Recovery of neutrophil functions by transfer factor therapy in Wiskott-Aldrich syndrome

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[Summary]

Granulocyte functions were studied in a case of Wiskott-Aldrich syndrome. In the agarose plate, granulocytes of this patient were shown to have moderately decreased responses in chemotaxis induced by N-formyl-methionyl-leucyl-phenylalanine (FMLP) and zymosan-activated human serum (ZAS), whereas they were shown to have a normal capacity of random migration. Phagocytosis and superoxide production by neutrophils were within normal limits. Chemotaxis of neutrophils in this patient was improved by transfer factor therapy. Clinical improvement of patients with Wiskott-Aldrich syndrome treated by transfer factor may be due to recovery of granulocyte functions.

Key words: Neutrophil chemotaxis
Superoxide anion
Transfer factor
Wiskott-Aldrich syndrome

【概 要】

Wiskott-Aldrich 症候群は感染性・湿疹・血小板減少を3主徴とし、伴性劣性の遺伝型式をとる原発性免疫不全症候群である。本症候群の免疫異常としては、血清 IgM の減少、抗体産生能の異常、神経抗原に対するリンパ球の幼若化現象や MIF 産生能の低下があげられる。一部の症例では好中球機能をともに遊走能の低下が報告されている。

著者らは、生後1ヶ月の男児の本症候群児に transfer factor (TF) を投与することにより臨床症状の改善とともに好中球機能の回復がみられたので報告する。

児児は生後3日に発症し、25日目に下痢・湿疹・出血斑で入院した。入院時血小板数 3.5万/mm³、血清 IgM
INTRODUCTION

The Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency disorder consisting of the triad of recurrent infection, eczema, and thrombocytopenia. Transfer factor therapy has been prescribed for management of patients with a high susceptibility to infection in WAS. However, despite subjective and objective clinical improvement, the cellular response of lymphocyte functions remained diminished in this syndrome. This report describes the granulocyte functions in a case of WAS before and after therapy with transfer factor.

CASE REPORT

A one-month-old male infant was admitted to our hospital with eczema and bloody stool. A brother who died of sepsis at 13 months of age had thrombocytopenia, eczema, and recurrent infections. The patient was born following a normal term delivery, and at 3 days of age after birth bloody stool was noted. He developed petechiae, diarrhea, and eczema at 25 days of age.

On admission he had scaly eczema, purpura and furuncles. The liver was palpable 2 cm below the costal margin. There was no enlargement of the spleen and lymph nodes. Red blood cells were 352×10⁶/cu mm, platelets, 3.5×10⁹/cu mm, and leukocytes, 22,500/cu mm. Bone marrow examination showed normal cellularity and numerous megakaryocytes. The stool was bloody and the urinalysis was normal. The serum immunoglobulin was normal for the age except hyper-IgE; IgG 1040 mg/dl, IgA 46 mg/dl, IgM 48 mg/dl, IgE 78 IU/ml. Delayed hypersensitivity skin reactivity to PHA was normal but secondary response to keyhole limpet hemocyanin (KLH) was negative. Lymphocyte blastogenesis measured by adding ³H-thymidine to phytohemagglutinin (PHA) or concanavalin A (ConA) was normal. T cell rosettes and EAC rosettes were normal. He showed negative antibody response to Diphteria Toxoid stimulation. Cervical lymph node biopsy revealed a decrease in the number of small lymphocytes in the T-zone.

He was diagnosed as having WAS in light of the clinical symptoms and laboratory data. The patient was given transfer factor 1 unit per a week subcutaneously from 2 months of age, because clinical improvement was not noticed by antibiotics and blood transfusion for 3 weeks. A total of 10 units of transfer factor were given over a 10-week period and his clinical and immunological status were evaluated.

TRANSFER FACTOR

Transfer factor was prepared by the Hokkaido Red Cross Blood Bank. The leukocytes from healthy adult donors were disrupted by 1 cycles of freezing and thawing, digested with deoxyribonuclease and then dialysed against distilled water. The dialysate was lyophilized and then reconstituted with sterile distilled water so that the extract from 10⁹ cells (one unit) contained two ml. The final products were passed through a 0.22 μ micropore filter and tested for sterility.

METHODS

Granulocyte function tests were performed under the condition of no infection and these tests of the patient and normal controls were studied at the same time. Neutrophil chemotaxis assays were done with the under-agarose method with FMLP and ZAS as chemoattractants. Chemotactic inhibitory activity of serum was studied by Boyden chamber system with the addition of 10% serum to the lower or upper
Table  Effect of transfer factor therapy on granulocyte functions

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. chemotaxis (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMLP $10^{-8}$M</td>
<td>1.3</td>
<td>2.8</td>
<td>(2.3–2.9)</td>
</tr>
<tr>
<td>ZAS</td>
<td>0.8</td>
<td>2.4</td>
<td>(2.3–2.6)</td>
</tr>
<tr>
<td>random migration (mm)</td>
<td>0.2</td>
<td>0.3</td>
<td>(0.2–0.5)</td>
</tr>
<tr>
<td>chemotactic inhibitory activity of serum (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>upper chamber + serum</td>
<td>10.2</td>
<td>1.4</td>
<td>(−10.3–14.5)</td>
</tr>
<tr>
<td>lower chamber + serum</td>
<td>−4.4</td>
<td>3.1</td>
<td>(−8.0–9.4)</td>
</tr>
<tr>
<td>2. phagocytosis (%)</td>
<td>82.5</td>
<td>86.0</td>
<td>(73.0–99.5)</td>
</tr>
<tr>
<td>3. superoxide anion production (nm/min/10$^5$ cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMLP $10^{-7}$M</td>
<td>0.47</td>
<td>0.64</td>
<td>(0.60–0.79)</td>
</tr>
<tr>
<td>cyto. D + ConA</td>
<td>0.71</td>
<td>0.85</td>
<td>(0.69–0.90)</td>
</tr>
</tbody>
</table>

The values of normal range are the data of age-matched healthy controls which were done in our laboratory in advance (N≥30). The values of normal controls simultaneously studied were within normal range.

Figure  Chemotaxis in transfer factor therapy

The values of chemotaxis are shown as the average of triplicate measurements, which were done two or three times. The values of controls studied at the same time were within normal range (N≥3). When the intervals of therapy were prolonged, the chemotaxis decreased moderately. However significant improvement of chemotaxis was noticed compared with before transfer factor therapy.

chamber and chemotaxis of normal neutrophils was determined. As for phagocytosis test, opsonized zymosan particles were added to a suspension of cells and after incubation smears were prepared. Phagocytosis was evaluated by microscopic examination. Superoxide anion ($\mathcal{O}_2^-$) production of neutrophils was
measured by an increase of the absorbance at 550 nm relative to that at 540 nm, which is due to the reduction of ferricytochrome C, with FMLP or with cytochalasin D (Cyto. D) and ConA as stimulants.

RESULTS
Transfer factor therapy resulted in a striking improvement of the clinical condition with less eczema and diarrhea, but with no evident change in the cellular and humoral immunological status.

Granulocyte functions, particularly chemotaxis, were impaired significantly before therapy with transfer factor, as compared to age matched controls (Table). Chemotaxis was decreased with either FMLP or ZAS as chemotactic factor. After therapy normal ranges were noted. There was no chemotactic inhibitory activity in the serum, as examined by the Boyden chamber system with the addition of 10% serum to the cell suspension or chemotactic factor. Phagocytosis of opsonized zymosan particles was normal before and after therapy. O2 production of the granulocytes was decreased moderately with FMLP as stimulants before treatment but was normal with Cyto. D and ConA. O2 generation with FMLP was normalized after therapy.

As the intervals of injection of transfer factor were prolonged for 2 weeks, 3 weeks, and 4 weeks which were continued for 3 months respectively, chemotaxis of the neutrophils decreased moderately (Fig). However, the chemotaxis was significantly improved at even one unit per 4 weeks than before the therapy and there was no evidence of infection.

DISCUSSION
The granulocyte functions in WAS have been only rarely documented. Spitler et al. stated that the nitroblue tetrazolium test and tests for killing of intracellular bacteria were normal while neutrophil adhesiveness was abnormal. Ochs et al. reported that chemotactic response of WAS showed a significant cellular defect and that the serum with a decreased chemotactic activity for normal granulocyte contained a heat stable inhibitor.

In our patient, the neutrophil chemotaxis was impaired before therapy with transfer factor. Although there was no change in the humoral and cellular immunological status, the chemotaxis was improved after the therapy. The recovery of chemotaxis followed definite improvements in the clinical condition, that is, he had less eczema, diarrhea, and furuncles. Prolongation of the interval of transfer factor therapy moderately depressed the neutrophil chemotaxis. These results indicated that the recovery of the granulocyte chemotaxis may be related to the transfer factor therapy.

The mechanism of chemotactic recovery by transfer factor is unknown. Transfer factor may not be directly related to the improvement of chemotaxis, as transfer factor used in this patient showed feeble chemotactic activity and his cells incubated with transfer factor were slightly depressed in chemotaxis. Chemotactic recovery may be due to improvement of the condition as described by Altman et al., associated with a humoral chemotactic inhibitor, possibly representing a lymphocyte-derived chemotactic factor that might deactivate circulating monocyte or neutrophil.

O2 formed by partial reduction of oxygen is an important component of bactericidal capacity. O2 production of the granulocytes stimulated by FMLP was decreased moderately before therapy, but it was normal with Cyto. D and ConA. The activation-system of O2 release with FMLP may differ from that Cyto. D and ConA, because O2 release induced by FMLP showed no lag time for activation and a short duration time while that with Cyto. D and ConA had a lag time and long duration time. The dysfunction of the neutrophils may not be attributed to defects in the activation-system for O2 generation itself, but rather to disturbance of the site of response to chemotactic factor, for example receptor, as FMLP is a chemoattractant. This hypothesis may be an explanation of chemotactic defect with normal random migration.

Measurement of granulocyte functions in patients with Wiskott-Aldrich syndrome may be useful for evaluating the efficiency of transfer factor therapy.

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[REFERENCES]


