Transfer factor therapy in hyperimmunoglobulinaemia E syndrome

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SUMMARY

The therapeutic effect of transfer factor (TF) from healthy donors was investigated in two children with extensive intractable atopic dermatitis, recurrent pyogenic skin infections, hyperimmunoglobulinaemia E, defective neutrophil chemotaxis and depressed cell-mediated immunity. Striking clinical improvement was noted in both patients with disappearance of skin infections, pruritus and eczema. No new lesions have occurred 13 months after the completion of therapy in the first patient but a few new atopic lesions have reappeared after 8 months in the second. Both patients are off steroids and antibiotics. Transfer factor administration did not influence the T cell rosette number or the lymphocyte blastic transformation response, but it did cause conversion of the skin-test reactivity in both patients and correction of polymorphonuclear chemotaxis in one of them. No clinical side-effects were noted but marked and persistent rise of serum IgE was observed in both patients.

Our data suggest that patients with hyper-IgE syndrome may be benefited by TF therapy and they lend further support to the notion that T lymphocyte deficiency may be the basis of the eczema in this syndrome.

INTRODUCTION

Hyperimmunoglobulinaemia E (hyper-IgE), chronic atopic dermatitis, defective neutrophil chemotaxis and recurrent pyogenic skin infections have been recognized as part of a symptom complex, referred to as hyper-IgE syndrome(s). (Buckley, Wray & Belmaker, 1972; Dahl, Green & Quie 1976; Hill & Quie, 1974; Rogge & Hanifin, 1976). A T cell defect has been associated with hyper-IgE syndrome. The presence of defective delayed type cutaneous reactivity in these subjects, as well as reduced numbers of circulating T lymphocytes and subnormal in vitro lymphocyte responses to mitogens and antigens tend to support this association. (Buckley & McGeady, 1974; Gottlieb & Hanifin, 1974; Rachelefsky et al., 1976; Thaler, Klansner & Cohen, 1977.) Transfer factor (TF), a dialysable extract of peripheral blood leucocytes has been shown to transfer to the recipient the specific cell-mediated immune responses of the donor (Lawrence, 1969). Transfer factor has been used with variable success in the therapy of a number of conditions with defective T cell function like Wiskott–Aldrich syndrome and chronic mucocutaneous candidiasis (Spite de et al., 1972; Schulkind et al., 1972).

Two children with high serum IgE levels, chronic intractable atopic dermatitis, defective neutrophil chemotaxis, recurrent pyogenic skin infections and depressed T-cell function were treated with TF. Their clinical and immunological parameters before and after the administration of TF are described in this report.

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CASE REPORTS

Patient No. 1 (D.H.). A 21-month old white girl with extensive atopic dermatitis and recurrent pyogenic skin infections since age 11 months was referred to us for further evaluation. Previous prolonged therapy with local and systemic steroids and antibiotics was unsuccessful in controlling numerous recurrences and hospitalizations. Marked pruritus interfered with sleep. Physical examination revealed positive findings limited to the skin. These included extensive areas of atopic dermatitis on the face, ears, trunk, diaper area and extremities. Evidence of pyogenic infection was present especially over the face and the ears. Administration of antibiotics selected on the basis of culture and sensitivity, while it resulted in clearance of infection, did not appreciably affect the course of atopic dermatitis. Routine laboratory evaluation was unremarkable. Immunological evaluation was performed before and after TF therapy.

Patient No. 2 (R.K.). An 11-year old white boy with whole body atopic dermatitis and pyogen skin infections was referred to us for further evaluation. His disease started from age 6 months and he has never been free of lesions with the exception of partial remissions. Over a period of years, all therapeutic efforts including local and systemic steroids had been of limited benefit. Prolonged courses of appropriate antibiotic therapy was followed by temporary clearance of areas of skin infection without altering the over-all course of eczematoid lesions. At age 15 months, he developed bronchial asthma requiring chronic bronchodilator therapy, systemic steroids and frequent hospitalizations for control. Allergy evaluation revealed multiple allergies. Immunotherapy first started at the age of 5 yr resulted in partial improvement of asthma with no effect on the skin lesions. Physical examination revealed a co-operative youngster with obvious growth retardation. Chronic whole body eczema was found with prominent lichenification, areas of active eczematoid lesions and evidence of localized skin infection. Chest examination revealed a barrel-shaped chest with occasional wheezing. The rest of the examination was unremarkable as were the routine laboratory data. Immunological evaluation was performed before and after TF therapy.

MATERIALS AND METHODS

Immunological evaluation. Complement, serum immunoglobulins and antibody studies. Total haemolytic complement activity (CH₅₀) was determined by the functional haemolytic assay. C₃ complement of component of complement and immunoglobulins (IgA, IgG and IgM) were determined by the radial immunodiffusion technique of Mancini, Carbonara & Heremans (1965). Serum immunoglobulin E (IgE) concentrations were measured by radioimmunosorbent technique. Serum isoagglutinin titers for anti-A and Anti-B were performed by routine blood-bank techniques. Neutralizing antibody to diphtheria toxin was determined by injecting 0.1 ml of active Schick toxin into the volar aspect of the patient’s forearm with diphtheria toxoid being used as the control in the other arm. The test was considered positive if erythema was detected at the injection site at the end of 5 days.

Skin tests. Delayed cutaneous reactivity was assessed by a number of skin test antigens including (a) candida albicans extract (Hollister–Steir Laboratories, Yeadon, Pennsylvania) in dilutions of 1 : 500 and 1 : 100 in sterile saline, (b) streptokinase-streptodornase (SK–SD, Varidase, Lederle Laboratories, Pearl River, New York) used in doses of 10–2.5 units diluted in sterile saline, (c) trichophyton extract (Hollister–Steir) in dilutions of 1 : 500 and 1 : 100 in sterile saline. These reagents were injected intracutaneously into the volar surface of the forearm in volumes of 0.1 ml and the largest diameter of induration measured at 48 hr. Induration of 4 mm or greater was considered a positive response.

T cell rosettes. T cell rosettes were assayed by the method of Wybran et al. (1973) using lymphocytes isolated on a Ficoll–Hypaque gradient from heparinized peripheral blood. The T cell rosettes were performed in the presence of pre-absorbed foetal calf serum. The eighteen squares of a haemocytometer (or at least 500 cells) were counted and the percentage of rosettes was calculated. A rosette was defined as a lymphocyte surrounded by at least three sheep red blood cells (SRBC). The normal values for our laboratory are 55 ± 12%.

In vitro lymphocyte response to phytohaemagglutinin (PHA-I). Lymphocyte reactivity in vitro was studied by using lymphocytes isolated on a Ficoll–Hypaque gradient from heparinized peripheral blood and culturing them in vitro in presence and absence of PHA concentrations ranging from 0.165 µg/ml to 5 µg/ml (MR 68, Burroughs–Wellcome, Research Triangle Park, North Carolina). After 72 hr of incubation at 37 C, the cultures were labelled with tritiated thymidine (New England Nuclear) for 4 hr at 37 C, and then harvested according to the method of Waithe & Hirschhorn (1971). The determination of tritiated thymidine incorporation was done by liquid scintillation spectrometry. The results are expressed as a ratio of counts per minute incorporated by stimulated cells over those of unstimulated control cells (PHA index). The normal value for PHA index in our laboratory is > 25.
Transfer factor in hyper-IgE syndrome

Bactericidal assay. Bactericidal assay was performed against Staphylococcus aureus by the method of Quie et al. (1967). The results were expressed as a percentage of intracellular and extracellular bacteria killed in the presence of fresh-pooled serum as well as patient's serum at 120 min. Normal values include killing of >90% of intra- and extracellular organisms.

Chemotactic assay. The chemotactic capacity of polymorphonuclear (PMN) leucocytes was assessed according to the method of Boyden (1971). Leucocytes were obtained from heparinized peripheral blood by gravity sedimentation. Zymosan (Sigma Chemical Company, St Louis, Missouri) activated serum was employed as the chemoattractant. The test was carried out in modified Boyden Chambers using 3.5 µ millipore filters (Millipore Corporation, Bedford, Massachusetts). Cell migration was assessed after staining and fixing of the filters. The results were expressed as the ratio of numbers of migrating cells in the presence of zymosan-activated serum over those of migrating cells in the presence of plain tissue culture medium 199 (Microbiological Associates, Bethesda, Maryland) and tissue culture medium containing serum. This ratio is referred to as the chemotactic index, with normal values for our laboratory 7–15.

Transfer factor preparation. Dialysable transfer factor (TF) was prepared according to the method of Lawrence & Al-Askari (1971). Heparinized blood (400 ml) was obtained from healthy adult donors with no history of atopic disease or allergy who demonstrated strongly positive delayed skin reactivity to at least one of the employed skin test antigens and were negative for hepatitis B surface antigen by radioimmunoassay. Before use, the lyophilized TF was reconstituted with 2 ml sterile distilled water, sterilized by filtration with 0.45 µ millipore filter and then injected i.m. or subcutaneously immediately. One dose of TF in these studies is equivalent to 1 × 10^9 lymphocytes. Each patient received two courses of TF, each course being defined as one dose per week for 4 consecutive weeks.

RESULTS

Pre-transfer factor immunological findings.

Results of the initial immunological evaluation of the patients are summarized in Tables 1 and 2. As seen in Table 1, both children were found to have normal serum immunoglobulins, CH₅₀ and C₃ component of complement. Serum IgE levels were increased (444 units/ml and 7120 units/ml in patients 1 and 2 respectively). Patient 1 belonging to blood group A demonstrated isoagglutinin titer of anti-B 1:4 and patient 2 belonging to blood group O showed anti-‘A’ 1:16 and anti-B 1:32. Both demonstrated neutralizing capacity for diphtheria toxin as indicated by negative Schick test.

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient 1 (D.H.)</th>
<th>Patient 2 (R.K.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal value</td>
<td>Pre-TF</td>
</tr>
<tr>
<td>Immunoglobulins (mg%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>26–147</td>
<td>57</td>
</tr>
<tr>
<td>IgG</td>
<td>538–1400</td>
<td>1080</td>
</tr>
<tr>
<td>IgM</td>
<td>69–264</td>
<td>133</td>
</tr>
<tr>
<td>IgE units/ml</td>
<td>11–61</td>
<td>444</td>
</tr>
<tr>
<td>C₃ (mg %)</td>
<td>55–120</td>
<td>92</td>
</tr>
<tr>
<td>CH₅₀c units/ml</td>
<td>34–42</td>
<td>35</td>
</tr>
<tr>
<td>Isoagglutinin titres</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-A</td>
<td>&gt;1 : 8</td>
<td></td>
</tr>
<tr>
<td>Anti-B</td>
<td>&gt;1 : 8</td>
<td>1 : 4</td>
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<tr>
<td>Schick test</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>


Cell-mediated immunity (CMI) evaluation revealed absent cutaneous reactivity to all skin test antigens* including diphtheria toxoid, and decreased numbers of T cell rosettes (patient 1 30%, patient 2 28%) in both patients. *In vitro* PHA responses showed a low PHA index for patient 1 and normal for patient 2 (Table 2).

* Dinitrochlorobenzene (DNCB) sensitization was not performed because of the extensive skin disease.
Polymorphonuclear leucocyte function evaluation showed normal PMN phagocytic and bactericidal capacity for both children but abnormal chemotactic response. Chemotactic index for patient 1 was 2.5 and for patient 2 was 1 (Table 2).

### Effect of transfer factor therapy

**Clinical response.** Each patient received two courses of TF, each course consisting of 4-weekly doses of $1 \times 10^8$ lymphocytes per week. In patient 1 relief from pruritus was noted after the second dose of TF resulting in the reduction of local steroid applications. By the end of the first course, the pruritus and associated infections were markedly reduced and progressive healing of eczematoid lesions became apparent. Improvement continued through the second course of TF. The patient has been followed for 13 months and is completely off steroids and antibiotics. No new active lesions of atopic dermatitis have developed within this period of time. She still has nasal symptoms of allergy and occasional hives. No side-effects of TF therapy have been noted. Patient 2 also received two courses of TF. Following the third dose of TF, significant amelioration of pruritus was noted. Subsequently, decreased frequency of skin infections and gradual disappearance of new atopic lesions was observed. Improvement continued through the second course of TF. Systemic steroid and antibiotic therapy was discontinued and there was no exacerbation of symptoms for the first 8 months following the completion of TF therapy. At the end of this period, few new atopic lesions appeared associated with less pruritus than before. He continued to have very dry skin with areas of chronic lichenification on the flexural surfaces and in ante-cubital and popliteal fossae. His school attendance has improved tremendously and no hospitalization has been necessary for the past year. His asthma is under better control than before as evidenced by the absence of hospitalizations, discontinuation of steroids and marked reduction in the use of bronchodilators. No side-effects of TF therapy have been noted.

**Immunological parameters.** Two weeks after the end of second course, the immunological evaluation was repeated. Results in comparison to pre-TF immunological evaluation appear in Tables 1 and 2. No change was observed in serum IgA, IgG, IgM and isoagglutinin titres in any of the children. The C₃ component of complement in patient 2 remained unchanged. However, serum IgE levels dramatically increased post-TF therapy in both children: 6120 units/ml from 444 units/ml in patient 1 and 115,000 units/ml from 7120 units/ml in patient 2. Repeat skin testing showed reactivity to Candida antigen but T cell numbers and in vitro lymphocyte responses to PHA remained unchanged. Polymorpho-
nuclear leucocyte function results are summarized in Table 2. As can be seen, post-TF polymorphonuclear chemotaxis remained abnormal in patient 1 (chemotactic index 2) but was completely corrected in patient 2.

DISCUSSION

Buckley et al., in 1972, first described two adolescent boys with very high serum IgE in association with recurrent cutaneous, pulmonary and joint infections, growth retardation, coarse faces, severe atopic dermatitis and depressed in vivo cell-mediated immunity and antibody formation. Since then, various clinical variants of this hyper-IgE syndrome have been described (Biorksten & Lundmark, 1976; Dahl et al., 1976; Hill & Quie, 1974; Hill et al., 1974; Rogge & Hanifin, 1976; Clark et al., 1973; DeCree et al., 1978). All have in common recurrent pyogenic (mainly staphylococcal) infections, chronic dermatitis indistinguishable from atopic dermatitis, high serum IgE, defective neutrophil chemotaxis and some degree of T cell dysfunction. Our patients resemble the patients reported with the exception that infections were mostly limited to the skin. Since infections were not as prominent a feature in the patients reported here, it is possible that severe atopic dermatitis with associated chemotactic abnormality may be the most appropriate diagnosis (Furukawa & Altman, 1978). Decreased T cell function and increased IgE levels have been associated with atopic disease (Rachelefsky et al., 1976).

During the past few years TF therapy has been used in a variety of clinical entities associated with defective T lymphocyte function (Spitler, Levin & Fundenberg, 1975; Berkel et al., 1977). Most encouraging results of TF treatment have been reported in patients with chronic mucocutaneous candidiasis (Schulkind et al., 1972; Kaffe et al., 1975) and Wiskott–Aldrich syndrome (Spitler et al., 1975). The beneficial effect of TF on the eczema of patients with Wiskott–Aldrich syndrome which is associated with IgE, depressed T cell function and increased incidence of pyogenic infections suggested to us that TF may be of benefit in patients with hyper-IgE syndrome. Both our patients responded favourably to the TF therapy with striking improvement of the skin lesions. This improvement could not be related to any other therapeutic regimen and spontaneous recovery is unlikely in view of the prompt response of a life-long disease, especially in patient 2. Similar results have been reported by Strannegard et al. (1975) and Alonso (1976). Each treated one patient with severe atopic dermatitis, hyper-IgE and evidence of depressed T cell function. These investigators also noted remarkable reduction of pruritus and skin improvement approximately 3–4 weeks after the initiation of TF therapy. Further, Alonso (1976) observed the disappearance of coexistent nasosinusal purulent infections in his patient which is in agreement with our finding of the clearance of skin infections.

In addition to the clinical response, the effect of TF in our patients was monitored by the evaluation of various immunological parameters. We observed that TF had no obvious influence on the level of serum C3 component of complement, IgA, IgG, IgM and isoagglutinin titres. Transfer factor therapy caused no significant increases in T cell rosettes or lymphoid blastogenic responses to PHA. In contrast to our findings, Strannegard et al. (1975) reported normalization of circulating T lymphocytes. Inconsistency in the effect of TF on T-rosette numbers has previously been reported (Kirkpatrick, 1975). Kirkpatrick (1975) suggested that TF effect might be exerted on non-circulatory lymphoid cells. Skin test conversion following TF administration occurred in both patients and this seemed to correlate with the clinical response, although the significance of the conversion of skin reactivity as an immunological parameter after TF is doubtful as reported by Neidhart et al. (1978) in a recent study. These investigators showed that equal numbers of patients receiving placebo vs TF converted following repeated skin testing. Polymorphonuclear leucocyte chemotactic capacity was corrected in one of the patients after TF therapy. This may have been the result of the disappearance of infections since transient chemotactic abnormalities have been reported in association with recurrent infection in certain atopic individuals (Hill et al., 1976). More likely, TF preparation may be responsible for the normalization of PMN chemotaxis since Gallin & Kirkpatrick (1974) have reported the presence of potent chemotactic activity in dialysable TF. The activity was found to be more pronounced with PMN leucocytes than with monocytes.

The pathophysiology underlying the PMN and T cell dysfunction of the hyper-IgE syndrome(s)
and the interaction with TF is not clear. Hill & Quie (1974) suggested that the IgE-mediated histamine release may be responsible for the defective chemotaxis in view of their in vitro finding of inhibition of PMN chemotaxis by histamine. In addition, Rocklin (1976) has shown inhibition of delayed type hypersensitivity (DTH) in the guinea-pig by histamine. One can speculate that abnormal histamine metabolism is responsible for the depressed phagocytic and T cell function in these patients. Histamine is known to increase the intracellular concentration of cyclic AMP (Plaut & Lichenstein, 1978) and substances which increase intracellular cAMP levels depress both phagocytic and T cell function (Bourne et al., 1974). Sandler et al. (1975) demonstrated that when suspensions of peripheral blood leucocytes were exposed to dialysable TF a four- to eleven-fold increase in intracellular cGMP content occurred. This finding suggests that enhancement of immunological responses by TF might involve changes in intracellular cGMP concentration. Transfer factor, therefore, may be able to restore PMN leucocyte and T cell function by antagonizing histamine effect on cyclic nucleotide metabolism. As it was already mentioned, TF preparation has been shown to increase both T cell function and chemotactic capacity of PMN leucocytes. It is of interest that De Cree et al. (1978) reported remarkable clinical improvement of a patient with defective neutrophil chemotaxis, raised serum IgE, recurrent bacterial infections and eczema by administration of levamisole. Levamisole, like TF, has been shown to decrease cAMP and increase cGMP levels in mouse lymphocytes (Hadden et al., 1975) and to maintain higher cGMP levels in human PMN leucocytes (Anderson et al., 1976). The improvement in the patient reported by De Cree et al. (1978) was transient which is similar to the experience we had with one of our patients. Khan et al. (1976a) reported a marked improvement of asthma in patients with asthma and T cell deficiency. In accord with these reports is the observation that our second patient showed a marked improvement of his asthma.

Significant side effects attributable to TF appear to be very uncommon. Polyclonal gammopathy was thought to be associated with chronic use of TF in a patient with combined immunodeficiency (Gelfand et al., 1973). Khan et al. (1976b) reported increasing level of serum IgE following TF therapy in a patient with Wiskott–Aldrich syndrome. We observed a marked increase of serum IgE in both of our patients following TF administration. In patient 2, the IgE level returned to almost a pre-treatment level 8 months after the completion of TF therapy. The aetiology of IgE rise is not clear. Okumura & Tada (1971a; 1971b; 1974) have presented evidence for a thymic-dependent regulatory system controlling the magnitude of the serum IgE response and, therefore, the absence of T cell regulatory activity would result in exaggerated IgE production. Lack of suppressor T cell activity has been implicated in atopic diseases (Takatsu & Ishizaka 1976). It is possible that TF in our patients acted on helper T cells only, thus augmenting IgE production; and that in normal individuals it would act on suppressor as well as helper T cells. An analogous phenomenon has been observed with TF effect on DTH responses (Kirkpatrick, 1975). Normal subjects were readily sensitized by adequate doses of TF from strongly positive donors but the results in patients with immune deficiency were variable. Thus it appears, as has been previously suggested (Kirkpatrick, 1975), that the response to TF is determined by the immunological status of the recipient rather than the informational molecules in the dialysates.

The favourable clinical response of eczema in our patients, following TF therapy, despite the increase in serum IgE, suggests that high IgE levels are not pathogenetically related to atopic dermatitis, whereas T cell function is. That abnormal T lymphocyte function is aetiologically related to the eczema is strongly suggested by the recent finding of Parkman et al. (1978) that the eczema of patients with Wiskott–Aldrich syndrome completely cleared after the establishment of only T lymphocyte grafts.

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