

LYMPHOCYTE TRANSFORMATION, IgE AND T-CELLS IN ECZEMA VACCINATUM TREATED WITH TRANSFER FACTOR

A Case Report

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Abstract. Transfer factor (TF) was given to intensify the cell-mediated immune reactions in an atopic patient with generalized vaccinia. The patient showed marked reactivity of peripheral blood lymphocytes to stimulation with phytohaemagglutinin and pokeweed mitogen, but also in non-stimulated cultures. However, later tests with mitogen stimulation of lymphocytes indicated a defective cellular defence mechanism. The addition of autologous plasma to lymphocyte cultures depressed the reactivity of PHA-stimulation considerably. Initially, the patient also showed a normal T-lymphocyte count in peripheral blood, but six months after her vaccinia, extremely high serum IgE levels and a decreased percentage of T-lymphocytes was observed. Although an evaluation of the clinical effect of transfer factor injection is difficult, it should be noted that the patient's temperature immediately fell to normal, and her general health improved following treatment.

Key words: Lymphocyte transformation; IgE; T-cells; Eczema vaccinatum; Transfer-factor; Atopic dermatitis

Recent investigations have shown that reaginic antibodies are confined to the IgE immunoglobulins (4, 6, 7). An increase of serum IgE has been found in allergic asthma (5), hayfever (1) and lately in atopic dermatitis (9, 13). Similar findings have also been made by us (2). Whilst studying this matter, we registered our highest serum IgE value in a 14-year-old girl, who had been hospitalized for generalized vaccinia 2 months previously. She also had symptoms of atopic dermatitis. The clinical appearance of our patient, when she was hospitalized for vaccinia, gave us a strong indication of a defect in her cell-mediated immune response against vaccinia virus. Furthermore, this conclusion was supported by recent observations made by Gottlieb & Hanifin (3), who found a decrease in the number of T-lymphocytes in peripheral blood in patients who had increased serum IgE levels.

Because of her severe vaccinia it was decided to treat this patient immediately with transfer factor (TF) before further investigations could be done. The present report presents our experience with the case, together with laboratory findings on T-lymphocytes and IgE-levels.

CASE REPORT

Our patient was a 14-year-old girl with a well-known predisposition to atopic dermatitis and asthma. She had always had dry skin and, periodically, dermatitis. She was vaccinated against smallpox at the age of six and developed in connection with this a minor outbreak of vaccinia. In October 1973 the patient's sister was vaccinated against smallpox. On November 3rd the patient developed blisters on her face, neck and upper part of the trunk. We first saw the patient in our department when she was in the vesicular stage of vaccinia. Her face was grossly oedematous and totally covered with yellowish encrustation, which partly closed her nose and mouth (Fig. 1). On her neck and upper part of her trunk there were numerous vesico-papules with typical umbilication. Scattered elements of similar character were observed at her left wrist. The patient had a temperature of 40°C, and her general health was affected. Her lymph nodes were enlarged.

The patient was treated with TF, which had been prepared according to a procedure previously described in detail (11). It was possible, by coincidence, to give her TF prepared from a normal donor who had been vaccinated against smallpox in the spring of 1973. The following day the temperature was normal and her general condition had improved pronouncedly. The patient was discharged after 4 weeks with healed skin dominated by scar formation. When re-examined 2 months later we found her with all signs of a typical atopic dermatitis with strong lichenification of the flexural sides of elbows, knees and wrists.

LABORATORY EXAMINATIONS

In two consecutive tests, vaccinia virus was found by inoculation in fertilized eggs. Before TF was given, venous blood

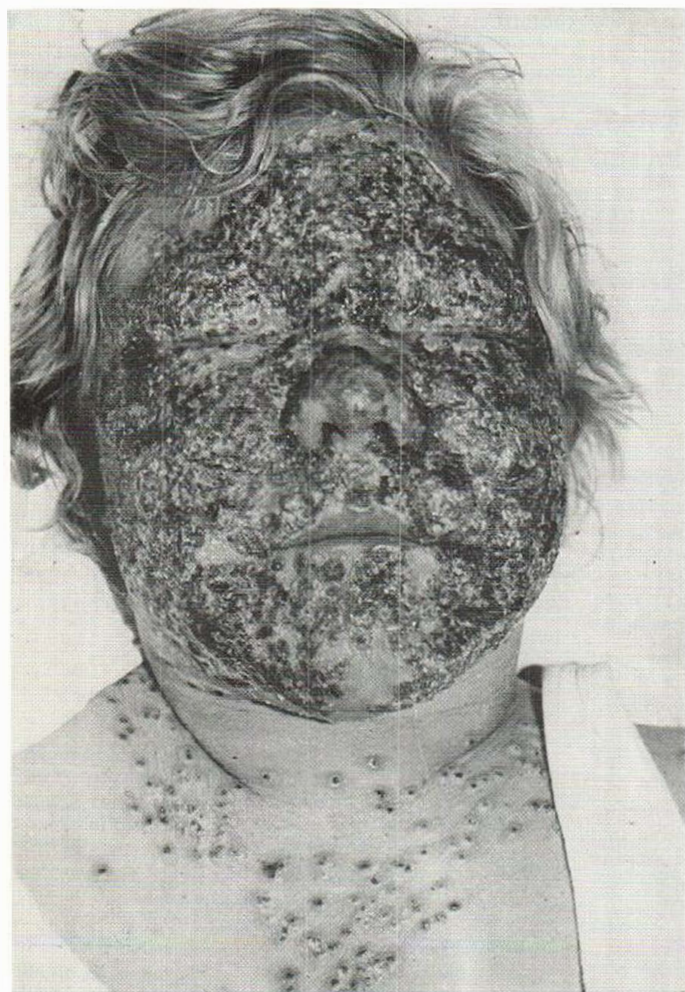


Fig. 1. Face of the patient on admission to hospital. The face was grossly oedematous and covered with encrustations.

was drawn for an investigation of the reactivity of the lymphocytes to stimulation with phytohaemagglutinin (PHA) and pokeweed mitogen (PWM). The technique of the lymphocyte transformation test (LTT) has been described elsewhere (12). The percentage of T-lymphocytes was measured by formation of E-rosettes according to the method of Jondal et al. (8), and the serum IgE level was determined. After TF had been given, blood was drawn at weekly intervals for LTT, and the results are shown in Table I.

The results of lymphocyte stimulation are expressed both as counts per minute (c.p.m.) and as a stimulation index, S.I. = counts per minute of mitogen-stimulated cultures/counts per minute of control cultures.

It is seen that the patient's lymphocytes from Nov. 8th showed a very pronounced synthesis of DNA in the control cultures, significantly higher than the normal mean value for control cultures in our system ($700 \text{ c.p.m.} \pm 300 \text{ c.p.m.}$). At the same time, a high reactivity of her lymphocytes to stimulation with PHA, $1 \mu\text{g/ml}$, was found, namely $152\,600 \text{ c.p.m.}$ (normal mean value = $121\,000 \text{ c.p.m.} \pm 36\,550$, $n = 27$).

During the following 2 weeks a decline was observed in c.p.m., both in cultures stimulated with mitogen, but more pronounced in the control cultures. An increase in S.I. was therefore seen. The low reactivity in the lymphocyte cultures from Nov. 20th is most probably due to the serum used in the culture medium, since low responses are found both in mitogen-stimulated cultures and in control cultures. Three weeks after TF had been given, the patient showed high reactivity of her lymphocytes to stimulation with PHA, $10 \mu\text{g/ml}$. Four weeks after TF the LTT showed depressed responses to PHA, the response to $1 \mu\text{g/ml}$ of PHA being significantly lower than the normal mean value. At that time the lymphocytes of the patient were also cultured in 15% autologous, inactivated plasma instead of 15% pooled AB serum. It is seen that the reactivity of the lymphocytes to stimulation with two concentrations of PHA was reduced by 52% and 90%, respectively, when cultured in autologous plasma.

Before TF treatment the percentage of T-lymphocytes in peripheral blood was found to be 54%, which is within the range of normal values. Six months later the percentage had

Table I. Results of lymphocyte transformation tests before and after transfer factor treatment on Nov. 8th, expressed as counts per minute (c.p.m.) and as a stimulation index, S.I.

S.I. = c.p.m. in mitogen-stimulated cultures/c.p.m. in control cultures; PWM = poke weed mitogen; PHA = phytohaemagglutinin; () = lymphocytes cultured in 15% autologous, inactivated plasma

Date	PWM, 10 μ l/ml		PHA, 1 μ g/ml		PHA, 10 μ g/ml		Control cultures c.p.m. $\pm$ 1 S.D.
	c.p.m.	S.I.	c.p.m.	S.I.	c.p.m.	S.I.	
8 Nov.	47 084	8.9	152 600	28.9	139 852	26.5	5 282 $\pm$ 261
13 Nov.	59 268	90.2	86 895	132.3	91 624	138.9	657 $\pm$ 82
20 Nov.	9 526	89.0	10 145	94.8	17 879	167.1	107 $\pm$ 35
27 Nov.	104 988	145.2	24 218	33.4	92 623	128.1	723 $\pm$ 134
4 Dec.	16 330	22.2	10 146 (4 054)	13.8 (5.5)	45 712 (4 995)	62.2 (6.8)	735 $\pm$ 125

fallen to 37%, which is considered to be subnormal. Serum IgE was found to be 2 880 μ g/ml on Nov. 8th. Two months and 6 months later the values were 23 200 and 16 220 μ g/ml. Haemoglobin was 11.4%. Sedimentation rate 84 mm/hour, leucocytes 7 200/ μ l. Differential count was normal. On Nov. 20th and 27th, immunoelectrophoresis showed normal values of IgG, IgA and IgM.

DISCUSSION

A development of generalized vaccinia in a vaccinated person is considered to depend mainly upon an inadequate cell-mediated immune response. O'Connelly et al. (10) observed that immunoglobulin therapy in a patient with vaccinia failed to achieve any improvement, whereas a transfusion of washed leucocytes induced a convalescence. TF treatment seems to be the best therapy at the moment.

The factors determining increased susceptibility to generalized vaccinia in atopics are unknown. Gottlieb & Hanifin have recently observed reduced numbers of T-lymphocytes in peripheral blood, simultaneously with increased IgE levels (3). This indicates an inverse relationship between T-lymphocytes and IgE, and it might provide an explanation for the defective cellular defence mechanism seen in atopic patients.

Our patient had previously shown a defective immune response to vaccinia virus, when she was 6 years old, but no in vitro investigations of her cell-mediated immune reactions had been made before her admission due to generalized vaccinia. During the acute attack of vaccinia our investigations showed a high reactivity of her lymphocytes to stimulation with PHA, coincident with a very high spontaneous reactivity in the control cultures. This might be due to the heavy demand on her immune system due to vaccinia virus.

The effect of TF treatment as measured with the LTT is difficult to evaluate. Previous experience with TF treatment in a patient with a depressed cell-mediated immune response (11) showed that TF induced an increased reactivity of the patient's lymphocytes to stimulation with PHA. This could be seen in the present patient, if the results are looked at as S.I., but not when expressed in c.p.m. The strong reactivity in the control cultures on Nov. 8th makes it difficult to compare the results from PHA-stimulation seen during the first 2 weeks. Nevertheless, the results from Nov. 27 show a normal response to mitogen-stimulation, followed by a decline to subnormal values on Dec. 4th. The clinical effect of TF treatment is also difficult to evaluate because the patient was in the later stage of her attack of vaccinia, when the treatment was given. However, it is remarkable that the temperature immediately fell to normal following the injection of TF.

The findings concerning IgE levels and T-lymphocyte count support the observations of Gottlieb & Hanifin (3). The initial values showed an IgE level on 2 880 μ g/l and a percentage of T-lymphocytes of 54%. Two months later, serum IgE showed a marked rise to 23 200 μ g/l and 6 months later serum IgE was 16 220 μ g/l. At that time the percentage of T-lymphocytes was found to be 37%. This inverse relationship between IgE and the percentage of T-lymphocytes also seems to be reflected in the function of the T-lymphocytes in that the addition of the patient's own plasma, containing high levels of IgE, reduced the reactivity of her lymphocytes to PHA-stimulation considerably. Further investigations are needed for the elucidation of the connections between IgE, T-lymphocyte population, and T-lymphocyte function.

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