Neutrophil function in infection-prone children

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SUMMARY Seven variables connected with the function of the neutrophil granulocyte were studied in 24 children who had undue susceptibilities to infections. The phagocytosis rate of IgG-coated latex particles by the patients' neutrophils was significantly reduced compared with an age-matched control group. The chemokinetic effect of patients' sera on normal polymorphonuclear neutrophil leucocytes was reduced too, especially in heated (56°C, 30 min) serum. Spontaneous chemotactic activity in the patients' sera was significantly lower than in sera from healthy adults and from the age-matched control group. A functional index based on the 7 variables of neutrophil function was constructed. The score of the functional index was correlated to the severity of the patients' clinical condition and discriminated well between normal children and those with undue susceptibility to infections.

Defence against bacterial infections mainly depends on the proper functioning of the neutrophil granulocyte. There are many reports that deal with defects of this function in adults. With the exception of the X-linked chronic granulomatous disease of childhood most reports on children have been limited to descriptions of single severely ill patients. However, frequent, but less severe, episodes of bacterial and viral infections are common in some children and an investigation of the neutrophil function in such children is therefore of interest. There have been two studies on this topic. In one, Hill et al. found a defective neutrophil chemotactic response in children with recurrent episodes of otitis media but only in those who also had chronic diarrhoea. The chemotactic activity improved when the patients became symptom-free, suggesting that the defect was secondary to the infection. In the other study Farhoudi et al. classified the defect into primary or secondary by studying the cell function of the parents of children with defective neutrophil mobility.

We have studied some aspects of the neutrophil function in a selected group of children with higher than normal incidence of infections. The purpose was to evaluate the clinical applicability of certain tests and to investigate whether the patients' clinical symptoms were correlated to one serious defect or to a combination of certain defects. We also tried to gather information about the underlying mechanisms for the undue susceptibility to bacterial infections in order to find the correct treatment.

Patients

Each of the 24 patients was chosen because he had an undue susceptibility to infections—that is, at least 6 bacterial infections during the previous year (Table 1). Most children, especially the younger ones (1–5 years), suffered from frequent upper respiratory infections with otitis. The total number of diagnosed infections with otitis varied between 8 and about 20. Many older children (6–13 years) had suffered from frequent middle ear infections earlier but their current problems were generally pharyngotonsillitis with fever every month, or every other month, and recurrent skin pustules. All children were investigated during a noninfectious period. The number of white cells was normal in all children. A slight hypogammaglobulinaemia (below the −2 SD limit for age) was found in one child (Case 18). This girl had been treated with monthly gammaglobulin injections for 6 months and during this time she had been free from any infection. Four children (Cases 3, 15, 23, 24) had an IgE value above the +2 SD limit for age. In all children serum concentrations of IgA and IgM were normal. Serum complement concentrations (C3 and C4) were found to be normal or slightly raised in all children.

The control group comprised 8 children with allergy to birch-pollen (blood-sampling was done during a symptom-free period) and 12 children who were admitted to the hospital for operations of noninfectious diseases—such as abdominal hernia. The
Table 1  List of patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Major clinical symptoms</th>
<th>Functional index score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>M</td>
<td>Recurrent tonsillitis</td>
<td>-4</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>M</td>
<td>Recurrent tonsillitis</td>
<td>-4</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>F</td>
<td>Chronic parotitis, otosialpingitis, recurrent bacteriuria</td>
<td>-4</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>M</td>
<td>Recurrent otitis media</td>
<td>-4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>M</td>
<td>Chronic respiratory infections, low IgA and IgM, mentally disturbed</td>
<td>-3</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>F</td>
<td>Recurrent otitis media</td>
<td>-3</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>M</td>
<td>Recurrent episodes of skin pustules, eosinophilia</td>
<td>-3</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>M</td>
<td>Recurrent episodes of respiratory infection with bronchitis</td>
<td>-2</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>M</td>
<td>Recurrent episodes of respiratory infection with bronchitis</td>
<td>-2</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>M</td>
<td>Recurrent otitis media, low IgM</td>
<td>-2</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>F</td>
<td>Recurrent otitis media</td>
<td>-2</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>M</td>
<td>Recurrent episodes of skin pustules</td>
<td>-1</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>M</td>
<td>Recurrent otitis media</td>
<td>-1</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>M</td>
<td>Recurrent otitis media</td>
<td>-1</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>M</td>
<td>Recurrent otitis media</td>
<td>-1</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>M</td>
<td>Recurrent otitis media</td>
<td>-1</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>M</td>
<td>Recurrent otitis media</td>
<td>-1</td>
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<tr>
<td>18</td>
<td>3</td>
<td>F</td>
<td>Recurrent otitis media, hypogammaglobulinemia</td>
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<tr>
<td>19</td>
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<td>Relapsing haemophilus meningitis</td>
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<td>M</td>
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<td>21</td>
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<td>22</td>
<td>6</td>
<td>M</td>
<td>Recurrent sinusitis</td>
<td>0</td>
</tr>
<tr>
<td>23</td>
<td>2</td>
<td>F</td>
<td>Recurrent otitis media</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>M</td>
<td>Recurrent otitis media</td>
<td>0</td>
</tr>
</tbody>
</table>

M = male  F = female.

mean age of children in the control group was 9 years (range 1 to 16). There were also 30 apparently healthy blood donors who acted as adult controls.

Methods

The white cell function tests were performed on heparinised blood within 2 hours after the sampling. From another blood specimen serum was separated after clotting and frozen at −70°C until analysed. IgG, IgA, C3, and C4 in serum were assayed by means of an immunological nephelometric method. IgM was assayed by the method of Mancini et al. and IgE by the Phadebas Prist method (Pharmacia, Uppsala, Sweden).

Phagocytosis of IgG-coated latex particles was measured kinetically by means of a Thrombocounter as described in detail. Briefly, 2 × 10⁸ isolated and washed polymorphonuclear neutrophil leucocytes (PMN) (purity about 90%) were mixed with 20 × 10⁶ IgG-opsonised latex particles (volume 4.06 × 10⁻¹⁴l) in a final volume of 1 ml in a siliconised glass tube. Incubation was made at 37°C during constant magnetic stirring (1600 rev/min). Aliquots of 100 μl were taken out from the mixture every minute for 6 minutes and latex particles not cell-associated counted in the Thrombocounter. The initial rate of disappearance of the latex particles was used as a measure of the initial rate of phagocytosis and expressed in minutes. When the effect of serum on phagocytosis was evaluated IgG-coated latex particles were incubated for 10 minutes in 20% serum at +37°C before mixing with normal PMN. The phagocytosis rate in these experiments was expressed as a percentage of the 100% control—that is phagocytosis in the presence of a freshly frozen (−70°C) pooled normal serum.

Migration. Migration of PMN was measured by the ‘leading-front’ method using a modified Boyden chamber. The micropore filter (Millipore, SA France) had a pore size of 3 μm and was about 150 μm thick. Isolation of the PMN was made by dextran sedimentation followed by hypotonic lysis of the erythrocytes. The PMN were washed twice in 0.15 mol/l sodium chloride and finally suspended in Gey's solution to the working concentration of 1.5 × 10⁶ PMN/l. Casein 1 g/l or fresh serum, in each case diluted in Gey's solution, was used as an attractant. Incubation time was 75 minutes at 37°C. The filters were fixed with 95% ethanol and stained with Mayer's Haemalun lö sung (Merck, Darmstadt, West Germany). The chemotactic activity of a patient's serum was expressed as a percentage of the activity of a freshly frozen (−70°C) pooled serum.

The effect of serum on PMN migration was evaluated after preincubation for 10 minutes at room temperature of normal PMN with 10% fresh or heat-inactivated (56°C, 30 min) serum. The serum was present throughout the incubation in the chemotaxis chamber. The results were expressed as a percentage of the migration of the same cells after preincubation with 10% heat-inactivated (56°C, 30 min) normal pooled serum. Reconstitution of patient's serum with normal serum was made by mixing 9 parts of patient's serum with 1 part of normal pooled serum. The mixture was allowed to incubate for 30 minutes at +4°C before it was diluted to 10% serum concentration and used in the experiments as described above. For practical purposes we have in this report used the following definitions for random migration, chemotaxis, and chemokinesis. Random migration was defined as the migration of PMN with Gey's solution below the filter. Chemotaxis was defined as the migration of PMN with casein or serum below the filter. Chemokinesis was defined as the alteration in the speed of migrating PMN, achieved in this case by the preincubation of PMN with serum and the presence of serum throughout the incubation. The chemokinetic effect was studied both with Gey's solution and with casein below the filter, as true chemokinetic factors by definition affect random migration and chemotaxis equally.
Student’s *t* test was used in the statistical evaluation of differences between groups.

**Results**

**Serum-independent phagocytosis of IgG-coated particles by patient PMN.** The ability of PMN isolated from the patient’s blood to ingest IgG-coated latex-particles was studied in the absence of serum. The results (Fig. 1) show that the group of infection-prone children as a whole had a significantly \((P<0.001)\) lower rate of ingestion compared with the healthy adults and the control children. In 7 children we were able to repeat the measurement 6 and 12 months later, and in all 7 patients the rate of ingestion of the IgG-coated particles was similar on both occasions.

**Phagocytosis of IgG-coated particles by normal PMN in the presence of patient serum.** Phagocytosis of IgG-particles in the presence of patient serum was performed in order to test the opsonic activity of serum by preopsonisation of the IgG-particles with serum, and to find out if there were inhibitors directed towards the cell. A reduced capacity to ingest particles in this system could thus imply either a lack of opsonins—that is primarily complement components—or the presence of inhibitors directed towards the opsonins or the neutrophil. To screen for these possibilities normal PMN were used and their capacity to ingest IgG-coated particles was studied with the patient serum. With the exception of one patient (Case 7) no difference could be found between the control group and the patients, so gross deviations from the normal were thus excluded.

**Migration of normal PMN in the presence of patient serum.** When fresh serum was used there were no differences between the two groups of children with respect to the effect of their sera on random migration or chemotaxis (Fig. 2). However, sera from 6 of the infection-prone children had an effect on random migration that was clearly inferior to that of the control group. In the presence of heated serum, random migration of normal PMN was as a whole significantly \((P<0.01)\) reduced in the patients (Fig. 2). The effects on chemotaxis were not very

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![Fig. 1 Phagocytic rate of IgG-coated latex particles by isolated PMN from two control groups (adults and children) and from the infection-prone children, the last group on two occasions. Horizontal lines indicate arithmetical means. The phagocytic rate of the infection-prone children (0.49 ± 0.12 (SD)) was significantly \((P<0.001)\) reduced compared with the children’s control group (0.63 ± 0.17 (SD)) and the adult control group (0.60 ± 0.12 (SD)).](http://adc.bmj.com/)

![Fig. 2 Chemokinetic activity of fresh and heated sera. Random migration and chemotaxis of normal PMN performed in the presence of 10% serum, from the children’s control group or from the patients with samples taken on two different occasions. Migration was expressed as a percentage of that in the presence of a heat-inactivated normal pooled serum. Horizontal lines indicate arithmetical means. Random migration in the presence of the heated patient’s sera (88 ± 16 (SD)) was significantly \((P<0.01)\) reduced compared with the children’s control sera (101 ± 15 (SD)).](http://adc.bmj.com/)
different, but the sera from 13 patients showed an activity which was below 84% that of the control sera; only 3 control sera had such a low activity. At follow-up between 6 and 12 months later, the activity was normal both for random migration and chemotaxis in most heated sera. However, the pattern for fresh sera taken at this time was more diverse. In some sera the activity was the same, but in others both higher and lower activities were found compared with the activity one year earlier.

**Migration of normal PMN towards patient serum.** The spontaneously existing chemotactic activity in serum from the infection-prone children was measured by allowing normal PMN, suspended in buffer, to migrate towards 10% fresh serum present below the filter. Fresh sera from healthy adults were used as the 100% control. Sera from the group of infection-prone children as a whole showed a significantly reduced (P < 0.001) chemotactic activity compared with sera obtained from healthy adults and with sera from the control group (Fig. 3). At follow-up 6 to 12 months later activity was normal.

**Effect of the addition of normal serum to some abnormal patient sera.** Normal serum at a final concentration of 10% was added to some sera from the infection-prone children which had shown a deviation from the control children in any of three different systems (Table 2). Spontaneous chemotactic activity of the fresh serum was made normal in 2 of 4 cases by adding normal serum; this probably indicates a deficiency in these 2 sera. However the other 2 sera could not be made normal and they therefore probably contained inhibitors of chemotactic activity. The effect of patient serum, fresh or heated, on random migration was made normal in each case by adding normal serum, thus indicating a deficiency in these sera.

**Scoring of granulocyte function (functional index).** In some variables the results overlapped considerably between controls and patients. In order to judge the collective function for each patient we constructed a functional index (FI) based on the 7 variables studied in this report. The patient was given one

![Fig. 3 Chemotactic activity of fresh serum. Isolated normal PMN were allowed to migrate towards 10% fresh serum obtained from the control groups (adults and children) or the infection-prone children, and from the last group on two different occasions. Migration was expressed as a percentage of that obtained towards 10% fresh pooled normal serum. Horizontal lines indicate arithmetical means of respective group. Migration towards patient's serum (75 ± 20 (SD)) was significantly (P < 0.001) reduced compared with the children's control group (97 ± 18 (SD)) and the adult control group (98 ± 7 (SD)).](http://adc.bmj.com/)

![Fig. 4 A functional index of the neutrophil function of the infection-prone children.](http://adc.bmj.com/)

### Table 2 Chemotactic and chemokinetic activity of patients’ sera after adding 10% normal serum

<table>
<thead>
<tr>
<th>Case</th>
<th>Normal serum</th>
<th>Chemotactic activity, fresh serum (%)</th>
<th>Chemokinetic activity, fresh serum (%)</th>
<th>Chemokinetic activity, heated serum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>15</td>
<td>58</td>
<td>99*</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>16</td>
<td>77</td>
<td>107*</td>
<td>8</td>
<td>43</td>
</tr>
<tr>
<td>14</td>
<td>37</td>
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<td>19</td>
<td>60</td>
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<td>11</td>
<td>51</td>
</tr>
<tr>
<td>16</td>
<td>77</td>
<td>107*</td>
<td>8</td>
<td>43</td>
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<tr>
<td>7</td>
<td>49</td>
<td>48</td>
<td>11</td>
<td>51</td>
</tr>
</tbody>
</table>

*The activity of normal serum was 100%.*
negative point for every variable that fell below the lower confidence limit of the 95% probability level. Theoretically, the chance of finding an individual in a normal population with an FI of -1 would be $0.025 \times 7 \times 100 = 17.5\%$, an FI of -2 ($0.025 \times 7)^2 \times 100 = 3.1\%$, an FI of -3 ($0.025 \times 7)^3 \times 100 = 0.5\%$, an FI of -4 = 0.09% etc. As shown (Fig. 4), the infection-prone children had a greater number of negative FI scores than expected. Individual scores of the patients are given in Table 1. There seems to be a relationship between the degree of negative FI scores and the diversity and severity of the infections.

Discussion

Impairment of the neutrophil function is a widely accepted cause of decreased resistance to bacterial infections. The impairment may have cellular or humoral causes. We chose to study 7 different aspects of neutrophil function. One aspect was a test of the cellular function and the other 6 were tests of the humoral conditions necessary for a proper cell function.

Phagocytosis was evaluated using a method which measures the kinetic uptake of opsonised latex particles by the cells. It has proved to be a very sensitive method and has been suitable for evaluating neutrophil phagocytosis in other groups of patients—such as those with systemic lupus erythematosus, rheumatoid arthritis, heat-burn, and uraemia. Our data show that the neutrophils of the infection-prone children have a reduced capacity to ingest IgG-opsonised latex particles, and that the reduction is a cellular defect as the patients’ sera did not inhibit normal cell phagocytosis. The reduction was probably not an acquired defect caused by the frequent infections, as the presence of an infection might have been expected to stimulate phagocytosis. Furthermore, the phagocytosis rate of the neutrophils in 7 children was unaltered after a period during which there was clinical improvement in the frequency of infections.

The finding that some of the patients’ sera were less effective in stimulating neutrophil migration, especially random migration, might be explained theoretically either by a lack of stimulating factors or by the presence of inhibitors. For example, high IgA concentrations have been shown to inhibit neutrophil chemotaxis. This possibility can however be excluded by the normal serum levels of IgA in all patients. The presence of inhibitors was probably excluded by the fact that the activity was restored in all patients’ sera by adding normal serum. The defect is more likely to be caused by a deficiency in these sera.

The cause of the deficiency could be a consumption of the ‘factor’ due to the presence of a subclinical infection. This notion is supported by the finding in heated serum that most children who had normal activity when followed up some time later also had an improved clinical condition. The tendency towards normalisation in fresh serum was much less clear; this probably indicates that several factors both heat-labile and heat-stable are active in the serum-mediated stimulation of PMN migration. There are other explanations for the less effective stimulation of migration by the patients’ sera. One such explanation is that the cells had been deactivated by spontaneously existing heat-stable chemotactically active components such as C5a. This explanation is unlikely since spontaneously existing chemotactic activity in the patients’ sera was significantly reduced compared with control sera, and in other experiments the existing activity was found to be heat-labile. The low spontaneous chemotactic activity in patients’ sera also tended to become normal in parallel to the improvement of the clinical condition, suggesting that the defect was caused by the infections. The nature of this particular defect is probably diverse. In 2 sera the addition of normal serum made the activity become normal, suggesting a deficiency similar to that described above. In the other 2 sera the activity was unaffected, suggesting the presence of inhibitors of chemotactic activity. Similar activity has been described in many other groups of patients and has also been isolated and characterised by Ward.

With the exception of a few children who had profound defects with respect to only one variable, the most severely ill children were generally to be found among those who had the highest FI scores. Thus there appears to be a relationship between the clinical condition of the children and the results of the tests made in this study. This also indicates that the major cause of decreased resistance to infections is more often the entire effect of several minor defects.

The reduced phagocytic activity of the PMN seems to be a primary defect whereas the defects found in the sera from the patients are likely to be secondary to the infections. One could therefore speculate that one mechanism operative in the development of an increased infection-propensity is initiated by a heavy challenge of a bacterial or viral infection in an individual with a reduced resistance. Due to the slow elimination of the pathogen either due to a defective phagocytosis or due to other defects—such as cellular defects of migration or killing capacity—the infection sustains in the organism producing secondary changes in other important systems. These secondary changes reduce the host’s ability to eradicate the pathogen or give rise to an increased susceptibility to the attack by further pathogens. So
the patient ends up in a vicious circle with one infection after another. Several other examples of secondary changes caused by the infection either viral or bacterial,27 have also been described, these include reduced bactericidal capacity,28 reduced content of bactericidal granule proteins,29 and migration defects.30

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References

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