NEW FAMILIAL DEFECT IN MICROBICIDAL FUNCTION OF POLYMORPHONUCLEAR LEUCOCYTES

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Summary

A family is described in which a defect in intracellular killing affected two, and probably three, siblings of both sexes. From an early age they have had recurrent severe infections. During these episodes their white-blood-cell count became very high. This familial disorder seems to differ from previously reported syndromes of abnormal leucocyte function.

Introduction

Phagocytosis and subsequent killing of ingested microorganisms by granulocytes and mononuclear phagocytes is one of the major mechanisms in host defence. Defects in phagocytic or microbial function predispose individuals to severe infections.1 2 We describe a familial disorder of intracellular killing in which one female and at least one (probably two) male siblings were affected.

Case-reports

Case 1 is a boy born in 1963 who first received medical attention because of recurrent otitis media. In 1967 and 1969 he was admitted to hospital because of stomatitis, gingivitis, and tonsillitis caused by Staphylococcus aureus, β-haemolytic streptococci, and Candida albicans. In 1970 skin lesions developed for the first time. These were diagnosed as pyoderma gangrenosum. In 1971 he was admitted to the Nijmegen University Hospital with a suppurative pericarditis caused by Staph. aureus which required pericardiotomy as well as antibiotic treatment. In 1972 he had a subperiosteal abscess of the right tibia caused by Staph. aureus from which he recovered after drainage and antibiotic therapy. In 1974 he was admitted to the same hospital with severe pneumonia. Although no causative microorganisms were found, he recovered with antibiotic treatment. During all these episodes of infection his white-blood-cell count (W.B.C.) became very high (25 000-55 000/mm3), the vast majority of cells being neutrophil leucocytes, with only a slight shift to the left. In the infections the W.B.C. was normal. When leucocyte function was studied (April, 1974) he had no infections, and, apart from scars from operations and from skin infections, no abnormalities were found on physical examination.

Case 2 is a female born in 1951, who has had severe recurrent pyoderma from her first year of life. These skin lesions were generally caused by Staph. aureus, but β-haemolytic streptococci (group A) or Proteus mirabilis organisms were sometimes responsible. In 1958 she had severe laryngotraheobronchitis of unknown cause, for which a temporary tracheostomy was necessary. Since 1958 she has been on corticosteroid therapy, which severely retarded her growth. In 1971 she had an uncomplicated pregnancy. In 1974 she was admitted to hospital on several occasions with erosive gastritis and esophageal candidiasis, which was treated with antifungal agents. Several attempts were made to withdraw corticosteroid therapy, but she always managed to obtain supplies from elsewhere. There was pronounced leucocytosis during episodes of infection. The W.B.C. became normal once the infection had subsided. When leucocyte-function was studied (April, 1975) her growth was found to be retarded (height 150 cm) and her appearance was cushingoid. Apart from scars of healed skin lesions and of a tracheostomy there were no other abnormalities.

Case 3 was a male born in 1949, who had had pyoderma from his first year of life. From 1952 until 1954 he was in a sanatorium with tuberculosis. From 1958 until 1964 he was on corticosteroid therapy. In 1960, 1963, 1965, and 1966 he had repeated attacks of pneumonia. He also had recurrent episodes of gingivitis, otitis, and sinusitis. In 1968 he had an abscess in his jaw and severe pneumonia with haemoptysis. A tracheostomy was performed, and despite all therapeutic measures (antibiotics, transfusions, and oxygen) he died in February, 1969. During this infection his leucocyte-count rose to 64 000/mm3. Leucocyte function was not tested. At necropsy, bronchiectasis and extensive pulmonary necrosis with abscesses were found.

The Pedigree

The family (fig. 1) belongs to a closed community living in caravans, leading a gipsy-like life. However, consanguinity is not likely because the mother married into this community, her family always having led a settled life.

The father has had perianal fistulas since 1959, but gave no history of pyoderma or of any serious infection apart from a wound infection after cholecystectomy. Apart from obesity, no physical abnormalities were noted. The mother was a healthy

Fig. 1—Family pedigree.
middle-aged woman, without a history of infections. Siblings A, B, C, and D were all in good health. Only D had been admitted to hospital because of some unknown skin disease and tiny areas of depigmentation could still be seen on his abdomen. The child of case 2 has had neither pyoderma nor any serious systemic infection. One of the father’s brothers has probably had recurrent infections, but no further details are available.

**Materials and Methods**

Phagocytosis and intracellular killing by leucocytes were studied with a modification of the method of Cohn and Morse, which allows both processes to be measured independently. The microorganism used was *Staph. aureus* type 421. The opsonic activity of the serum was investigated by the same method as for phagocytosis, but using patient’s serum instead of donor serum and normal leucocytes instead of those from the patient.

Peroxidase staining using Kaplow’s method was performed on blood-smears. The glucose-6-phosphate-dehydrogenase (G-6-P.D.) activity of the red blood-cells was assayed by Dr. L. N. Went. Lymphocyte transformation was studied using phytohaemagglutinin, pokeweed mitogen, and *C. albicans* extract as stimulants.

Immunoglobulins were measured in all family members by turbimetric immunoassay (performed by Dr P. van Munster, Nijmegen). Total haemolytic complement was measured as the CH<sub>50</sub> value in a sheep-red-cell/rabbit-antibody assay with fresh pooled human serum as a standard. Clq, C3 C4, and C5 were assayed by immunodiffusion using specific antisera (assayed by Dr K. Pondman, Amsterdam) HL-A and blood-groups were determined by Prof. J. J. van Rood.

**TABLE I—LEUCOCYTE FUNCTION**

<table>
<thead>
<tr>
<th>Family members</th>
<th>Recurrent infections</th>
<th>Phagocytosis (%)</th>
<th>Intracellular killing (%)</th>
<th>Opsonic activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
<td>120 min</td>
<td>60 min</td>
<td>120 min</td>
</tr>
<tr>
<td>Father</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibling A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibling B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibling C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibling D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal subjects*</td>
<td>+</td>
<td>96.3</td>
<td>90.4</td>
<td>96.3</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2.8</td>
<td>9.1</td>
<td>3.2</td>
</tr>
</tbody>
</table>

*Mean ± S.D.

**FIG. 2—Rate of intracellular killing of Staph. aureus.**

Number of viable intracellular bacteria is expressed as percentage of viable intracellular bacteria + S.D. is given for 17 healthy individuals. Intracellular killing was significantly reduced at 90 and 120 minutes (p<0.01) in cases 1 and 2.

**TABLE II—QUANTITATIVE SERUM-IMMUNOGLOBULIN STUDIES**

<table>
<thead>
<tr>
<th>Family members</th>
<th>IgG (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>IgM (mg/dl)</th>
<th>IgE (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>967</td>
<td>15</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>977</td>
<td>284</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>Sibling A</td>
<td>899</td>
<td>152</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Case 2</td>
<td>495</td>
<td>168</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Sibling B</td>
<td>1102</td>
<td>183</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Sibling C</td>
<td>967</td>
<td>168</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>Sibling D</td>
<td>964</td>
<td>168</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>1236</td>
<td>295</td>
<td>94</td>
<td>236</td>
</tr>
<tr>
<td>Normal subjects*</td>
<td>800-1500</td>
<td>90-300</td>
<td>70-250</td>
<td>&lt;300</td>
</tr>
</tbody>
</table>

*Mean ± S.D.

**TABLE III—HL-A AND BLOOD GROUPS**

<table>
<thead>
<tr>
<th>Family members</th>
<th>HL-A</th>
<th>ABO</th>
<th>Rhesus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td></td>
<td>A</td>
<td>CcDee</td>
</tr>
<tr>
<td>Mother</td>
<td>1.8</td>
<td>7</td>
<td>W6/W6</td>
</tr>
<tr>
<td>Sibling A</td>
<td>2.7</td>
<td>7</td>
<td>W6/W6</td>
</tr>
<tr>
<td>Case 2</td>
<td>2.7</td>
<td>6</td>
<td>W6/W6</td>
</tr>
<tr>
<td>Sibling B</td>
<td>2.7</td>
<td>6</td>
<td>W6/W6</td>
</tr>
<tr>
<td>Sibling C</td>
<td>2.7</td>
<td>6</td>
<td>W6/W6</td>
</tr>
<tr>
<td>Sibling D</td>
<td>2.7</td>
<td>6</td>
<td>W6/W6</td>
</tr>
<tr>
<td>Case 1</td>
<td>1.8</td>
<td>8</td>
<td>W6/W6</td>
</tr>
</tbody>
</table>

**Studies on Leucocytes**

In all family members the morphology of granulocytes, monocytes, and lymphocytes was normal by light microscopy. Peroxidase staining was positive in granulocytes and monocytes from cases 1 and 2. The total and differential W.B.C. of all family members were normal when the leucocyte functions were studied. The results of the studies on phagocytosis, intracellular killing, and opsonic activity (table I) show that the granulocytes of all members of the family phagocyte normally. Intracellular killing of bacteria was defective in at 90 and 120 minutes (p<0.01) (fig. 2). The opsonic activity of the serum of cases 1 and 2 and their father was normal (table I), despite the low immunoglobulin levels in case 2 and the father (see below). The G-6-P.D. activity of the red blood-cells was normal in cases 1 and 2. The in-vitro response of lymphocytes from case 1 to phytohaemagglutinin, pokeweed mitogen, and *C. albicans* was normal.

**Humoral Immunity**

The results of quantitative serum-immunoglobulin studies (table II) were normal in most members of the
family, but in the father the IgA concentration was low and case 2 had low IgG and IgM concentrations (which she had had from an early age). All family members had normal total haemolytic complement levels. Cases 1 and 2 had normal levels of C1q, C3, C4, C5. After an attack of influenza in 1971, case 1 demonstrated a significant rise in influenza-B-antibody titre. Tests for rheumatoid factor (Rose test and latex fixation) and antinuclear factor were negative in all family members.

**HL-A and Blood-group Typing**

The HL-A haplotypes and blood-groups are given in table III. Cases 1 and 2 have HL-A 1 and HL-A 8 in common. Their father also has these antigens. All three affected siblings are blood-group O.

**Discussion**

This report describes a bactericidal defect of leucocytes in male and female siblings. A brother who died before this study was done, probably had the same defect of leucocyte function. The affected individuals have recurrent hypergranulocytosis. In both patients the defect of intracellular killing was detected when no infection was present and the leucocyte-count was normal. This is important, since it is known that that intracellular killing may be depressed in acute infection.8, 9 and extreme leucocytosis.8

How are our cases related to previously reported syndromes? The most extensively investigated microbicidal defect is chronic granulomatous disease (C.G.D.) which only affects boys and is usually carried by their mothers and sisters.10-12 A variant of this syndrome has been described in females12-15 for which an autosomal mode of inheritance has been suggested. To our knowledge there is no report of a family with C.G.D. in which both male and female siblings are affected. The clinical pattern of C.G.D. is different from the patients we studied. Our patients had no recurrent lymphadenitis, no typical granulomas, and no liver and spleen enlargement. In addition biochemical studies of the leucocytes of our patients by Weening et al16 demonstrated no stimulation of oxygen consumption, superoxide production, and hexose-monophosphate-shunt activity during phagocytosis of latex particles without opsonins, whereas, in contrast with the findings in C.G.D., stimulation of cell metabolism was normal after ingestion of IgG-coated latex particles. They also found that zynosan was not iodinated. In familial lipochrome histiocytosis the clinical signs are also different (e.g., arthritis, splenomegaly) and this disease has only been described in females.20, 21

It is unlikely that the defect reported here is due to G-6-P-D. deficiency, since in all reported cases of G-6-P-D. both leucocytes and red blood-cells showed G-6-P-D. deficiency.22-24 Myeloperoxidase deficiency,25, 26 which is not a sex-linked defect, was excluded in our patients by a positive peroxidase stain. Unlike the patient with a bactericidal defect reported by Strauss et al.27 the morphology of the leucocytes in our patients is normal. Comparison of our findings with other reports of sporadic cases of microbicidal defects is difficult because it is not known whether these conditions are inherited or acquired.28-30

The defect of intracellular killing in the family described here probably has a genetic basis. A relation between this disorder and HL-A antigens could not be established since the antigens which were common to the affected individuals (HL-A 1,8) are very widespread in the general population.

We thank Mr Peter C. J. Leijh for statistical analysis; and Dr Richard W. Crofton, Dr L. N. Went, and Dr J. W. Bruning for their helpful comments.

Requests for reprints should be addressed to R.F.

**REFERENCES**

1. Lancet, 1974, i, 438
7. McCaill, C. E., Caves, J., Cooper, R., Dechatete, L. R., Infect. Dis. 1971, 124, 68.

**IgA DEFICIENCY, EPILEPSY, AND PHENYTOIN TREATMENT**

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**Summary**

In a prospective study of thirty-two children with seizures treated with phenytoin (phenylhydantoin), five had low levels of serum-IgA before treatment. All of these were among the fifteen who had had febrile convulsions in infancy. IgA levels fell significantly during 6 months treatment in the fourteen patients studied sequentially. Treated children with low serum-IgA had normal numbers of lymphocytes with surface IgA. This suggests that phenytoin causes failure of terminal differentiation of B lymphocytes without opsonins, whereas, in contrast with the findings in C.G.D., stimulation of cell metabolism was normal after ingestion of IgG-coated latex particles. They also found that zynosan was not iodinated. In familial lipochrome histiocytosis the clinical signs are also different (e.g., arthritis, splenomegaly) and this disease has only been described in females.20, 21

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