representatives of the immunoglobulin population is corroborated by the results obtained in terms of the total number of M-components. The different results obtained in the separate immunoglobulin classes are not inconsistent with this but merely indicate that the $\varkappa/\lambda$ ratio is not the same for all classes. In this respect, these results confirm the observations of Bernier and

Cebra[30]. Using the values reported by these authors as test ratios for the IgG and IgA classes, no statistically significant deviation was found. Nor did the $\varkappa/\lambda$ ratio for Bence Jones proteins show a statistically significant difference from that in normal urine. All these results are consistent with the assumption that paraproteins result from a random dysregulation of immunoglobulin-synthesizing cells. For the IgM and IgD classes, the ratio between cells which synthesize type K and those which synthesize type L molecules is unknown. If we accept the ratio established between type K and type L M-components as a yardstick, then it is justifiable to conclude that at the cellular level the ratio for the IgM class must be greatly in favour of the type K-synthesizing cells, while that for the IgD class should be greatly in favour of the type L-synthesizing cells.

Immunoglobulin levels

As is known from the immunofluorescence studies, monoclonal gammopathies of the immunoproliferative type are associated with a marked reduction in the number of cells which synthesize immunoglobulins other than the monoclonal component. This has its reflection in the serum concentrations of these proteins[32,33,34]. The low serum levels, particularly in the terminal stage of the disease, may explain the high incidence of infections of the skin, the respiratory tract and urinary tract in these patients. Hobbs[35] has been able to study two patients with solitary myeloma in which the tumours were removed and it was found that the immunoglobulin levels returned to normal. The author suggests that the tumours release some humoral substance which inhibits the synthesis of the residual normal immunoglobulins, but not that of the M-component. This intriguing finding merits a more detailed systematic study.

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