Acquired T Cell Specific Deficiency Other than Acquired Immunodeficiency Syndrome (AIDS)

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Evidence of an acquired T cell-specific deficiency distinct from acquired immunodeficiency syndrome (AIDS) in a 63-yr-old Japanese female is provided. Recently, this patient suffered from primary invasive pulmonary aspergillosis. Skin tests to purified protein derivative of tuberculin (PPD) and Aspergillus antigens were negative. Upon admission to our hospital, her lymphocytes were exclusively unresponsive to T cell mitogens (concanavalin A, phytohemagglutinin, and OKT 3). The level of cells defined by monoclonal antibodies (CD1, CD2, CD3, CD4, WT31, and CD5) was less than 3%. In contrast, no decrease in the number of red blood cells, platelets, neutrophils or B cells was apparent. Five years ago, the patient had a normal white blood cell and lymphocyte count. However, over the following 4yr, she developed lymphopenia. With medication, her pulmonary disease recovered, while lymphopenia still continued. The levels of immunoglobulins, complements and enzyme activities (adenosine deaminase and purine nucleoside phosphorylase) were normal. Moreover, several tests for HIV (ELISA and Western blot) were negative suggesting that the T cell-specific deficiency was not a congenital immunodeficiency or AIDS but rather a new type of acquired immunodeficiency.

Key words: ID different from AIDS

Introduction

Immunodeficiencies are diseases characterized by an easy susceptibility to viral, bacterial or fungal infections. In most cases, the onset of immunodeficiency is congenital, although acquired immunodeficiency diseases have also been reported. Of these, acquired immunodeficiency syndrome (AIDS) is one of the most life threatening.

In 1981, clinical reports on AIDS recorded a severe depletion of CD4 phenotype of peripheral blood lymphocytes in affected patients (1, 2). Subsequently, a family of viruses etiologically associated with AIDS, termed human immunodeficiency virus (HIV), was isolated (3, 4). The virus is tropic for lymphocytes bearing the CD4 antigen, and infected cells lose their functional capacity and die prematurely (5–7). Most patients with AIDS suffer a progressive depletion of CD4 lymphocytes (8–10). In contrast to the significant reduction in CD4 cells, the number of CD8 cells generally increases. As a result, the CD4/CD8 ratio is strongly reduced and the absence of a delayed-type hypersensitivity reaction to antigens, such as to a purified protein derivative of tuberculin (PPD), is common among these patients (11, 12).

Here we report a patient who developed T cell-specific deficiency. She tested negative for HIV antibodies. She has never received a transfusion or used intravenous drugs or engaged in risky sexual behavior, suggesting the possible presence of a new acquired T cell-specific deficiency distinct from AIDS.

Patient and Methods

Patient profile. The patient, a 63-yr-old Japanese female, suffered from recurrent infections, including primary invasive pulmonary aspergillosis, from the age of 60. She has not, however, experienced any skin disorder such as Kaposi's sarcoma. She was a healthy
female of normal height and weight with no previous episodes of severe infection. She has never experienced lymph node swelling or splenomegaly. Her children (two sons and one daughter) were examined and the clinical history of her relatives was inquired, however, none had an episode of severe infection or lymphopenia. When she was admitted to our hospital (March 1988), she had an abnormal lung shadow on her chest X-ray. Her white blood cell count was 3,100/mm³, and neutrophil and leukocyte counts were 76.0 and 12.5%, respectively. The cell number and analyses of bone marrow aspirates were normal. The patient had normal levels of complement and serum immunoglobulins (IgM 132, IgG 1,645, IgA 244 mg/dl), and natural killer (NK) activity and nitroblue tetrazolium test (NBT) results were also normal. Enzyme activities of adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) were also within the normal range. Moreover, specific antibodies for several antigens such as Epstein-Barr (EB) virus and cytomegalovirus were positive. However, antibodies to hepatitis B (HB) virus, HIV, and human T cell leukemia virus (HTLV) were negative. Serum samples were tested for HIV seropositivity using commercially available ELISA kits (duPont, Wilmington, DE) and Western blot analysis (duPont).

Isolation of lymphocytes and cell culture. Peripheral blood mononuclear cells (MNC) of the patient and healthy controls were obtained from centrifugation of heparinized venous blood on Ficoll-Hypaque and three washes in Hank's balanced salt solution (HBSS). The MNC were cultured in flat bottom microtiter plates (Falcon Plastics Co., Oxnard, CA) in triplicate with each microwell containing 1 × 10⁵ cells in 0.2 ml of culture medium. Mitogens dissolved in HBSS or an equivalent volume of HBSS as control were added to each well. The cells were incubated for 3 days at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. All cultures were carried out in RPMI-1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% FCS and antibiotics. Incorporation of ³H-thymidine was assayed on day 3 of culture (13).

Mitogens. Phytohemagglutinin (PHA), concanavalin A (Con. A) (Difco Lab., Detroit, MI), OKT 3 (Ortho Diagnostic Systems, Raritan, NJ), and staphylococcus aureus strain Cowan I (SAC, 10% v/v formaldehyde and heat-killed; Calbiochem-Behring Corp., San Diego, CA) were used (14, 15).

Flow cytometrical analysis. Fluorescence-activated cell sorter (FACS) analysis was performed as previously described (14) using FACS II (Becton Dickinson Co., Mountain View, CA). For two-color analysis, cells were incubated for 30 min at 4°C with a fluorescein isothiocyanate-coupled monoclonal antibody (FITC-mAb) and a phycoerythrin-coupled (PE)-mAb, washed, and resuspended. The data are displayed as a counter diagram in which log intensities of FITC are plotted on the x-axis and log intensities of PE on the y-axis. Constant values of the percentage of total cell number on the z-axis were selected to draw the rings or contours around peaks of cells correlating FITC and PE fluorescence. In most experiments, FITC- or PE-coupled Leu series mAb (Becton Dickinson) such as Leu 6, 5b, 4, 3a, 1 and 2a, were employed. In some experiments, PE- or FITC-coupled OKT 3, 4, and 8 mAb (Ortho Diagnostic Systems) were also used to confirm the results.

Results

Stimulation of mononuclear cells (MNC) by mitogens

The skin tests to PPD, Candida, and Aspergillus antigens were negative. Therefore, responsiveness to T and B cell mitogens were examined. Peripheral blood MNC were stimulated with PHA, Con. A, OKT 3, and SAC at varying concentrations for 72 h. MNC from the patient did not proliferate in response to PHA at any concentration tested (Fig. 1). Responsiveness to the other T cell mitogens, Con. A, and OKT 3 was also significantly low. In a healthy control on the same day of assay, responsiveness to these T cell mitogens was normal. In contrast, the patient’s MNC proliferative response to the B cell mitogen SAC was the same as healthy controls. These results suggest that the patient’s MNC are exclusively unresponsive to T cell mitogens.

FACS analysis of MNC

In order to determine whether the unresponsiveness to T cell mitogens was due to a decrease of T cells or T cell subsets, a FACS analysis was conducted. Most of the MNC from the patient were both CD2 and CD3 negative and log intensities of PE on the y-axis.

![Fig. 1. Proliferative responses to T and B cell mitogens.](image-url)
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and the percentage of CD2 or CD3 positive cells was less than 3% (Fig. 2A). In a healthy control, the percentages of CD2 and CD3 positive cells were as high as 70% (Fig. 2B). Other T cell markers, such as CD1, CD4, WT31, and CD5 were also significantly low in the patient (Table 1). In contrast, the ratio of B cells, macrophages, and NK cells defined by CD11, CD20, CD21, and CD57 were significantly high (Table 1). The percentage of CD8 positive cells was 18.9% but the percentage of both CD3 and CD8 positive cells was less than 2% (Fig. 2C) and that of CD8 and CD57 (Leu7) positive cells was about 10% (Fig. 2D, Table 1). In some analyses, OKT 3, 4, and 8 were used in place of Leu series antibodies. The difference between these antibodies was less than 2% (data not shown). These results demonstrated that the number of T cells was significantly decreased in the patient’s MNC. Further, CD8 positive cells were not T lineage cells but rather large granular lymphocytes (LGL) or NK lineage cells (16). This suggests that the lack of a response to T cell mitogens may be due to a lack of T cells.

**Total cell number of peripheral blood cells**

A FACS analysis showed that the number of T cells was significantly low. The total number of circulating lymphocytes and T cells was measured. From the time of admission to our hospital (March 1988), the number of the patient’s white blood cells, especially lymphocytes, was low (Table 2). The total number of T cells, obtained

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**Table 1. Fluorescence-Activated Cell Sorter (FACS) Analysis of Peripheral Blood Lymphocytes**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Percentage (%)</th>
<th>Cell number (/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mar.16 '88</td>
<td>Mar. 7 '89</td>
</tr>
<tr>
<td><strong>T cell</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD1</td>
<td>0.6 ( &lt; 1)*</td>
<td>2.4 (under 60)</td>
</tr>
<tr>
<td>CD2</td>
<td>3.8 2.9 (78-88)</td>
<td>15.2 9.5 (940-1,800)</td>
</tr>
<tr>
<td>CD3</td>
<td>2.7 2.6 (68-82)</td>
<td>10.8 8.5 (820-1,600)</td>
</tr>
<tr>
<td>CD4</td>
<td>2.1 2.2 (35-55)</td>
<td>8.4 7.2 (420-1,100)</td>
</tr>
<tr>
<td>CD5</td>
<td>3.3 (65-79)</td>
<td>19.8 (780-1,600)</td>
</tr>
<tr>
<td>CD8*</td>
<td>18.9 19.2 (19-37)</td>
<td>75.6 63.0 (230-700)</td>
</tr>
<tr>
<td>WT31</td>
<td>2.4 (68-82)</td>
<td>9.6 (820-1,600)</td>
</tr>
<tr>
<td><strong>B cell</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD19</td>
<td>13.3 (5-15)</td>
<td>53.2 (60-250)</td>
</tr>
<tr>
<td>CD20</td>
<td>28.0 26.7 (5-15)</td>
<td>112 87.6 (60-250)</td>
</tr>
<tr>
<td>CD21</td>
<td>20.1 (5-15)</td>
<td>80.4 (60-250)</td>
</tr>
<tr>
<td>s-IgM</td>
<td>24.3 (5-15)</td>
<td>97.2 (60-250)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>39.6</td>
<td></td>
</tr>
<tr>
<td>CD11**</td>
<td>45.4 (13-27)</td>
<td>119 (90-440)</td>
</tr>
<tr>
<td>CD16</td>
<td>29.8 (8-22)</td>
<td>192 (160-540)</td>
</tr>
<tr>
<td>CD57**</td>
<td>48.0 47.3 (13-27)</td>
<td>155 (160-540)</td>
</tr>
</tbody>
</table>

FACS analysis was carried out on March 16, 1988. Each cell number is given by the multiplication of the lymphocyte number and each percentage. * Normal range. ** Two-color analysis was carried out and the percentage of CD8+ and CD11+ cells was 9.8 and that of CD8+ and CD57+ cells was 10.3.
Hematological examinations were carried out and each cell number was given by multiplication of lymphocytes number and each percentage. * Normal range.

by multiplication of the number of lymphocytes and the percentage of T cells, was less than 10/mm³ (Table 1). Her severe, selective T cell deficiency has lasted for more than a year. On the other hand, the number of erythrocytes, platelets, neutrophils, B cells, monocytes and NK cells were within the normal range. These results suggest that her lymphopenia was due to a deficiency of T cells. An increase in the percentage of B cells, macrophages, and NK cells (Table 1) was merely due to the T cell deficiency.

**Time course of the decrease in lymphocytes and the clinical course**

The selective T cell deficiency of the patient could be either congenital or acquired. Therefore, the patient's medical history was examined. Five years ago she had flu symptoms. Upon consulting a doctor, she received aspirin and vitamin supplements. At that time, the patient had a normal count of white blood cells (4,600/mm³) and lymphocytes, (1,700/mm³) as well as platelets, neutrophils, and red blood cells. During the subsequent 4 yr, she suffered from flu-like syndromes more than 10 times. Blood cell count results revealed that the number of white blood cells, especially lymphocytes, decreased year by year (Fig. 3). In contrast, the number of red blood cells, platelets, and neutrophils did not decrease. At the beginning of 1988, after complaining of a severe cough with serous sputa, she was found to have an abnormal shadow on her chest X-ray and was subsequently admitted to our hospital. Aspergillus was isolated from among her lung ailments and the diagnosis of primary aspergillosis of the lung was made. Since then, she has been treated...
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In the present study, a case of acquired T cell-specific immunodeficiency distinct from AIDS is discussed. A 63-yr-old Japanese female lacked delayed-type hypersensitivity and exclusive responsiveness to T cell mitogens. Her total T cell count was less than 10/mm³ as defined by CD2, 3, 4, 5, and WT31 (TCR) monoclonal antibodies. In contrast, the total number of other cells such as B cells, macrophages, NK cells, neutrophils, erythrocytes, and platelets were within the normal range. The activities of NK, NBT, and complement and the enzyme activities of ADA and PNP were all within the normal, healthy range. B cell functions of proliferation and differentiation to Ig secreting cells were not affected. In preliminary experiments, her B cells produced as much IgM, IgG, and IgA as healthy controls when cultured with allogenic T cells in the presence of SAC (17). The levels of serum Igs were within the normal range. Specific antibodies such as EB virus and cytomegalovirus were also positive. From birth to the age of 60, she was not susceptible to infections and had no history of severe viral, bacterial, or fungal disease. At the age of 58 when she consulted a doctor about a flu, her total white blood cell and lymphocyte counts were within the normal range. Then, unexplainably, she gradually developed lymphopenia. This lymphopenia was probably a T cell specific deficiency, although her total T cell count was not investigated prior to her admission to our hospital (March 1988). Her diagnosed condition of T cell deficiency (<10/mm³) has remained for more than 15 months. Although she has recovered from her lung ailments, the lymphopenia-linked T cell specific deficiency has persisted. None of her relatives has sustained similar symptoms or has exhibited T cell specific deficiency. Taken together, these results suggest that her T cell deficiency is acquired and is not due to a congenital immunodeficiency such as severe combined immunodeficiency, Nezelof syndrome, DiGeorge syndrome or ADA and PNP deficiencies (18–20).

Her present condition superficially resembles AIDS. However, she has never received a transfusion, used intravenous drugs or engaged in risky sexual behavior. She is HIV negative. All T cell counts, including CD8 T cells, were low. A diagnosis of HIV infection was rigorously analyzed and was excluded on the basis of multiple serologic tests including Western blots. Her peripheral blood mononuclear lymphocytes contained 18.6% CD8-positive cells. However, most CD8-positive cells did not bear phenotypical T cell markers such as CD2, CD3, or CD5. In AIDS, it is suggested that CD8 positive T cells are not affected (7, 8). Her CD8 cells did bear CD57 and CD11, suggesting that these CD8 cells are not T lineage cells but are NK or LGL lineage cells (16).

In addition to cases of congenital immunodeficiency diseases and AIDS, there have been a few cases that show profound cellular immunodeficiency. In these cases, the number of T cells defined by CD2 or CD3 did not decrease significantly. The defects reside in their intrinsic T cell abnormalities such as a lack of production of IL-2, a deficiency of T cell receptors or defective expression of class I or II antigens (15, 21–24). The present patient was deficient of pan T cells defined by CD2, CD3, and CD5 mAbs suggesting that her condition is quite different.

The reason as to why the present patient developed lymphopenia, especially T cell deficiency, is not clear at present but several possibilities can be postulated. It is possible that circulating antibodies for lymphocytes, especially T cells, may decrease the number of these cells (18). However, this is not likely because we could not find such antibodies in her serum. Peripheral blood T cells and T cell lines, Molt 4 and SKW 3, were cultured with the patient’s serum, washed, and cultured with FITC-conjugated anti-human Ig. No positive cells were found (data not shown).

T cell deficiency due to drug side effects can also be considered. When she had flu symptoms, she took the typical, small dose of aspirin and vitamins. She had not taken any other particular drug in the last 10 yr. Before using amphotericin B, she had already developed lymphopenia. It seems unlikely that these medicines could cause selective T cell deficiency. There seem to be no report in the lifetime that these drugs cause severe T cell deficiency, but this possibility cannot be completely dismissed.

T cell deficiency due to another viral infection is likely. Several viruses such as measles, Epstein-Barr, and cytomegalovirus are known to cause transient or long-lasting immune suppression (25–27). However, we are unaware of any report that these viruses cause selective T cell deficiency for more than 2 yr, and moreover, the titers of such viral antibodies are within the normal range in the present patient, suggesting that her T cell deficiency is not due to such viral infections. In addition to HIV, lymphotrophic viruses which cause selective T cell deficiency have been reported in mice (28, 29). An increase in CD8/CD57 or CD8 positive cells is considered a distinguishing characteristic of several viral infections as a part of the immunological response to viruses in general (8). Indeed, the possibility cannot be denied that an etiologically unknown virus has caused this patient’s selective T cell deficiency, however it seems...
that this case is an example of a new type of T cell deficiency.

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References


