Transfer Factor and Repeated Otitis Media

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The effect of transfer factor (TF) was investigated in 12 children with repeated otitis media. These patients were immunologically compared to a control group of 23 age-matched healthy children. Levels of immunoglobulins, total and “active” T-cells, and phagocytic activity of granulocytes and monocytes were evaluated in the 12 children prior to, during, and after TF therapy. Percentages of “active” T cells and absolute numbers of “active” T and total T cells, which were initially low in the patient group, increased significantly after TF therapy to statistically match those of the healthy control group. The percentage of phagocytic monocytes in patients after therapy did not differ from healthy children; however, the percentage of phagocytic granulocytes remained depressed significantly. The levels of IgG, IgA, and IgM were unaffected by the therapy although the IgA and IgM were higher in the patient population throughout the study. After therapy, one-half of the patient population remained asymptomatic for a 1 year period and the others had markedly reduced attack rates.

INTRODUCTION

In the Department of Otolaryngology, Hospital Bulovka, Prague there are a group of patients classified as having “repeated otitis media.” This designation is applied to patients who, though apparently recovered from their initial disease, go on to have at least three more attacks within a 1-year period. It has been shown (1, 2) that some children apparently had both depressed cell-mediated immunity and phagocytic activity of granulocytes and monocytes. Based on repeated immunologic investigations during the steady state of their disease, a group of 12 patients were selected to further investigate this observation. These patients were treated with a transfer factor preparation and the effect of such treatment was followed by clinical and immunological evaluations.

MATERIALS AND METHODS

Study Group

The study group consisted of 12 children (Dept. of Otolaryngology, Hospital Bulovka, Prague) with an average age of 6 years. In all of them, values for “active” T cells, total T cells, and phagocytic activity of peripheral blood leucocytes were repeatedly found to be depressed. The protocol for the transfer factor therapy called

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for an immunological evaluation prior to initiation of therapy, a second evaluation prior to the third injection of transfer factor, and a final evaluation 2 months after finishing the treatment. No significant complications were observed in any patient during therapy. Twenty-three healthy children of the same age served as the control group.

Immunological Investigations

Separation of lymphocytes. One milliliter of peripheral venous blood was collected in sterile glass test tubes containing 100 μl of 5% EDTA solution and diluted 1:3 with cold phosphate-buffered saline (PBS). The peripheral blood lymphocytes (PBL) were isolated on a layer of Ficoll–Vero graftin 35% (Spofa, Prague, CSSR) by centrifugation at 400g for 30 min at room temperature. The layer of PBL was washed two times in cold PBS and one time in medium M 199 (USOL, Prague, CSSR) with 1% polyvinylpyrrolidone 10 (Sigma Chemical Co., St. Louis, Mo.) (3). Final concentration of cells was adjusted to 2 × 10⁸/ml of medium.

Sheep red blood cells (SRBC). SRBC were obtained fresh every 7 days and stored as a 50% suspension in Alsever’s solution at 4°C.

Total T cells. Lymphocytes bearing receptors for SRBC were determined according to Hoffman and Kunkel (4) employing our modification (3), where 100 μl of washed PBL (2 × 10⁵ lymphocytes) was mixed with 100 μl of 0.5% SRBC (SRBC:PBL ratio 50:1) in small plastic test tubes (12 × 75 mm) and immediately centrifuged for 10 min at 100g at room temperature. The test tubes were placed on ice and refrigerated at 4°C for 18 hr. The next day the cell pellets were resuspended by hand by very gently rolling tubes and one drop of each suspension was transferred onto a microscope slide. The number of E rosettes (three or more SRBC surrounding a lymphocyte) were counted employing an Opton Universal microscope at 640×. All tests were performed in duplicate and 400 lymphocytes were counted to determine the percentage of total T cells.

“Active” T cells. The method of Wybran and Fudenberg (5) in our modification (1) was used to determine “active” T cells. Briefly, 2 × 10⁵ PBL were mixed with SRBC at a final ratio 1:8. Cells were immediately centrifuged for 5 min at 200g at room temperature. “Active” T cells were counted immediately after centrifugation employing the same medium and criteria as used for total T cells.

Test for phagocytosis. Fresh samples of complete blood with 5 IU of heparin per ml were employed in a simple micromethod with synthetic hydrophobic particles based on 2-hydroxyethylmethacrylate copolymer (2). Briefly, 100 μl of heparinized fresh blood was added to 50 μl of diluted particles and incubated at 37°C with agitation every 15 min. After 60 min incubation of the suspension, a standard microscope slide smear was prepared for each specimen and stained using the May–Grunwald–Giemsa procedure. At least 200 leucocytes were evaluated and leucocytes with 3 or more engulfed particles were considered phagocytic cells.

Levels of serum IgG, IgA, and IgM. The method of Mancini et al. (6) was used for quantitative analysis of serum immunoglobulins utilizing the radioimmuno diffusion (RID) plates for quantitative analysis of plasma proteins from SEVAC (USOL, Prague, CSSR).
Transfer Factor Treatment

Transfer factor (TF) employed in these studies was prepared from leucocytes of peripheral blood of healthy donors (prepared and supplied by Dr. Pekarek, USOL, Prague, CSSR). Briefly, the leucocytes were isolated from noncoagulating blood by centrifugation. A homogenate of leucocytes was prepared by freezing and thawing ten times. The lysate was dialyzed against distilled water (Aqua Pro injection). Releasing of low-molecular-weight substances was monitored continuously spectrophotometrically. The dialysate obtained was then lyophilized and each injection was solubilized and adjusted with Aqua Pro injection to contain the equivalent of $2 \times 10^8$ leucocytes. Transfer factor was administered to children in four injections according to the following scheme: the first three injections at 1-week intervals, the last injection 1 month later. The transfer factor was applied subcutaneously into the gluteal area. During this treatment no clinical complications were observed.

Statistical Analysis

The student $t$ test was used for statistical analysis.

RESULTS

The following parameters were evaluated in the control group and in patients receiving therapy: the number of leucocytes, the percentage and absolute number of lymphocytes, “active” T cells, total T cells, the percentage of phagocytic granulocytes and monocytes, and the levels of serum IgG, IgA, and IgM. It was established (see Table 1) that the ill children before the treatment had significantly elevated number of leucocytes and lymphocytes ($P < 0.01$). Absolute number and percentages of “active” and total T cells were significantly reduced ($P < 0.01$). In the ill children, a significant defect of phagocytic activity of leucocytes, especially in granulocytes was found ($P < 0.01$). The levels of serum IgA and IgM were slightly elevated in ill children whereas the IgG levels were normal.

During therapy, the evaluation of “active” total T cells performed before the application of the third injection showed that the percentage of “active” T cells and the absolute number of “active” and total T cells had increased significantly. The overall values did not now differ from the values in healthy children. However, the percentage of total T cells, while elevated slightly over the prior-to-therapy status, was still depressed compared to the control group.

During the final investigation, performed 2 months after completion of therapy, it was observed that the number of leucocytes, the percentage and absolute numbers of “active” T cells, and the absolute number of total T cells were further increased to equal or to higher numbers than those of the control group. These final differences, however, were not statistically significant. It was observed that the percentage of total T cells was still depressed.

The percentage of phagocytic monocytes in patients after therapy also did not differ from healthy children, however, percentages of phagocytic granulocytes remained significantly depressed. Of note, the levels of serum IgG, IgA, and IgM were not affected by the therapy.
TABLE 1

Immunological Parameters Before, During, and After Therapy with Transfer Factor

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Healthy children (n = 23)</th>
<th>Otitis media patients (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td>Leucocytes/mm³</td>
<td>5,409 ± 430</td>
<td>7,118 ± 470</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>48 ± 233</td>
<td>48 ± 337</td>
</tr>
<tr>
<td>Lymphocytes/mm³</td>
<td>2,487 ± 173</td>
<td>3,536 ± 415</td>
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<tr>
<td>&quot;Active&quot; T cells (%)</td>
<td>25.9 ± 3.92</td>
<td>6.3 ± 0.95</td>
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<tr>
<td>&quot;Active&quot; T cells/mm³</td>
<td>666 ± 112</td>
<td>220 ± 48</td>
</tr>
<tr>
<td>Total T cells (%)</td>
<td>70.4 ± 1.2</td>
<td>26.3 ± 3.1</td>
</tr>
<tr>
<td>Total T cells/mm³</td>
<td>1,752 ± 113</td>
<td>931 ± 69</td>
</tr>
<tr>
<td>Phagocytes monocytosis (%)</td>
<td>33.8 ± 76.6</td>
<td>20.2 ± 8.0</td>
</tr>
<tr>
<td>Phagocytes granulocytes (%)</td>
<td>31.3 ± 6.4</td>
<td>13.8 ± 3.5</td>
</tr>
<tr>
<td>IgG g/liter</td>
<td>9.70 ± 2.50</td>
<td>10.68 ± 3.09</td>
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<tr>
<td>IgA g/liter</td>
<td>1.99 ± 0.34</td>
<td>7.43 ± 0.87</td>
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<tr>
<td>IgM g/liter</td>
<td>0.93 ± 0.25</td>
<td>1.18 ± 0.28</td>
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* ND: Not done.

Table 2 compares the attack rate incidence in the patient population for a 1-year period prior to transfer factor therapy to a 1-year incidence after. Clearly a marked improvement to total asymptomatic status was achieved for all these patients.

DISCUSSION

Otitis media belongs to a group of frequent diseases in childhood; in spite of the fact that we can use a number of effective antibiotics, it is observed that the otitis media has a tendency to reoccur. The solution to the question of repeated diseases in children is not simple because the disease may have a variety of pathogenic onsets. In recent years, it has often been observed that in the pathogenesis of many diseases any defect of the immune system may play a very important role. Disease with such defects of immunity become a serious problem because any therapy not accompanied by reestablishment of a normal immune system is of limited effectiveness. Since the whole immune system is an extremely complex system of mutually coupled reactions, any imprudent intervention into it may result in a serious derangement of the whole system and therefore in an exacerbation of the original defect.

Many immunomodulators are now in use, most of them having different clinical effects. In our work, transfer factor was chosen, because this natural substance has an ability to transfer cell-mediated immunity, elevate the number of mature
functional T lymphocytes, activate macrophages, and induce production of interferon (7, 8). The substance has been successfully applied in this manner as an immunomodulator in therapy of many diseases (9–14). Even with these documented studies, the use of transfer factor is still experimental. In part this is reflected in the schema of therapy of different authors which differ not only in dosing of the active substance, but also in the manner of application and duration of the therapy. The effectiveness of the treatment of various diseases has been discussed elsewhere (15, 16). Transfer factor, prepared using the original Lawrence method (9), is presumed to be a heterogeneous substance, with a well known ability to stimulate cell-mediated immunity, as well as a series of other biological activities (17). With our patients, children with proven depression of T cells, it was established in all of them that the therapy had a positive effect not only on the population of T lymphocytes, but also on the phagocytic activity of peripheral blood leucocytes. The most substantial changes were observed within a T-lymphocyte subpopulation, the “active” T cells. As early as the second injection of transfer factor, a normalization of percentage, as well as absolute number, of “active” T cells was established. On the other hand, an interesting discrepancy between the percentage and absolute number of total T cells was found. This normalization of absolute numbers was achieved after the second injection, while the percentage, though rising during the treatment was lower 2 months after the end of the treatment than the corresponding percentage in healthy children. Of note, changes in the number of leucocytes and lymphocytes were not observed. Moreover, TF application affected positively the phagocytic activity of monocytes and granulocytes. The percentage of phagocytic monocytes increased significantly and final values were the same as those of healthy children. The percentage elevation of phagocytic granulocytes was not so marked; even after 2 months, the percentage was lower than in healthy children. These results correspond to the observations published previously (18) in which the target cells for the TF activity were primarily T lymphocytes and monocytes.

No increase in levels of serum Ig was observed during and after the TF therapy. This is in accordance with the experience of other authors (19). However the patient
group did have a higher level of IgA and IgM than the control group, indicating their intact B-cell immunity and possibly even an initiating T-cell-directed normal humoral IgA response.

It is presumed that the subpopulation of “active” T cells represents cells that are highly active to direct reactions with antigens. These cells may play a critical role of supervision in the host and form a kind of intermediary stage in a differentiation of T cells (20). It is further presumed that this subpopulation might provide more precise information about immunological status of a patient than the number of total T cells (21). Thus any reestablishment of normal “active” T-cell profiles in a patient would argue for a favorable prognosis.

The described TF therapy in this report led to a substantial reduction of attacks of disease in all the treated children. Some of the children (see Table 2) were without any difficulties throughout the following year. Because we do not know all of the biological functions of TF, we believe that very close attention must be paid to the selection of patients, and TF should be administered prudently in conjunction with repeated immunological evaluation. Additionally, the patients must be monitored even during the therapy keeping in mind that the transfer factor is an interventional form of therapy. The success reported here argues for the use of TF in the type of patient population described with otitis media.

ACKNOWLEDGMENTS

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REFERENCES