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A New Class of Immunoglobulin in Human Serum

S. G. O. JOHANSSON, H. BENNICH AND L. WIDE

The Blood Centre, University Hospital; Institute of Biochemistry, University of Uppsala; Department of Clinical Chemistry, University Hospital, Uppsala, Sweden

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Summary. A new class of normal immunoglobulin corresponding to a myeloma protein (myeloma-IgND), which fails to react with specific antisera to IgA, D, G and M (Johansson and Bennich, 1967) was detected in serum from sixty-two blood donors using a radio-immunosorbent technique (Wide and Porath, 1966).

IgND in normal sera corresponds to myeloma-IgND on electrophoresis, gel filtration and DEAE-chromatography.

Isolated IgND gave a reaction of identity with myeloma-IgND in Ouchterlony gel diffusion analysis.

The concentrations of IgND in 93·5 per cent of the samples was within the range of 100–700 ng/ml.

Normal levels of IgND were found in four samples apparently lacking IgA and/or IgD as determined by single radial immunodiffusion.

Elevated levels of IgND were found in four samples one of which was from a subject with previously undiagnosed extrinsic asthma.

It is concluded that myeloma-IgND represents a new class of human immunoglobulin.

INTRODUCTION

An atypical (myeloma) immunoglobulin (subsequently called myeloma-IgND) which fails to react with antisera specific to the immunoglobulins A, D, G or M was described in a previous paper (Johansson and Bennich, 1967). An antiserum specific to the Fc fragment of myeloma IgND (myeloma-FcND) was used in an attempt to demonstrate a normal counterpart to the myeloma protein. Studies on 300 sera from blood donors and patients with various clinical disorders using the single radial immunodiffusion test (SRD) (Mancini, Carbonara and Heremans, 1965) indicated that if such a protein were present, its level was less than 0·01 mg/ml serum.

In the present report further investigations are described using a radio-immunosorbent technique (Wide and Porath, 1966), which is about 1000 times more sensitive than SRD. Protein carrying the antigenic determinants specific for myeloma-IgND could be detected in low concentrations in all the sera investigated. The distribution of this protein (subsequently called IgND) and the immunoglobulins A, D, G and M has been investigated in normal sera subjected to zone electrophoresis, gel filtration and DEAE-chromatography. From the results presented we conclude that myeloma-IgND belongs to a previously unrecognized class of human immunoglobulins.

MATERIALS AND METHODS

Venous blood was collected from sixty-two apparently healthy donors, and sera kept frozen at −20° until subjected to analysis.

Protein solutions were concentrated by ultrafiltration in collodion bags or Visking tubing (8/32) as previously described (Johansson and Bennich, 1967). Antisera specific to immunoglobulins A, D, G, M, myeloma-IgND and Fc fragment of myeloma-IgND (myeloma-FcND) were prepared as previously described (Johansson and Bennich, 1967).

Immunoelectrophoresis, single radial immunodiffusion (SRD), (Mancini *et al.*, 1965) and double diffusion according to Ouchterlony were performed as previously described (Johansson and Bennich, 1967).

A radio-immunosorbent assay (RISA) (Wide and Porath, 1966) was modified for the determination of IgND. Myeloma-IgND (Preparation B, Johansson and Bennich, 1967) was iodinated with ^{125}I utilizing chloramine T to oxidize iodide to iodine and sodium-metabisulphite to reduce unlabelled iodine (Hunter and Greenwood, 1962). The [^{125}I] IgND was purified by gel filtration. Myeloma-IgND (25 μg) labelled with 2 mc of ^{125}I, gave a specific activity of about 100 μc/μg. Antibodies specific to myeloma-FcND were

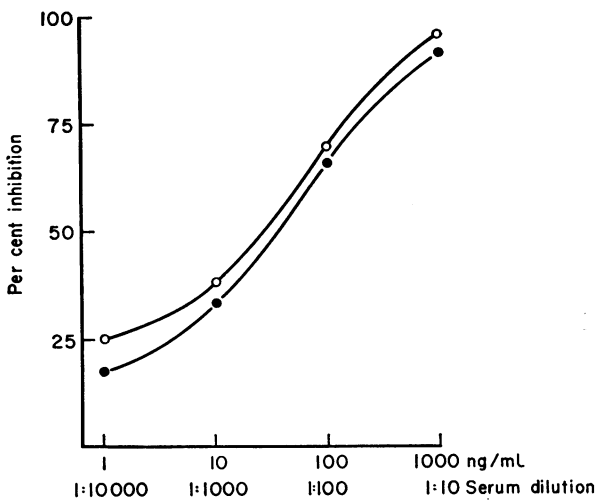

FIG. 1. Dose–response curves illustrating the inhibition of the binding of myeloma-IgND-^{125}I to the immunosorbent by unlabelled myeloma-IgND (●) and normal serum (○). Each point represents the mean of two determinations.

covalently coupled to an insoluble dextran derivative activated by thiophosgene (Axén and Porath, 1966). In order to make the RISA specific 0·05–0·1 mg immunosorbent was suspended in 1 ml of a buffered solution containing normal pooled serum (diluted 1:250), IgG (11 μg/ml), Bence Jones protein ND (8 μg/ml) and Fab fragment of myeloma-IgND (myeloma-FabND) (0·1 μg/ml). The concentration of IgND in a sample was measured by comparing its capacity to inhibit the binding of [^{125}I]antigen (myeloma-IgND) with the inhibition produced by standard myeloma-IgND solutions of known concentration. By this method concentrations of 0·005–0·01 μg IgND/ml could be measured.

Starch block electrophoresis was performed at pH 8·6 as described (Kunkel, 1954).

Gel filtration on Sephadex G-200 (Pharmacia, Uppsala) was performed in Tris–HCl buffers at pH 7·7 or 8·0 (for details see Figures).

Ion exchange chromatography was performed on DEAE-Sephadex (Pharmacia,

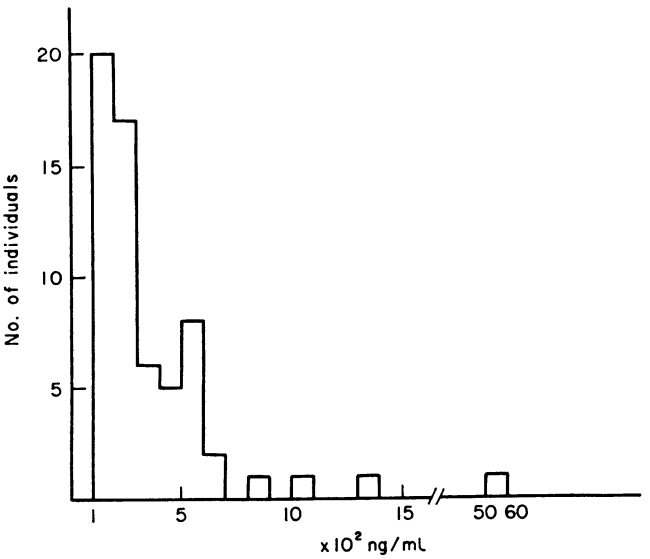

FIG. 2. Distribution of IgND concentrations in sera from sixty-two apparently healthy blood donors.

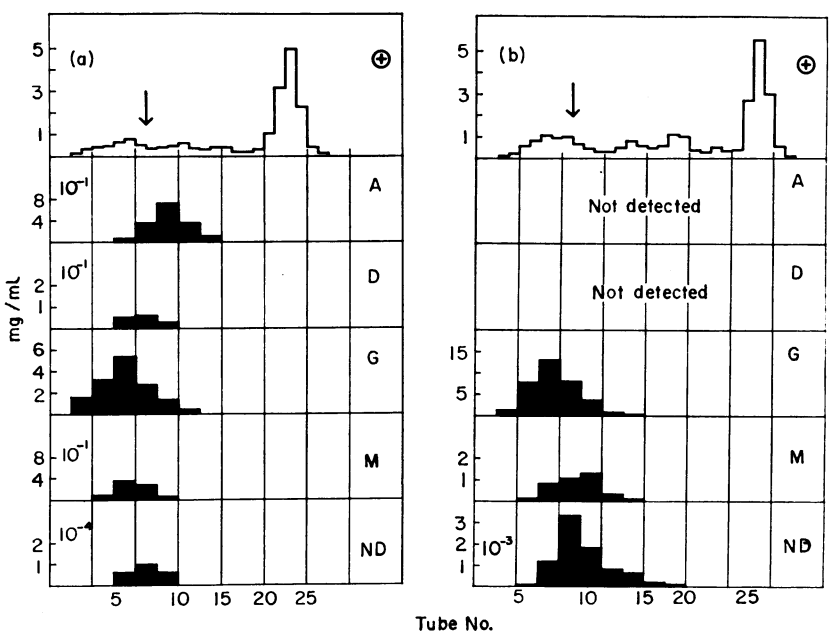

FIG. 3. Electrophoretic distribution of immunoglobulins A, D, G, M and ND in two normal sera of which one (sample G.S.—b) was lacking IgA and IgD. Starch block electrophoresis (50 × 30 × 1 cm), barbital buffer (I = 0·1) pH 8·6, 350–400 V, 100–110 mA, 20–24 hours, 4°; 1·25 cm cuts were eluted for protein determination by a modified Folin method (Eggstein and Kreutz, 1955) and subsequently pooled and concentrated ten times by ultrafiltration. The immunoglobulins A, D, G and M were determined by SRD and IgND by RISA (see 'Materials and methods'). The concentrations of the immunoglobulins in the normal serum (a) were: 1·1 mg/ml A, 0·05 mg/ml D, 10·5 mg/ml G, 0·49 mg/ml M and 510 ng/ml ND and in sample G.S.: <0·01 mg/ml A, <0·01 mg/ml D, 12·8 mg/ml G, 1·20 mg/ml M and 210 ng/ml ND.

Uppsala) A-50 in 0·1 M Tris–HCl, pH 8·0, at 20° using an increasing Tris concentration at a constant pH, obtained with a Varigrad (Technicon).

RESULTS

A sample of normal serum analysed by RISA gave a dose–response curve similar to that obtained with myeloma-IgND (Fig. 1). By appropriate dilution of the serum sample the two curves will overlap.

The concentrations of IgND and immunoglobulins A, D, G and M were determined

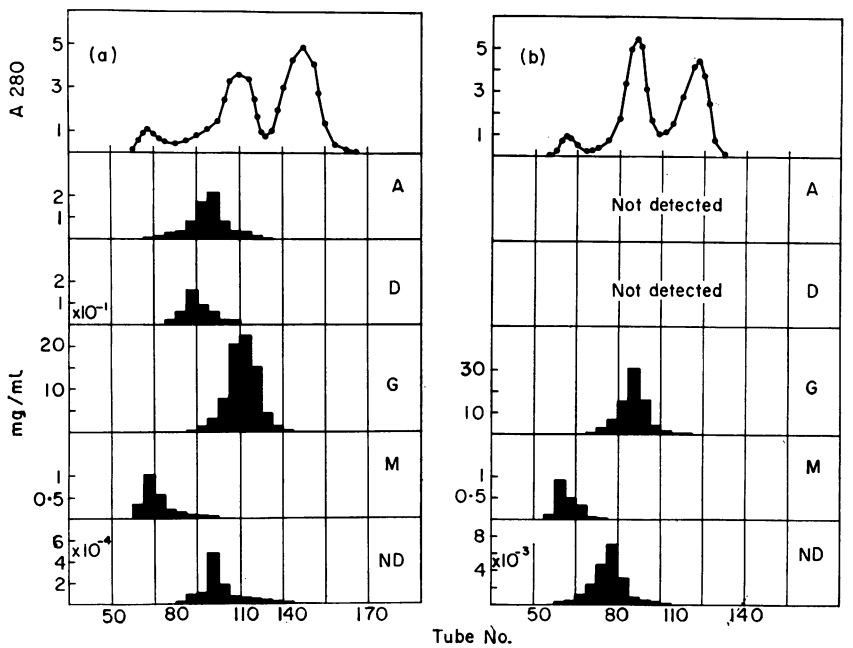

FIG. 4. Distribution on gel filtration of immunoglobulins A, D, G, M and ND in two normal sera of which one (sample L.J.—b) was lacking IgA and IgD. Gel filtration on Sephadex G-200 (91·4 x 7·2 cm) equilibrated with 0·1 M Tris–HCl–0·5 M NaCl, pH 8. Fifteen-millilitre fractions were collected for determination of the absorbancy at 280 nm and subsequently pooled and concentrated ten times by ultrafiltration. Immunoglobulins A, D, G and M were determined by SRD and IgND by RISA (see 'Materials and methods'). The concentrations of the immunoglobulins in the normal serum (a) is given in legend to Fig. 3. and in sample L.J. the concentrations of the immunoglobulins were <0·01 mg/ml A, <0·01 mg/ml D, 30·2 mg/ml G, 1·10 mg/ml M and 400 ng/ml ND.

in sera from sixty-two apparently healthy donors. The distribution of IgND concentrations in the samples is shown in Fig. 2. The concentration range was 110–5900 ng/ml. The average concentration of IgND was 430 ng/ml; fifty-eight of sixty-two samples were within the range 100–700 ng/ml.

Elevated concentrations of IgND (840, 1100, 1400 and 5900 ng/ml) were found in four sera. Clinical re-examination revealed that the donor (sample UA) with the highest concentration of IgND presented symptoms consistent with a state of asthma of extrinsic type (a detailed report will be published elsewhere). No allergic symptoms were evident in the other three donors with high IgND concentrations.

In four sera IgD could not be detected by SRD (0·01 mg/ml or less) and in one serum IgA also was not detected. The levels of IgND in these four cases were similar to the levels found in all samples studied.

One serum with normal levels of the immunoglobulins A, D, G, M and ND and two sera apparently lacking immunoglobulins A and D and with normal levels of G, M and ND were subjected to zone electrophoresis, gel filtration and DEAE-chromatography. The distribution of IgND (RISA) and of the immunoglobulins A, D, G and M (SRD) were determined in the fractions as described in Figs. 3–5. Fig. 3 shows that IgND migrates in the β–γ-region on electrophoresis at pH 8·6. On gel filtration (Fig. 4) IgND elutes similarly to IgA, earlier than IgG and later than IgM and IgD. By ion exchange

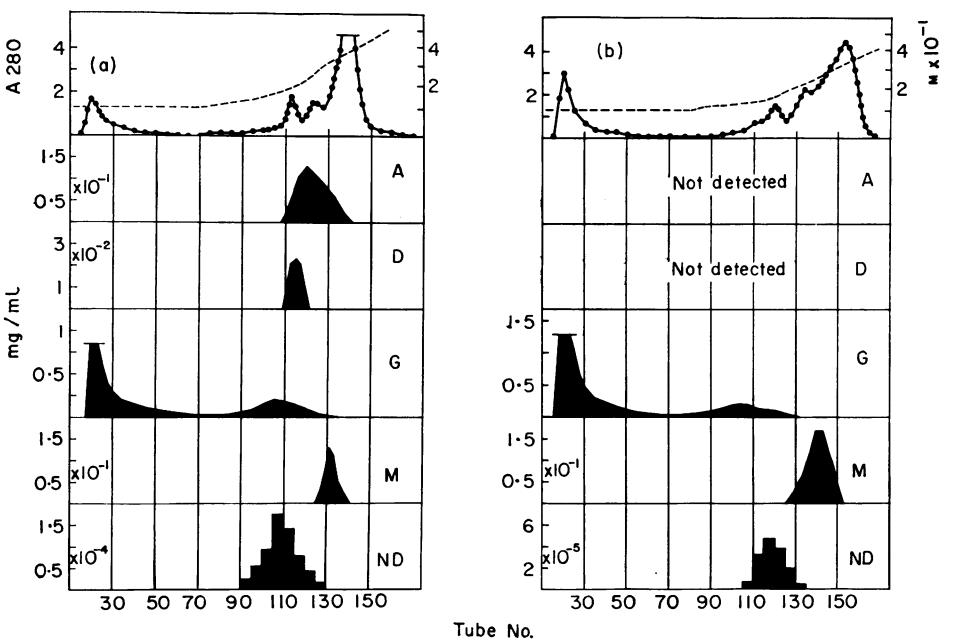

FIG. 5. Distribution of immunoglobulins A, D, G, M and ND in two normal sera of which one (sample G.S.—b) was lacking IgA and IgD. Chromatography on DEAE-Sephadex A-50 (3·2 × 29 cm) equilibrated with 0·1 M Tris–HCl, pH 8·0. An increasing concentration of Tris–HCl, pH 8·0 (- - - -) was used for elution. 10 ml of serum dialysed against the starting buffer was applied and 4·3-ml fractions were collected for determination of the absorbancy at 280 nm and the immunoglobulins A, D, G and M by SRD. For determination of IgND by RISA the fractions were pooled and concentrated ten times by ultrafiltration. The immunoglobulin concentrations in the sera are given in the legend to Fig. 3.

chromatography (Fig. 5) IgND could be separated from the immunoglobulins A, D, G and M. The behaviour of IgND on gel filtration and DEAE-chromatography was similar to that of myeloma-IgND.

The ND-determinants of IgND in normal serum were compared with those of myeloma-IgND and myeloma-FcND using IgND isolated from serum UA by gel filtration on Sephadex G-200 (Fig. 6). The pooled fractions were concentrated about fifty times and analysed by double diffusion using an antiserum specific for myeloma-IgND determinants. Fig. 7(a) shows that a reaction of identity was obtained between IgND and myeloma-IgND and between IgND and myeloma-FcND. Fig. 7(b) shows that the antiserum used was specific for IgND.

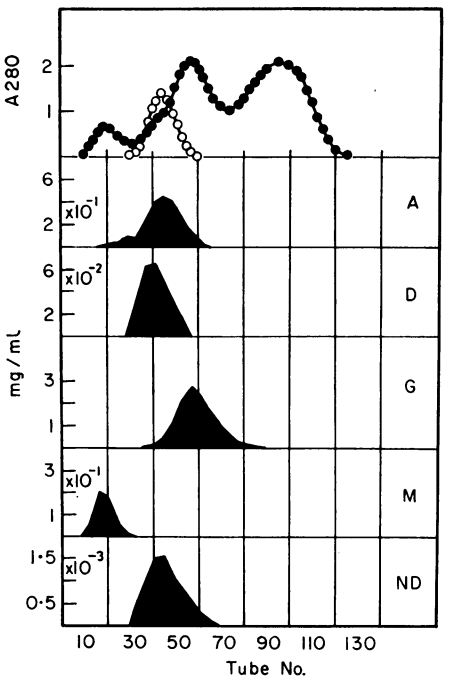

FIG. 6. Distribution of immunoglobulins A, D, G, M and ND in a serum (sample UA) with elevated IgND concentration following gel filtration on Sephadex G-200 (3·2 × 93·5 cm) equilibrated with 0·1 M Tris–HCl–0·2 M NaCl–0·002 M $EDTANa_2$, pH 7·7. For determination of the absorbancy at 280 nm 3·6-ml fractions were collected. The immunoglobulins A, D, G and M were determined by SRD and ND by RISA. The immunoglobulin concentration in serum UA was 2·2 mg/ml A, 0·03 mg/ml D, 12·9 mg/ml G, 0·86 mg/ml M and 5900 ng/ml ND. The elution pattern of purified myeloma-IgND run on the same column is indicated (○). The corresponding fractions were pooled and concentrated about fifty times by ultrafiltration before double diffusion analysis (see Fig. 7a).

DISCUSSION

It is now generally agreed that M-components found in human serum reflect pathologically increased concentrations of normal serum immunoglobulin. Consequently, every M-component may be 'typed' as belonging to one or other of the known classes of immunoglobulins. In those cases in which class specific determinants cannot be revealed, the M-component might represent a pathological immunoglobulin, in which class specific determinants are either absent or hidden. Alternatively the untypable M-component might represent a previously unrecognized class of immunoglobulin, e.g. the recently described IgD (Rowe and Fahey, 1965).

A previous report (Johansson and Bennich, 1967) described an M-component having a molecular weight of 196,000 ($S^2_{20,w}$7·9) and a total carbohydrate content of 10·7 per cent, which could not be classified as immunoglobulin A, D, G or M. From a study of 300 normal sera by the SRD method, it was concluded that a normal counterpart to the myeloma component was not usually present in a concentration higher than 0·01 mg/ml which corresponds to the lower limit for detection by the method used.

The radio-immunosorbent assay (RISA) (Wide and Porath, 1966) used in the present study allowed the detection of immunoglobulins at concentrations of 0·01 μg/ml, or about

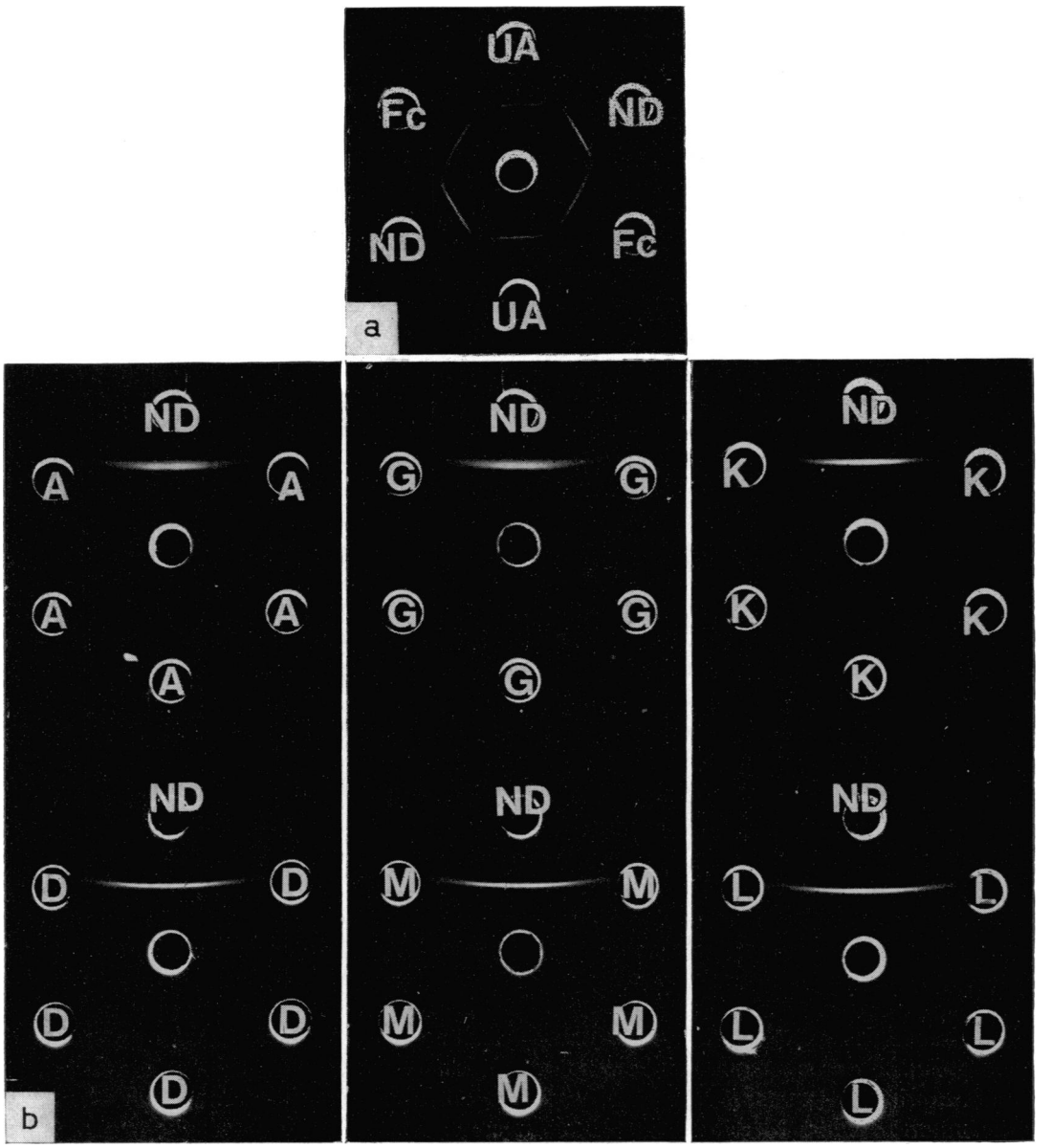

FIG. 7 (a) Double diffusion analysis of the IgND containing fraction isolated from serum UA (see Fig. 6), (UA); myeloma-IgND, 0·5 mg/ml (ND); myeloma-FcND, 0·3 mg/ml (Fc); using an antiserum to myeloma-IgND (centre well) absorbed per millilitre with 0·25 ml normal serum, 5 mg Bence Jones protein ND and 0·3 mg myeloma-FabND. (b) Double diffusion analysis of myeloma-IgND 0·5 mg/ml: (ND), immunoglobulins A, G, M and a serum rich in IgD; light chains of type K and L. All samples except myeloma-IgND were tested clockwise in two-fold dilutions from 0·5 to 0·03 mg/ml. Centre wells: Antiserum to myeloma-IgND absorbed per millilitre with 0·25 ml normal serum, 5 mg Bence Jones protein ND and 0·3 mg myeloma-FabND.

1/1000 of that detected by the SRD method. All sera investigated inhibited the reaction between the labelled myeloma-IgND and the specific immunosorbent. The inhibiting protein exhibited a dose–response curve which followed the same slope as that obtained with myeloma-IgND. It was, therefore, concluded that the protein detectable in normal sera carries specific ND-determinants.

The concentration range of IgND was 100–6000 ng/ml and in 93·5 per cent of samples the range was 100–700 ng/ml. Different values for the IgND level might be obtained, however, when purified IgND is available as a standard preparation. In four cases where IgA or IgD or both could not be determined by the SRD method a normal level of IgND was found.

From the results of zone electrophoresis, gel filtration and DEAE chromatography of normal serum, it was evident that a protein carrying ND-determinant is different from the immunoglobulins A, D, G and M and that its physico-chemical behaviour is very similar to that of the myeloma-IgND. IgND isolated from a normal serum by gel filtration showed a reaction of identity with the myeloma-IgND in double diffusion analysis.

From the present study it is concluded that the atypical myeloma immunoglobulin (myeloma-IgND) previously described (Johansson and Bennich, 1967) represents a new class of human immunoglobulins. The significance of this immunoglobulin present in such small concentrations is difficult to evaluate at present. It is however interesting that one of the donors with a serum IgND level about fifteen times the average showed clinical signs of extrinsic asthma. Studies in progress show that elevated levels of IgND are found in about 30 per cent of patients with asthma (Johansson, to be published).

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