Recurrence Infectious Diseases in Human CD53 Deficiency

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We report a familiar syndrome of recurrent heterogeneous infectious diseases, caused by bacteria, fungi, and viruses, which has as its only detectable defect the lack of CD53 antigen expression in neutrophils. All other assays ruled out known causes of recurrent infectious diseases due to either leukocyte adhesion or phagocytosis defects. CD53 belongs to the transmembrane-4 superfamily of proteins, which are a novel group of membrane proteins implicated in growth regulation and cell motility and possibly cell adhesion. We postulate that defects in these membrane proteins can be clinically manifested as complex recurrent infections.

Clinical syndromes characterized by recurrence of heterogeneous infectious diseases with several pathogenic agents are frequently the result of two major types of deficiencies that affect different aspects of leukocyte biology (2, 9). Members of the first group of disorders are usually the result of defects in leukocyte adhesion and cell motility properties, which are mediated by several proteins such as the integrins, CD11a, CD11b, and CD18 (2). In the second group, the defects are in the mechanisms of phagocyte killing related to oxidative properties, such as superoxide anion production in neutrophils, causing recurrent-infection syndromes (9). This latter group includes chronic granulomatous diseases (CGD) (9).

We have identified three members of a family, a 46-year-old woman and her two sons, 18 and 20 years old, that have similar clinical pictures, more severe in the mother, of heterogeneous recurrent infectious diseases since early childhood affecting several organs and caused by viruses, bacteria, and fungi. These three patients have tuberculosis as a common infection. All of them have to be frequently treated with antibiotics, in addition to tuberculosis treatment, to have normal lives. They usually do not feel well and get tired easily even in the absence of an active infection.

The first patient is a woman, a daughter of first cousins. She was diagnosed with pulmonary tuberculosis when she was 3 months old. Now her tuberculosis affects the intestine and kidney, with lesions detectable by computerized axial tomography scan; however, there is little lung damage. From early infancy, she has had frequent upper respiratory tract infections. From 19 to 21 years of age, she suffered from chronic diarrhea, with some bleeding. She has had recurrent esophagitis and vaginitis caused by Candida albicans that have responded to antifungal drugs. Occasionally she has had crises of arthritis or vasculitis that have responded very well to antibiotic treatment. There is calcification in her soft tissues, particularly near the backbone. There has been a loss of bone density. She suffers from a recurrent herpes infection. Her basal temperature dropped to 35°C at age 40. She has mild allergies to milk, pollen, and dust.

From 2 months to 11 years of age, the elder son had very frequent intestinal and upper respiratory tract infections that responded to antibiotic treatment. At 12 years of age, he had salmonellosis and a mild crisis of meningitis that responded to treatment; since then, his basal temperature has been 35°C. At 19 years of age, he was diagnosed with renal-intestinal tuberculosis. He continues to experience recurrent respiratory infections.

Like his brother, the second son had frequent upper respiratory tract infections until he was 7 years old; at that age, his basal temperature dropped to 35°C. At 3 years of age, he had a case of pneumonia that required hospitalization and responded to antibiotic treatment. At 13 years of age, intestinal tuberculosis was diagnosed, with a new crisis at age 16. From 15 years of age, there have been recurrent new episodes of upper respiratory tract infections. He has mild allergies to milk, pollen, and dust. He has an enlarged spleen and liver. He has experienced frequent episodes of infectious arthritis, affecting the knee and ankle, which improves with antibiotics but does not disappear. He has also had frequent viral infections, including Epstein-Barr virus infections.

The general situations of these three patients improve considerably when they receive chronic treatment with antibiotics. However, these antibiotics have to be changed frequently due to the appearance of resistance.

The heterogeneity and type of infectious agents suggested that most of them were opportunistic infections or reactivation of chronic silent infections in patients with an unidentified underlying immunodeficiency.

Based on the suspicion of a likely immunodeficiency, we determined several parameters to try to identify a possible pathogenic factor for the diseases in these patients. The only antigen we detected to be different in these three patients from those in controls was CD53 (3, 14). We determined the CD53 surface antigen levels in neutrophils from several healthy individuals and these three patients. Two monoclonal antibodies (MAb) against human CD53 antigen, MEM53 and HI29, recognizing different epitopes (12), were used. These antibodies were obtained from Serotec (Oxford, United Kingdom) or Pharmingen (San Diego, Calif.). Neutrophils were prepared by standard procedures (20). The percentage of positive human neutrophils isolated from healthy individuals (n = 16) for CD53 was over 85% in all cases. However, neutrophils from these three patients were negative for this antigen, with <5% positive cells. The immunofluorescence flow cytometry patterns are shown in Fig. 1. None of the patients appeared to...
have significant levels of CD53 antigen in neutrophils (Fig. 1B, C, and D), whereas neutrophils from a healthy individual used as a control showed high-level cell surface expression of this antigen (Fig. 1A). Furthermore, in CD3-positive T cells, there was no detectable CD53 antigen (not shown). In humans, all mature cells of the lymphoid-myeloid lineage are positive for CD53 antigen (3, 14). CD53 antigen is a member of the transmembrane-4 superfamily (also known as TM4SF or tetraspan proteins), a novel group of glycoproteins whose physiological function is unknown despite widespread cellular distribution (23). However, this antigen has been shown to be involved in cellular signaling in B (21, 22) and T and NK (4, 5) cells, implicating both tyrosine kinases and protein kinase C (4, 21). In human B cells and monocytes, cross-linking of CD53 antigen triggers a respiratory burst (21). In mice, CD53 antigen also appears to be implicated in thymic selection of immunocompetent cells (17). In rat macrophages, CD53 antigen regulates the inducible nitric oxide system (6). Furthermore, the CD53 antigen surface level appears to be downregulated after neutrophil stimulation in a manner similar to the levels of glycophorin (CD43) and hyaluronic acid receptor (CD44) (7, 19). This regulation suggested that CD53 antigen was implicated in the modulation of cellular adhesion, although its natural ligand is still unknown. In this context, the clinical histories of these patients are similar in their heterogeneity to the disease phenotypes reported for defects in cellular adhesion (2).

FIG. 1. Immunofluorescence flow cytometry of CD53 antigen cell surface levels in human neutrophils from a control individual (A), the affected mother (B), the elder son (C), and the younger son (D). Cells were analyzed with MAb MEM53 (anti-human CD53) (solid lines). The determination of CD11b antigen with MAb Bear1 was used as the positive control (broken lines). The background and negative control were determined with P3X63 myeloma supernatant (dotted lines).

To rule out other potential defects, we also determined the cell surface expression of a number of leukocyte antigens in these patients. Thus, different cell surface markers were analyzed by immunofluorescence flow cytometry using distinct MAb in a Becton Dickinson FACStar Plus flow cytometer. Cellular staining and analyses of different cell subpopulations were performed by standard techniques. All other MAb used in this work were from Becton Dickinson (San José, Calif.), Immunotech (Westbrook, Maine), or Pharmingen. The specific reactivities of MAb markers for different cell populations and subpopulations were within normal limits and similar to those from control individuals. These other markers included CD2, CD3, CD4, CD5, CD7, CD8, CD11a, CD11b, CD11c, CD14, CD16, CD18, CD19, CD20, CD21, CD25, CD56, CD57, Leu 4, RO, RA, T-cell receptor αβ, T-cell receptor γδ, HLA-DR, DQ, and DP. The immunoglobulin levels were within the lower limits of normal values (Table 1). We expected the immunoglobulin G (IgG) values to be significantly higher because of chronic infections. Their mitogenic responses to enterotoxins A and C1, interleukin-2 (IL-2), MAb OKT3, and several lectins, including concanavalin A, phytohemagglutinin, and pokeweed, were also normal.

NK cell cytolytic activity was tested by using different ratios of effector to target cells (ranging from 100 to 12.5); in these three patients, the values were in the middle of the normal range.

In the T-cell population, CD53-positive cells represented <1% of cells, regardless of the type, which is in contrast to the situation in a healthy individual, where all populations are positive (3). This observation suggested that the CD53 deficiency was a general phenomenon and was not restricted to neutrophils. The CD4\(^+\)/CD8\(^+\) lymphocyte ratios for two patients were within the lower limit (Table 1).

The production of several cytokines after intravenous stimulation with 3 ng of endotoxin per kg was determined during a 24-h period. In these three patients, the levels of tumor necrosis factor alpha (TNF-α), small TNF-α receptor, granulocyte-macrophage colony-stimulating factor, IL-8, IL-1β, IL-6, IL-4, gamma interferon, and lactoferrin and changes in temperature were within normal control variation.

Recurrent infections are also part of the clinical phenotype of CGD (9). Therefore, we tested for neutrophil functional properties affected in CGD. However, all markers for CGD were negative. Neutrophil cellular immunity appeared to be normal, as assessed by several functional studies of phagocyte activity (9), such as Escherichia coli and C. albicans phagocytosis, and biological mechanisms of cell toxicity, such as generation of superoxide anion and the Nitro Blue Tetrazolium

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Value for*:</th>
<th>Normal values</th>
</tr>
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<tbody>
<tr>
<td>IgG level (mg/dl)</td>
<td>491, 430</td>
<td>1.250 ± 300</td>
</tr>
<tr>
<td>IgA level (mg/dl)</td>
<td>68, 79</td>
<td>210 ± 50</td>
</tr>
<tr>
<td>IgM level (mg/dl)</td>
<td>68, 70</td>
<td>125 ± 50</td>
</tr>
<tr>
<td>IgE level (IU/ml)</td>
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<td>5–200</td>
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<tr>
<td>C3 level (mg/dl)</td>
<td>99</td>
<td>70–160</td>
</tr>
<tr>
<td>C4 level (mg/dl)</td>
<td>16</td>
<td>20–40</td>
</tr>
<tr>
<td>CD4(^+) cells (%)</td>
<td>57</td>
<td>37–61</td>
</tr>
<tr>
<td>CD8(^+) cells (%)</td>
<td>20</td>
<td>19–31</td>
</tr>
<tr>
<td>CD4(^+)/CD8(^+) ratio</td>
<td>2.8</td>
<td>1.5–2.6</td>
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*In the cases of IgG, IgA, and IgM levels, data are two determinations performed at a 2-year interval.

TABLE 1. Laboratory parameters
The human leucocyte surface antigen CD53 is a protein structurally similar to CD37 and MRC OX-44 antigens. Immunogenetics 32:281–285.


