

Clinical and Immunological Aspects of a Case of Monoclonal Hyper-IgE

Isolation of IgE-Protein, Estimation of Basophil Cell Bound IgE and Histamine Release

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A case of monoclonal IgE type lambda with IgE levels about 1 mg/ml has been followed for 6 years. Except for a slight asthma no signs of malignancies, parasitic infestations or other known diseases compatible with hyper-IgE have been found. By the combination of fractional ammonium sulphate precipitation, gel filtration, chromatofocusing, and subtraction affinity chromatography the IgE protein was isolated in an immunochemically pure and homogeneous form. Immunofluorescence of bone marrow cells showed about 1% IgE plasma cells. The amount of basophil bound IgE was 42 ng/10⁶ cells, and histamine release from basophils challenged with anti-IgE was not different from that in atopic control persons, indicating a within-allergic-patients normal amount of IgE receptors. The protein-A reactivity was 0.4% equivalent to well-known IgE myeloma proteins. No antigen specificity of the IgE protein was found. Only a few cases of asymptomatic hyper-IgE are known, and it cannot be ruled out that this represents a premyleoma condition, since a similar case terminated in a malignant lymphoma.

Key words: immunoglobulin E; multiple myeloma; histamine release; basophil cell bound IgE.

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A new class of immunoglobulins (IgE) was initially described by Ishizaka et al. (15) who later demonstrated that IgE was identical with reagenic antibodies involved in skin sensitizing activity in allergic diseases. Johansson & Benich (16) independently described a myeloma protein (ND) that was antigenically identical to IgE.

About a dozen new IgE myeloma have been described since 1967 (25). Most patients present with classical features of multiple myeloma. Immunological studies have shown that most cases have an M-component of kappa type. Only very few cases of benign hyper-IgE

have been described (4, 8, 14, 21, 22). An unusual case of presumably benign IgE M-component of lambda type is presented.

CASE REPORT

The patient (M.K.) is a 74-year-old woman without atopic manifestations until 56 years old. At that time the patient was admitted to hospital because of progressive cough and dyspnoea. Sedimentation rate was 50 mm/h, the white blood cell count normal, but blood smear showed 33% eosinophils. A complete allergological evaluation showed no sign of atopic

disease. The lung symptoms disappeared spontaneously.

1978 (68 years old) admitted to hospital after 3 months of progressive asthma. Because of the severity of bronchial symptoms treatment with prednisone (30 mg daily) was initiated. On admission the sedimentation rate was 14, the haemoglobin level 7.6 mmol/l (7.0–9.4), the white blood cell count 5.6 mia/l (3.0–8.5) with normal blood smear. Physical examination revealed no abnormal findings.

Routine allergological examination showed negative skin prick test to ordinary pollen, animal dander, house dust mite, and moulds. The total IgE level was routinely estimated 15,000 U/ml (Phadebas IgE, Pharmacia Diagnostics, Uppsala, Sweden). Studies were performed to search for known diseases which might explain the elevated IgE. Results of the following laboratory examinations were within normal limits: erythrocyte sedimentation rate, haemoglobin, total leucocyte count, thrombocytes, bilirubin, liver parameters, calcium. ANA, smooth muscle antibodies, rheumatoid factors, anti-mitochondrial antibodies. IgG, IgA and IgD were within normal limits. X-ray examination of the entire skeleton showed no sign of osteolytic or osteosclerotic lesions. The bone marrow smear taken a few days after admission revealed no invasion of plasma cells. No proteinuria and a negative test for Bence Jones protein was found. There was no sign of other malignancies. A thorough clinical and laboratory investigation was performed to exclude known diseases compatible with hyper-IgE, especially nematode infestations and Buckley's syndrome (9).

The patient has been followed during the last 6 years and no sign of multiple myeloma has appeared. The patient still has a normal blood

smear, only 1% of plasma cells in the bone marrow, no Bence-Jones proteinuria, normal haemoglobin and sedimentation rate. Scintigraphy of the entire skeleton revealed no osteolytic bone lesions. Determinations of IgE and serum electrophoresis are evident from Table 1. The only medical treatment is beta₂-agonist aerosol for the asthma.

MATERIAL AND METHODS

Isolation of IgE protein

The IgE protein was isolated by fractional ammonium sulphate precipitation (18) followed by gel filtration, chromatofocusing (23), and subtraction affinity chromatography (2).

Fractional ammonium sulphate precipitation

Forty ml serum from the patient were mixed with 40 ml 66% (500 g/l) saturated ammonium sulphate (SAS) in 0.1 M phosphate buffer, 0.15 M NaCl, pH 7.4. The mixture was kept at room temperature for 2 h and then centrifuged at 6,000 *g* at 4°C for 30 min. The supernatant was made 50% saturated with ammonium sulphate by addition of 0.105 g/ml solid salt. After 2 h, the mixture was centrifuged as described above and the precipitate was dissolved in 10 ml 0.1 M Tris-HCl, 0.2 M NaCl, 20 mg/l chlorhexidine acetate, pH 7.7.

Gel filtration

The redissolved precipitate was applied to a calibrated 2.5 × 98 mm Sephacryl S-300 (Pharmacia) column. (Calibration was performed with myoglobin (MW 16.8 kdal), ovalbumin (MW 44 kdal), IgG (MW 150 kdal), and thyroglobulin (MW 670 kdal) as markers. The correlation coefficient for the linear regression of low MW on the elution volume was -0.999). Elution was performed at room temperature with 0.1 M Tris-HCl, 0.2 M NaCl, 20 mg/l chlorhexidine acetate, pH 7.7. The flow rate was 0.25 ml/min, and 5 ml fractions were collected after the first 180 ml had passed. The fractions were examined for IgE content and

Table 1
Serum IgE values

		Normal values
1978	0.36 mg/ml	(2–250 × 10 ⁻⁶ mg/ml)
1978	0.86 mg/ml	
1979	1.08 mg/ml	
1982	1.02 mg/ml	
1983	1.21 mg/ml	

for presence of other serum proteins by fused rocket immunoelectrophoresis (12) against monospecific rabbit anti-human IgE (Behringwerke, Marburg, W. Germany) (5 μ l antiserum/cm² gel) and rabbit anti-human serum (Dakopatts, Copenhagen, Denmark) (12.5 μ l/cm² gel), respectively. The fractions corresponding to middle of the IgE peak were pooled and concentrated by precipitation with 60% SAS.

Chromatofocusing

A 1.5 $\times$ 50 cm Polybuffer exchanger PBE 94 (Pharmacia) column was equilibrated with 0.025 M imidazol-HCl, pH 7.4. The precipitated IgE was dissolved in a small amount of this buffer and freed of ammonium sulphate on a PD-10 column (Pharmacia), equilibrated with the same buffer. The redissolved IgE was applied to the column. Elution was performed at room temperature with Poly buffer PB 74 (Pharmacia), diluted 1:8 with distilled water and adjusted to pH 4.0 with 1 N HCl. The flow rate was 1.0 ml/min, and 10 ml fractions were collected after the first 160 ml had passed. The fractions were examined for IgE and other serum proteins by fused rocket immunoelectrophoresis as described above. The IgE-containing fractions were pooled and precipitated by 60% SAS.

Subtraction affinity chromatography

To 6 g CNBr-activated Sepharose 4B (Pharmacia) were coupled 110 mg rabbit IgG anti-human serum as described by the manufacturer. The anti-human serum immunosorbent was packed in a 1 $\times$ 20 cm column. The lambda-chain reactivity of the immobilized anti-human serum was blocked by perfusion with a lambda-chain Bence-Jones protein. The column was extensively washed with 0.1 M borate buffer, 0.5 M NaCl, 20 mg/l chlorhexidine acetate, pH 8.0 (BB). The lambda-chains were then covalently coupled to the immunosorbent by perfusion with 0.015 M dimethyl suberimidate dihydrochloride (Sigma, St. Louis, Mo., U.S.A.) in 0.01 M borate buffer, pH 9.2, as described in (19).

The precipitated IgE was redissolved in a small amount of BB and applied to anti-human serum column. Elution was performed at room temperature with BB. The flow rate was 0.25 ml/min, and fractions of 5 ml were collected. The elution was monitored by measurement of the absorbance at 280 nm. The protein-containing fraction was concentrated to 1.6 ml by ultrafiltration, using an immersible CM 30 ultrafiltration unit (Millipore, Bedford, U.S.A.).

Characterization of the isolated IgE protein

The immunochemical purity of the isolated IgE protein was assessed by crossed immunoelectrophoresis with intermediate gel (3) as described in Results. The homogeneity of the protein was examined by agarose electrophoresis (10). Furthermore, the isolated IgE protein was labelled with ¹²⁵I and the labelled protein gel filtered as described in (11). The concentration of the isolated IgE was calculated by measurement of the absorbance at 280 nm, using $E_{1\%}^{1\text{cm}}$ for IgE = 14.1 (5). The IgE concentration in the isolated product was measured by the PRIST-Kit (Pharmacia). This assay was also used for determination of the IgE concentration in the patient's serum.

Immunofluorescence technique

The bone marrow plasma cells were investigated by a direct immunofluorescence technique (13). Rabbit anti-human IgE specific for epsilon chain (Behringwerke, W. Germany) and polyspecific antisera (Dakopatt, Denmark) were used. Isolated IgG fractions from the antisera were labelled with fluorescein isothiocyanate. The IgE antiserum showed monospecific reaction in crossed immunoelectrophoresis. The specificity was tested on monoclonal bone marrow plasma cells with IgA, IgM, IgG, IgD, and IgE multiple myeloma and macroglobulinemia.

Histamine release and estimation of surface IgE

Histamine release and surface IgE were estimated as previously described (24). Briefly,

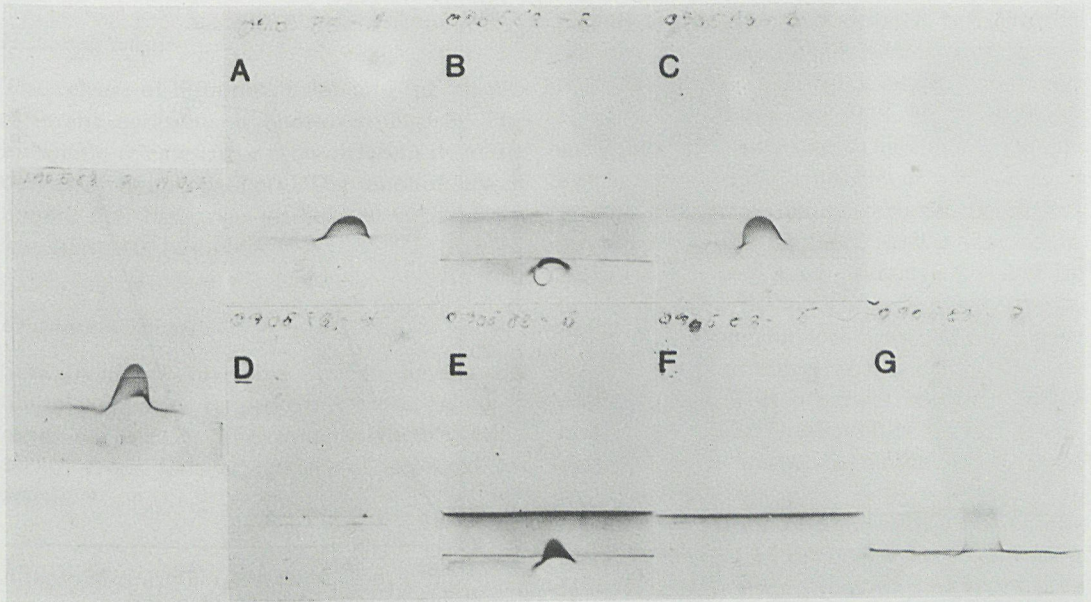


Fig. 1. Crossed immunoelectrophoresis (with intermediate gel) of the isolated IgE protein. Plate: 5×7 cm. Reference gels: A–C: $5 \mu\text{l}/\text{cm}^2$ of rabbit anti-human IgE; D–G: $12.5 \mu\text{l}/\text{cm}^2$ of rabbit anti-human serum. Intermediate gels: A: empty; B + E: $15 \mu\text{l}/\text{cm}^2$ of rabbit anti-human lambda-chains (Dakopatts, code No. A 194); C + F: $15 \mu\text{l}/\text{cm}^2$ of rabbit anti-human kappa-chains (Dakopatts, code No. A 192); G: $15 \mu\text{l}/\text{cm}^2$ rabbit anti-human IgE. 1. dimension: $5 \mu\text{l}$ purified IgE; $10 \text{ V} \times \text{cm}^{-1}$. 2. dimension: $2 \text{ V} \times \text{cm}^{-1}$. Insertion at left: Crossed immunoelectrophoresis of the patient's serum against rabbit anti-human IgE (empty intermediate gel).

mononuclear cells were isolated on a Ficoll-Hypaque density gradient and incubated with 10-fold dilutions of anti-IgE (Behringwerke) and the release determined.

Basophil cell bound surface IgE was eluted from 40 ml venous blood, anticoagulated with EDTA. Cells were isolated on Ficoll-Hypaque and elution performed with 10 ml Tris buffer adjusted to pH 3.8 with HCl for 5 min (4°C). The supernatant was readjusted to pH 7.6 with 5N NaOH and concentrated to 500 μl on a Minicon-B 15 (24).

Surface IgE was measured in polystyrene tubes (Nunc, Denmark). Anti-IgE (Behringwerke) was adsorbed to the tubes and the supernatant incubated overnight at room temperature. After washing bound IgE was measured by ^{125}I -labelled anti-IgE (Pharmacia). IgE from Pharmacia PRIST-kit was used as standard and the result in U/ml was multiplied by 2.4 to convert to ng IgE (5).

RESULTS

Serum IgE concentration

In the dilutions 1:10,000 and 1:100,000 the IgE concentration in the patient's serum from 1982 was 425,000 and 400,000 U/ml, respectively, when measured by the PRIST assay. When the IgE concentration was measured by the PACIA technique (20), or by rocket immunoelectrophoresis against rabbit anti-human IgE (Behringwerke), almost identical concentrations were found.

Purification of the IgE protein

After the chromatofocusing step no contaminating serum proteins could be demonstrated by fused rocket immunoelectrophoresis against rabbit anti-human serum (results not shown). Neither could contaminating proteins be seen

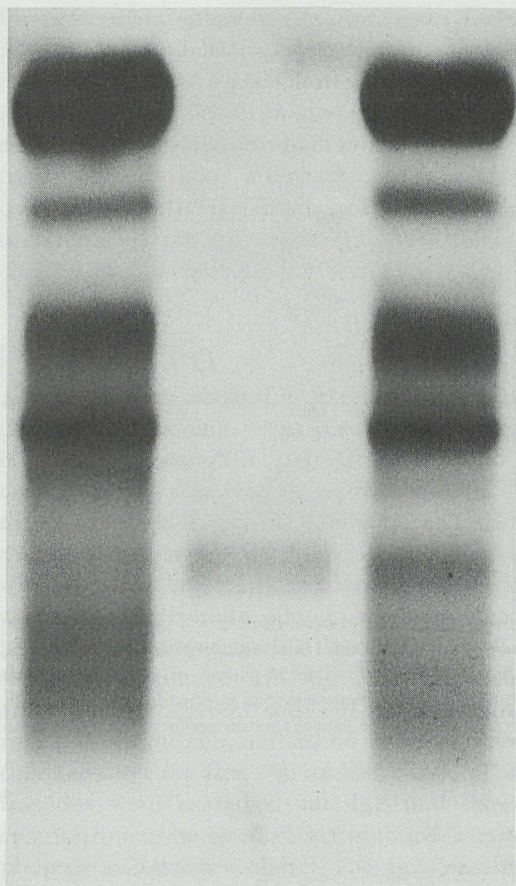


Fig. 2. Agarose electrophoresis of normal human serum (left), purified IgE (centre), and patient's serum (right).

after the subtraction affinity chromatography by crossed immunoelectrophoresis (Fig. 1) against rabbit anti-human serum. The crossed immunoelectrophoresis with intermediate gel furthermore demonstrated that the isolated protein was IgE (lambda)-protein (Fig. 1). As shown in (11) the ^{125}I -labelled protein eluted as a single symmetrical peak by Sephacryl S-300 gel filtration. The molecular weight of the ^{125}I -labelled protein was estimated from the elution volume obtained by gel filtration in non-dissociating buffer on a calibrated column as described in (11). The molecular weight was found to be 230 kdal. Agarose gel electrophoresis of the isolated protein (Fig. 2) showed

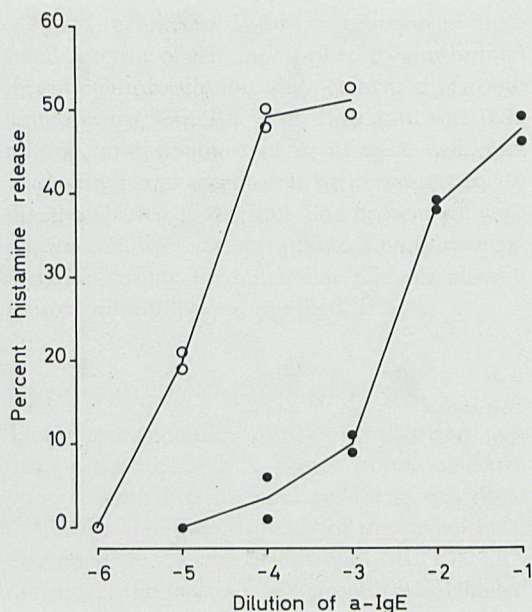


Fig. 3. Histamine release in percentage of the total content as a function of a-IgE concentration (Behringwerke, stock solution 400,000 U/ml). Histamine release was performed in 1979 (●) and in 1982 (○).

only one band. The IgE only reacted with antiserum to IgE and to lambda-chains in immunoelectrophoresis (results not shown). Assuming that the isolated protein was pure IgE, the protein concentration was calculated from the absorbance at 280 nm, using $E_{1\text{cm}}^{1\%} = 14.1$. The IgE concentration was found to be 1.2 mg/ml. By the PRIST assay the IgE concentration in the isolated product was 565,000 U/ml. Thus, 1 U IgE was equivalent to 2.1 ng IgE.

Immunofluorescence

Approximately 1% of bone marrow cells stained specifically with anti-IgE (13). All the IgE-producing cells were light chains of lambda type. One third of the IgE plasma cells had Russel bodies. In Ficoll-Hypaque isolated cell suspension about 1% of the cells showed a very intense fluorescence with anti-IgE. After elution of IgE a few cells (< 0.1%) still showed a very faint staining.

Histamine release

The release of histamine using anti-IgE on two different occasions is shown in Fig. 3. The histamine release curve is no different from the curves of atopic patients. The amount of cell bound IgE based on acid-eluted supernatant was 42 ng/10⁶ basophils.

Protein-A reactivity

Sera from IgE myeloma patients have a low binding capacity to protein-A from *Staphylococcus aureus* (17). The protein reactivity of a serum from 1979 was 0.4% of expected reactivity.*

Antigen specificity of IgE-protein

Based on skin prick test and histamine release with common pollen, animal dander, house dust, and moulds the IgE-protein did not show antigen specificity. Specific IgE in serum diluted 1+4 estimated by RAST (Phadebas®, Pharmacia) showed RAST class 4 to all tested allergens.

DISCUSSION

Neither the clinical history nor the haematological tests give evidence of a multiple myeloma (7, 25) as the cause of the M-component in this patient which must be considered as benign. Only very few cases of hyper-IgE not associated with myeloma have been published, and in most cases the M-component has been found in malignancies (4, 8). Furthermore, no signs of Buckley's syndrome (9) nor parasite infestations were found.

A case of hyperimmunoglobulinemia E and eosinophilia has been presented (21). Contrary to our patient the IgE concentration was about the half and polyclonal. At the presenting of lung symptoms in 1966 a massive eosinophilia was found and it cannot be ruled out that the

patient at that time had elevated IgE. On the other hand, no eosinophilia was observed at the time of elevated serum-IgE levels. Since the first IgE determinations in 1978 serum-IgE has shown a slow increase. The first routinely estimated total IgE is probably too low, due to insufficient dilution of the serum. The clinical implication of the high IgE level is very scant. Except for a very mild asthma controlled by beta₂-inhalation the patient is in good health. Probably the antigenic specificity of the IgE is of no clinical significance from an allergological point of view. This is supported by SPT and histamine results. The RAST results are probably caused by unspecific binding due to the high IgE level (26).

By a combination of four well-known separation techniques we succeeded in isolating the IgE-production in an immunochemically pure and homogeneous form. Only light chain-type lambda was found. The finding that 1 U IgE is equivalent to 2.1 ng is in close agreement with the results presented by Bazara & Hamburger (5), indicating that the isolated IgE-protein is not significantly contaminated by other serum proteins. The estimate of the molecular weight of the isolated IgE protein (230 kdal) is higher than previously reported values (188–196 kdal) for another monoclonal IgE protein (IgE(ND)) (6). The latter values were determined by ultracentrifugation techniques, whereas we estimated the molecular weight by gel filtration under non-dissociating conditions. As IgE is a glycoprotein (6), the estimate obtained by gel filtration of this protein is probably too high (1). The gel filtration behaviour of the ¹²⁵I-labelled IgE molecule did not differ significantly from that of unlabelled, polyclonal IgE (11). The immunofluorescence results indicate a within normal number of IgE-plasma cells, and both the histamine release investigation and the amount of basophil bound IgE is not different from that in control allergic persons. The protein-A reactivity of this monoclonal IgE is similar to the reactivity demonstrated by other IgE myeloma proteins (17).

The present case is similar to a patient briefly commented on by Johansson & Inganäs (17). This patient had benign monoclonal IgE type

* Protein-A reactivity kindly performed by Prof. S. G. O. Johansson, Uppsala, Sweden.

lambda in concentrations from 0.5–1 mg/ml. After about 8 years the disease progressed to a malignant lymphoma and the IgE value increased 5- to 10-fold (S. G. O. Johansson, personal communication). The possibility that the presented case represents a pre-myeloma state has not been ruled out. Furthermore, serum IgE levels in the IgE myeloma range might be compatible with very few clinical manifestations.

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