

Plasma Separation in Patients with Bronchial Asthma, Atopic Dermatitis and Hyperimmunoglobulinaemia E

H. NIELSEN,¹ B. THOMSEN,¹ R. DJURUP,¹ I. SØNDERGAARD,¹ E. KAPPELGAARD,¹
A. BERKOWICZ,² H. KJERSEM,² J. I. DAHLAGER,³ W. SZPIRT,³ S. ULLMAN,⁴
N. H. VALERIUS⁵ and B. WEEKE¹

¹Laboratory of Medical Allergology and Medical Immunology, Dept. TA, ²The Bloodbank,

³Medical Department of Nephrology (P), ⁴Dept. of Dermatology (H), and ⁵Dept. of Clinical Microbiology, Rigshospital, Copenhagen, Denmark

The effect of intensive plasma separation performed eight times within 5 weeks in four patients with atopic dermatitis, bronchial asthma and hyperimmunoglobulinaemia E was followed as regards clinical symptoms and changes in the concentrations of serum (S) IgE, S IgG, S IgA, S IgM, plasma complement C3 split products, S transferrin, blood eosinophils, chemotaxis of neutrophil cells and histamine metabolites in urine in samples obtained consecutively during the period of observation. The occurrence of circulating immune complexes (IC) was analysed by a polyclonal rheumatoid factor (pRF) agglutination inhibition assay and an IgE IC specific technique. IgE IC were demonstrated in three of the patients prior to plasma separation, complexed IgE was 2-3% of the total concentration of S IgE. In one patient complexes were detected by the pRF agglutination inhibition assay, also. In the three patients with IgE IC, the complexes disappeared during treatment, but recurred in two of the patients shortly after the last plasma separation. Shortly after eight separations the S IgE was reduced in all patients to a mean level of 46% of the pre-exchange concentrations. During the following 3 weeks the relative increase of S IgE in three of the patients was similar to the values obtained for S IgG. Serum IgG was subnormal in all patients during the period of treatment. Increasing numbers of eosinophils were observed in three of the patients after the fifth separation procedure. The histamine metabolite 1,4-methylimidazoleacetic (1,4-MIAA) in urine was increased in all patients, but no significant changes were observed during the treatment. The increase of complement C3c split product in two of the patients during the separation procedure indicated that complement activation took place. Questionable clinical improvement of dermatitis and asthma was observed during the period of plasma separation, and the effect was short lasting. For the time being plasma separation in patients with combined hyperimmunoglobulinaemia E, asthma and atopic dermatitis cannot be recommended as a tool in the management of the disease.

Key words: atopic dermatitis; bronchial asthma; hyperimmunoglobulinaemia E; plasma separation.

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The occurrence of increased S IgE concentration has been described in a variety of diseases including atopic disorders (12), Hodgkin's disease (1), paraproteinaemia (18), and in patients with recurrent staphylococcal infection and hyperimmunoglobulinaemia E syndrome (23).

Hyperimmunoglobulinaemia E syndrome is associated with pronounced blood and sputum eosinophilia, suppressor T-cell deficiency and impaired chemotaxis (2, 15). In the majority of patients with very high concentrations of S IgE the clinical hallmarks are atopic dermatitis,

asthma and positive immediate weal-and-flare skin reactions to different antigens (5, 10). In some tropical populations with hyperimmunoglobulinaemia E, however, a low prevalence of atopic disease has been observed. This indicated that several factors besides the S IgE levels might be of importance for the clinical manifestations (13).

In patients with atopic dermatitis different groups have demonstrated the occurrence of circulating immune complexes (5, 21). In one of the groups food antigens were demonstrated as possible constituents of the complexes (21). The reduction of S IgE or immune complexes by a plasma separation has been reported in one case with increased S IgE and asthma (8) and in one case with hyperimmunoglobulinemia E syndrome (11). In both cases clinical improvement was observed. In the present study the effect of intensive consecutive plasma separations in four patients with increased concentration of S IgE and atopic phenomena was followed by monitoring of clinical symptoms, including possible side effects secondary to plasma separations, and changes in serum and urine components, especially S IgE and IgE IC.

MATERIAL AND METHODS

Patients

Four patients, mean age 30 years (range 25–42 years) with elevated S IgE, asthma and atopic dermatitis without any signs of staphylococcal infections underwent plasma separation. All patients showed positive skin prick hypersensitivity to horse and dog antigens. Furthermore, three of the patients (except patient M.M.) showed skin hypersensitivity to *Phleum pratense*, *Artemisia*, cats, and *Dermatophagoides pteronyssinus*. None of these allergens seemed to have decisive importance for the course during plasma separation. All patients were on treatment with oral β_2 -stimulators, steroids in spray and slow-release theophylline tablets. Various dermal treatment had been tried, but was of short benefit.

In all patients clinical status regarding changes in asthma, dermatitis and general con-

dition, as well as changes in medications, were followed. By use of a score system, changes in these parameters were followed for each patient during the period of observation. The score indices were calculated weekly for the patients as indicated in Figs. 2 and 3. The clinical state of the patients at the starting point was indicated by 100%, each parameter (asthma, dermatitis, general condition and medication) were equally weighed in the score system. Before plasma separation all the patients had eosinophilia corresponding to a mean value of $690 \times 10^6/l$ (range 330 – $1420 \times 10^6/l$), normally below $350 \times 10^6/l$. An SIgE mean value of 8.9 (range 5.7 – 14) $\times 10^3$ U/ml was observed at the same time, the range of controls was $(0$ – $0.3) \times 10^3$ U/ml.

Sampling

Blood and urine samples were obtained before, during and 20 h after each plasma separation. Serum was collected and after centrifugation, stored at -20°C until analysis. For analysis of complement split products EDTA-plasma (10 mmol/l EDTA) was stored at -80°C until assayed. The urine samples were acidified by addition of 6 ml 6 N HCl to the collection bottles and kept frozen until analysis.

Plasma separation procedure

PlasmafloTM from Asahi Medical, Japan, was used as plasma separating filter. Vascular access was obtained by a femoral catheter introduced *ad modum* Seldinger and for return flow a canula in a peripheral vein was used. The volume of discarded plasma was replaced by an adequate amount of 3% albumin in Ringer's lactate and dosed automatically by a Gambro[®] Hæmofiltration monitor. Anticoagulation was obtained by 5000 iu heparin initially, followed by continuous infusion of 1000 iu/h. A blood flow of 100–150 ml/min gave a filtration rate of 30–50 ml/min, thus, the whole procedure was completed in approximately 90 min. The test design used included eight plasma separations in each patient during a period of 5 weeks. During the first week three filtrations were

done, the following week two filtrations and the last 3 weeks one filtration per week. On each occasion the volume of exchanged plasma was 2–3 l, amounting to a total of approx. 20 l/plasma per patient.

Measurement of histamine metabolites

The amount of the histamine metabolite (1,4-MIAA) was analysed by a high performance liquid chromatographic procedure (27). Reference interval 0.72–2.34 mg/24 h of 1,4-MIAA.

Measurement of chemotaxis

Chemotaxis of polymorphonuclear leukocytes (PMN) was determined using an invertible Boyden chamber as previously described by Valerius (28). The chemotactic factors used were casein dissolved in Gey's solution at 1 mg/ml and 5 mg/ml, and a culture filtrate of *E. coli*, diluted 1:3 in Gey's solution. All experiments were performed in triplicate and the median value used as the result.

Gel filtration

Approximately 1.5 ml of serum was heated to 37°C for 45 min to dissolve any cryoglobulins (24). Particulate material was removed by filtration through a Millipore filter with pore size 0.8 μ m. The filtered serum (approx. 1 ml) was then applied to a calibrated 2.5 $\times$ 98 cm Sephacryl S-300 (Pharmacia) column. Elution was performed with 0.1 M Tris-HCl, 0.2 M NaCl, pH 7.7, with a flow rate of 15 ml/h, and 5-ml fractions were collected. The IgE concentration in the fractions was determined with an automated particle-counting immunoassay for S IgE as described in detail elsewhere (19). Dilutions of an IgE reference serum (Behring Institute, Marburg/Lahn, West Germany) were used as standards.

Measurement of complement C3c/d

Quantitative determination of C3c and C3d were performed by means of rocket immunoelectrophoresis as described elsewhere (3, 4).

Measurement of circulating immune complexes

IgE-containing immune complexes were measured by precipitation with 3.5% (w/v) PEG 6,000 followed by quantitation of IgE in the redissolved precipitates. Based on *in vitro* studies of PEG precipitation of radiolabelled monomeric human IgE, significant precipitation of IgE did not occur until concentrations of 4–5% PEG were reached. We therefore selected 3.5% PEG for further studies (7). Circulating immune complexes were also measured by a polyclonal rheumatoid factor agglutination inhibition assay (pRF) described by Kappelgaard et al. (14).

Measurement of serum proteins

The concentration of S IgE was measured by Enzygnost IgE (Behringwerke). S IgA, IgG, IgM and transferrin were measured by use of an immunoelectrophoresis technique (17).

RESULTS

With the PEG precipitation assay, IgE IC were detected in three of four patients before treatment. By gel filtration of the sera (7) it was estimated that 2–3% of total serum IgE existed as IgE IC (Fig. 1). After the last plasma separation, the IgE IC had disappeared in all three patients. In two of the patients, the complexes reappeared a few weeks after the final separation. By use of a pRF agglutination inhibition assay IC containing IgG and/or IgA were demonstrated in one of the patients (B.B.). These IC disappeared after the second filtration procedure.

Within the first 5 weeks the response to treatment concerning the changes in the concentrations of S IgE were similar in all patients. Data obtained in patient A.A. (Fig. 2) represent the general response to treatment. S IgE, as measured 20 h after the final filtration, decreased to a mean value of 46% (range 32–69%) of the pre-exchange concentrations in all four patients (Table 1). In three of the patients the mean value of S IgE was raised to 80% of the pre-exchange level 3 weeks after the final separation procedure. In the last patient

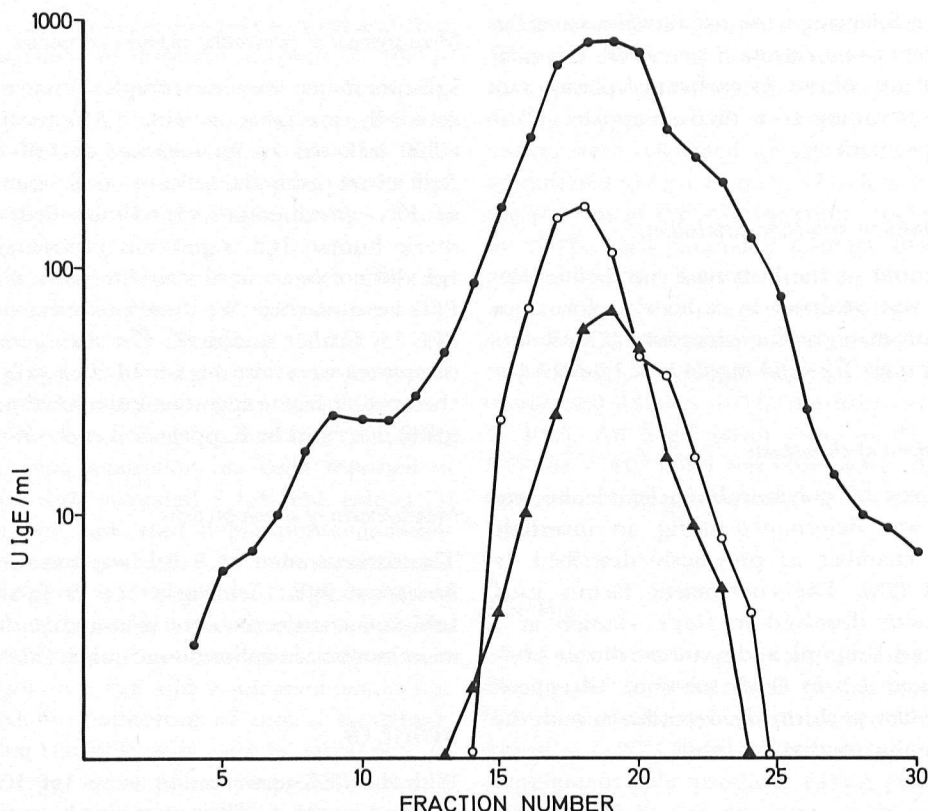


Fig. 1. Sephacryl S300 elution profile of monomeric 125 I-IgE ▲—▲ as compared with the IgE profile from patient AA, before ●—● and after plasma separation ○—○.

(M.M.) the change in S IgE was different: a rebound phenomenon was observed less than 3 weeks after the final separation procedure. As indicated in Fig. 3, the concentration of S IgE exceeded the pre-exchange value by approximately 70%. A spontaneous fall in the S IgE was observed about 4 months after the final plasma separation procedure in this patient. The total amount of S IgE removed during the therapy observed in patient A.A. was approximately 96,000 K units. The amount of IgE retained in each filter was calculated to approximately 3–4% of the IgE amount removed. The pretreatment level of blood eosinophils was increased in all patients. In two of the patients, however, a further increase was observed after the fifth plasma separation, most pronounced in patient M.M. (Fig. 4). A rebound phenomenon of IgE was observed in

this same patient approx. 1 month later, as indicated in Fig. 3. The urine, 1,4-MIAA concentration was measured consecutively in each patient. Increased values were found in all, the pre-exchange mean value was 2.7 mg/day and the post-exchange mean value after eight separations was 3.0 mg/day. However, no systematic changes were found in the patients during the period of observation. Finally, all patients had a normal chemotactic neutrophil response *in vitro*, and three consecutive samples showed no changes during treatment.

As shown in Table 1 the relative values of S immunoglobulins G, A, M was decreased during therapy. The most dramatic reduction was observed in S IgG in which the absolute serum concentration was below the range of the controls (29) in all patients already after the first separation procedure. After the fourth plasma

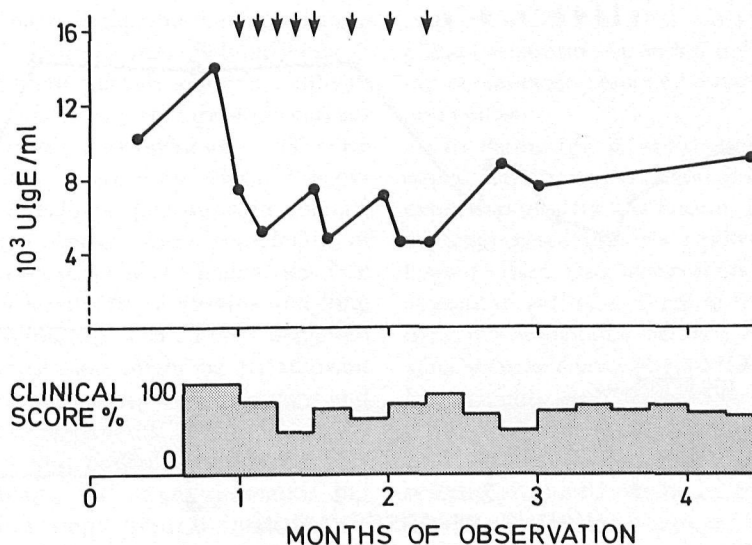


Fig. 2. Changes in serum concentration of IgE during plasma separation (↓) in patient AA compared with time and clinical course. Clinical scores of 100% indicate maximal disease activity in each patient – a decrease in the scores indicates improvement.

separation S IgA was reduced to subnormal levels in two of the patients. In all patients the S IgM and S transferrin were within the reference levels during the therapy. In all cases S IgA, S IgM and S transferrin were similar to the pre-exchange values 3 weeks after the final separation, while S IgG was still below (Table 1). No increase in complement split products (C3d and

C3c) between the plasma separations was observed. During plasma separation complement C3 split products (C3d and C3c) were followed for technical reasons only in two of the patients. These patients showed a significant increase of C3c approx. 30 min after start of the separation procedure and during the rest of the period of treatment (Fig. 5). A similar increase in the concentration of plasma C3d was not observed. The increase in plasma C3c levels in these patients was accompanied by a slight decrease in blood pressure. Three of the patients had attacks of asthma within 1 h of the initiation of plasma separation on one or more occasions. Medical treatment produced immediate symptom improvement in all patients, and no bronchial asthma of delayed type was registered. On two occasions a local skin infection at the site of the femoral catheter was observed and effectively treated with antibiotics.

Clinically, three patients showed modest improvements (Figs. 2 and 3) and one (N.N.) was unchanged during the separations. Only patient A.A. showed improvement in his dermatitis during the observation period. No long-term effect was observed. Patient B.B. had distinct rhinoconjunctivitis and asthma related to high

Table 1

Relative changes in different serum components based on pre-exchange concentrations compared with post-exchange values obtained 20 h and 3 weeks respectively after eight plasma separations

Serum components	Before plasma separation	20 h after final plasma separation	3 weeks after final plasma separation
IgE	(1.0) ¹	(0.46) ¹	(0.80) ²
IgG	1.0	0.32	0.86
IgA	1.0	0.64	1.12
IgM	1.0	0.54	1.05
Transferrin	1.0	0.67	0.95

¹ Relative mean value obtained in four patients.

² Relative mean value obtained in only three patients – results of MM are given in Fig. 3.

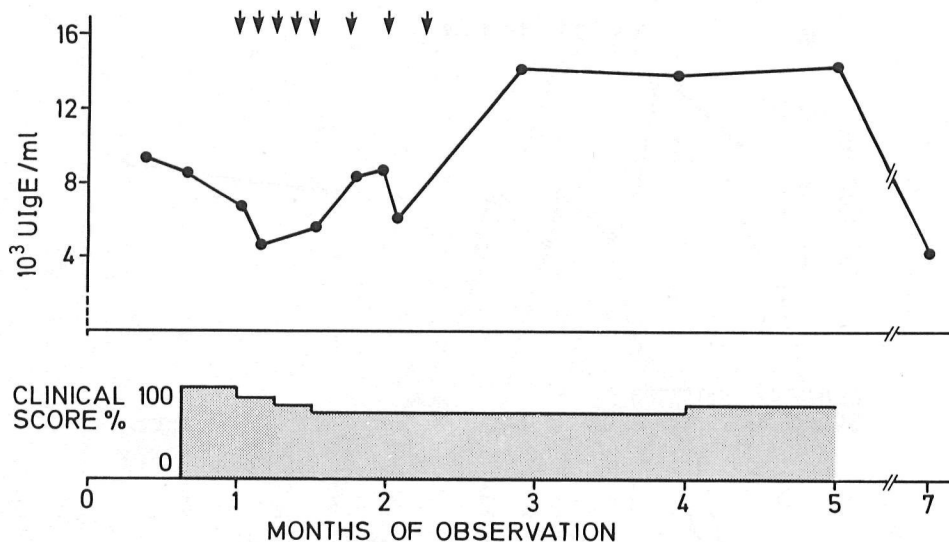


Fig. 3. Changes in serum concentration of IgE during plasma separation (↓) in patient MM compared with time and clinical course. Text as Fig. 2.

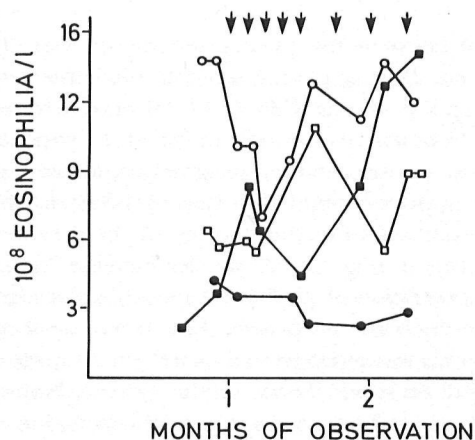


Fig. 4. Changes in blood eosinophils during plasma separation (↓) in patients: AA ○—○, BB ●—●, NN □—□, MM □—□.

grass pollen counts 1 month after the last separation. No exacerbation secondary to increased numbers of blood eosinophils or IgE rebound phenomena was observed.

DISCUSSION

The effect of plasmapheresis in severe asthma has been studied previously in one patient

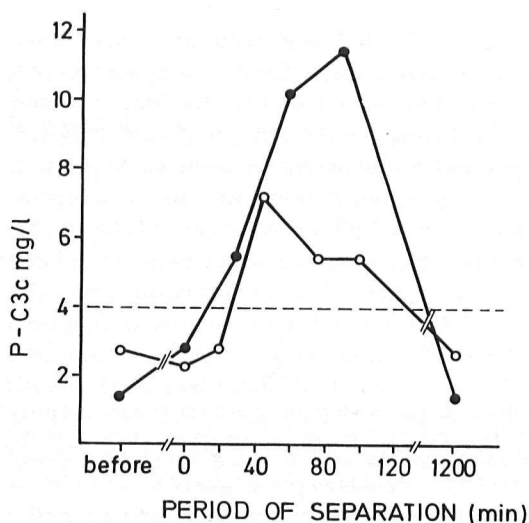


Fig. 5. Changes in the occurrence of plasma C3c split products during plasma separation in two patients. Upper limit for spontaneous activation of C3 is indicated by -----, based on 20 healthy controls.

whose symptoms had failed to respond to drug therapy (8). The authors reported a beneficial effect after removal of approx. 6 l of the patient's plasma over a period of 4 weeks. Clinical evaluation was based on lung function tests.

Recently, plasma exchange has been applied to a patient with hyperimmunoglobulinaemia E syndrome and recurrent non-systemic staphylococcal skin infections, chronic tonsillitis and recurrent otitis media and pneumonia (11). Five plasma separations were done within 3 weeks and the skin infections, pruritus and eczema, improved for 2 weeks. After completion of plasma separation therapy the patient was free from new skin lesions for 2 months and lung infections for 6 months. The authors suggested that plasma separation might be an adjuvant therapy in controlling recurrent infections and skin itching in these patients.

Our study of four patients with hyperimmunoglobulinaemia E and atopic dermatitis and no complicating staphylococcal infections or decreased chemotactic response, demonstrated that the beneficial effect of plasma separation was marginal, and possibly liable to bias, and comparatively time consuming and expensive. In contrast to the other studies, in which exchanges were done by centrifugation technique, side effects were observed during the plasma separations. Asthmatic attacks and slight decreases in blood pressure during the separation procedure were observed on one or several occasions in all patients. Complement activation was observed in two of the patients, and also two skin infections. However, no uncontrollable or hazardous situations arose and side effects disappeared immediately with adequate medical treatment.

The mechanism by which complement activation occurs during plasma separation remains to be determined. Aggregation of immunoglobulins on nylon fibres and generation of plasmin which has C1 cleaving activity could initiate classical pathway activation (22). Complement activation occurs in blood within the extra corporal circuit (26). The occurrence of complement split products and especially increased plasma concentrations of anaphylatoxins could be responsible for the transient episodes of decreased blood pressure, chills and respiratory symptoms observed in our and other patients (6, 9). In general, no late onset symptoms were observed in the four patients. No complement activation based on consecutive

analysis of C3c and C3d was seen between the plasma separation episodes, nor any changes in the chemotactic response during the period of observation.

Circulating IgE IC are thought to be of pathogenic importance in atopic diseases (20). From experimental data it is known that non-specifically aggregated IgE has a potent histamine releasing effect. Our observation of an increased amount of IgE IC in three of four patients with hyperimmunoglobulinaemia E and atopic dermatitis, increased level of 1,4-MIAA in urine of all four patients, and increased blood eosinophilia, could indicate a possible pathogenic role of IgE IC in the manifestation of dermatitis and the severity of asthma. As noted by Brostoff et al. (5) the skin lesions in atopic dermatitis do resemble the delayed hypersensitivity skin reactions characteristic of immune complex lesions (25). Although the amount of IgE IC found in our patients (7) did not correspond to the amount of IgE IC found in patients with atopy by Brostoff et al. (5), we undertook the present study to analyse the effect of intensive plasma separation. As stated, the IgE IC disappeared during treatment. Although a great amount of S IgE was removed from each patient, no decrease of urinary 1,4-MIAA or blood eosinophils was observed. It is important to stress that the serum concentration of IgE is the result of production, release from cell surfaces and from deposits in the tissue, as well as removal from circulation. IgE in monomeric or aggregated form on the mast cell surface is probably the major factor responsible for activation of these cells and hence immediate-type hypersensitivity reactions. Therefore the clinical response is likely to depend on the degree of removal of IgE from mast cell surfaces, which however, cannot be monitored at present.

As indicated in Table 1 the relative changes in the mean value of S IgE were of the same order as S IgG. A prolonged survival of IgE has been observed in patients with markedly increased S IgE, especially multiple myeloma. It is possible that fixation of IgE to the receptors of cell membranes may be involved in the normal metabolism of IgE. In patients with high IgE levels, a limited amount or alter-

ed binding affinity of IgE receptors could result in a prolonged survival of free IgE molecules. The observed relative mean changes in S IgE (Table 1) correspond to the above mentioned theory. The mean value of IgE compared with IgG in three of the patients 3 weeks after the final filtration procedure indicates similarities in the metabolism between these proteins. According to other studies the half-life of IgG in normal individuals varies between 15–21 days while that of IgE is about 3 days (29). Our study does not prove, but indicates a closer similarity between the half-life of IgG and IgE in these patients with hyperimmunoglobulinaemia E. The relative changes between S IgA, S IgM and S transferrin secondary to plasma separation in this study were in agreement with the observation by Keller et al. (16).

The pathological mechanisms involved in atopic dermatitis are unknown. An increased release of histamine from, e.g. mast cells in the dermis might be one mechanism of importance. According to this assumption, plasma separation could be a rational therapeutic tool. After eight separations during a 5-week period there was marginal clinical improvement in itching and degree of atopic dermatitis, but the effect did not last after the separations terminated. Plasma separation is both expensive and cumbersome and as it does not seem to give any lasting effect it cannot, for the time being, be recommended in the treatment of atopic dermatitis and hyperimmunoglobulinaemia E.

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Address:

Dr. Henrik Nielsen

Medical Dept. TA

Rigshospital

Tagensvej 20

DK-2200 Copenhagen N

Denmark

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