Dissociated Impairment of Neutrophil Functions and Recurrent Infections

Introduction
An increased susceptibility to infection, especially staphylococcal infections, seems to be caused by dysfunction of the neutrophil leukocyte more frequently than has been realised up until now. As emphasised by Segal (1), most commonly used techniques are probably too insensitive for the mechanisms underlying neutrophil disorders to be accurately identified. In addition, these tests are so poorly assorted that no real pathophysiologic classification is currently available. We therefore feel that the following case is important and interesting to report.

Case Report
A 43-year-old woman was referred for medical treatment because of a four-year history of recurrent skin infections caused primarily by staphylococci. Since the onset of her problems, treatment had consisted of repeated administrations of various antibiotics, either alone or in combination. Neither the patient nor her immediate family had any documented problems of atopy. She had not been given any drugs other than the antibiotics she had taken intermittently. Physical examination revealed no abnormality. Cultures repeatedly yielded Staphylococcus aureus. The erythrocyte sedimentation rate was 26 mm/h (Westergren technique), haemoglobin was 7.4 mmol/l, and the platelets and the reticulocytes reached $350 \times 10^9$ and $37 \times 10^9$, respectively. The white cell count rose to $5.8 \times 10^9$, with 68% mature granulocytes; no myeloid cells less mature than a metamyelocyte were found in leuko-concentration. The values for leukocyte alkaline phosphatase and peroxidase activity were 34 (normal range: 75-125) and 288 (normal range: 250-340), respectively. Serum electrophoresis was normal. No evidence was found of an underlying systemic illness (e.g. diabetes mellitus, hypogammaglobulinaemia, rheumatoid arthritis or uraemia). The immunoglobulins (including IgE) and the total haemolytic complement were normal. The levels of Clq, C3, factor B, C5 and C9, measured by radial immunodiffusion plates made in our laboratory or purchased from Behringwerke, West Germany, were all within the normal ranges. Examinations for antineutrophil antibodies (complement consumption) were repeatedly negative. The total lymphocyte count was $2.7 \times 10^9$ with 61% T-lymphocytes measured as cells forming rosettes with sheep erythrocytes (normal range: 64-72%, 101 controls), and 17% B-lymphocytes measured by direct immunofluorescence (normal range: 11-15%, 50 controls).

The patient was given levamisole (2.5 mg/kg for three days every other week), and then re-examined three months later. There was a clear clinical improvement.

Methods
Leukocyte suspensions containing over 90% neutrophils were prepared from heparinized blood by Dextran T500 (Pharmacia, Sweden). Sedimentation was carried out by centrifugation on Ficoll-Hypaque (Eurobio, France), and hypotonic lysis of erythrocytes with ammonium chloride. Phagocytosis was examined according to the technique of Park and Good (2) using polystyrene particles (0.81 μm diameter, Difco, U.S.A.); the results were expressed as the percentage of neutrophils containing at least ten latex particles. A minimum of 200 cells were counted. Results of the nitroblue tetrazolium

Received: 9 October 1981
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Infection 10 (1982) Nr. 3
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Table 1: Phagocytosis (%) and chemotaxis (chemotactic index) before and after levamisole.

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<tr>
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<th>Autologous serum</th>
<th>AB serum</th>
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<tr>
<td><strong>Phagocytosis</strong></td>
<td></td>
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<tr>
<td><strong>Patient</strong></td>
<td><strong>before</strong></td>
<td><strong>after</strong></td>
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<tr>
<td></td>
<td>43.33 ± 4.72**</td>
<td>58.00 ± 5.35</td>
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<td></td>
<td>62.49 ± 6.04**</td>
<td>67.71 ± 4.11</td>
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<tr>
<td><strong>Controls (n = 48)</strong></td>
<td>83.31 ± 7.40</td>
<td>84.63 ± 7.29</td>
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<tr>
<td><strong>Chemotaxis</strong></td>
<td></td>
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<tr>
<td><strong>Patient</strong></td>
<td><strong>before</strong></td>
<td><strong>after</strong></td>
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<tr>
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<td>1.12 ± 0.04**</td>
<td>1.12 ± 0.07</td>
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<td></td>
<td>1.20 ± 0.02**</td>
<td>1.28 ± 0.09</td>
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<tr>
<td><strong>Controls (n = 33)</strong></td>
<td>1.77 ± 0.11</td>
<td>1.81 ± 0.08</td>
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* Mean values ± 1 S.D. from triplicate assays
** p < 10⁻³ between patient and controls

NBT reduction was 98 ± 3% (normal range: 91.1 ± 8.4%, 57 controls). Adhesiveness was 96 ± 2% (normal range: 88.4 ± 7.1%, 53 controls).

Discussion

Minor boils and persistent superficial folliculitis in our patient correspond closely to the dermatologic picture classically associated with neutrophil dysfunction (6), where in most cases, recurrent infection is due to S. aureus.

We have described an association between decreased phagocytosis and impaired chemotaxis and bactericidal capacity. A criticism of the above-mentioned phagocytosis assay is that it does not distinguish the binding of particles from true phagocytosis; this would overrate the results if a reduction was found. On the other hand, adhesiveness and NBT reduction were unaffected. NBT (NBT) reduction were expressed as the percentage of neutrophils containing black formazan deposits among those which had ingested latex particles.

Adhesiveness was studied according to MacGregor et al. (3) using a 1 ml tuberculin syringe packed with 20 mg of nylon fibre and pre-wetted with 1 ml of warm Eagle's minimal essential medium. Results were expressed as the percentage of neutrophils adhering to the nylon column.

Bacterial killing was assessed using S. aureus 502A (Institut Pasteur Production, France) according to Alexander et al. (4), bacteria killed and the results were expressed as the percentage of bacteria killed at 20, 40 and 60 min.

Chemotaxis was studied in agarose by the method of Nelson et al. (5) using zymosan-activated serum (Sigma Chemical Company, U.S.A.) as a chemotactic stimulus. Results were expressed as the ratio (termed chemotactic index) between directed and spontaneous migration.

Each experiment was performed in triplicate; the results are expressed by the mean ± 1 standard deviation. A healthy individual, matched for age and sex, was taken as a control for chemotaxis and to study the effect of the patient’s serum on phagocytosis. The significance was evaluated by the Student's t-test.

Results

Phagocytosis (Table 1) in autologous and AB serum was much lower before levamisole had been administered (43.33 ± 4.72% and 58.00 ± 5.35%, respectively), as opposed to 83.31 ± 7.30% and 84.63 ± 7.29% in controls; it was significantly modified after treatment with levamisole (62.49 ± 6.04% and 67.71 ± 4.11%).

Chemotaxis in autologous and AB serum was likewise reduced (1.12 ± 0.04 and 1.12 ± 0.07, respectively), as opposed to (1.77 ± 0.11 and 1.81 ± 0.08 in controls), and was not improved by levamisole (1.20 ± 0.02 and 1.28 ± 0.06). It is worth noting firstly that spontaneous migration (the denominator of the chemotactic index) was absolutely normal (therefore only the directed migration was affected), and secondly that chemotaxis of the control neutrophils in the patient’s serum did not decrease at all (1.70 ± 0.15).

Bacterial killing (Figure 1) slowed down significantly before levamisole (50 ± 1%, 57 ± 2% and 79 ± 2% at 20, 40 and 60 min, respectively, as opposed to 58 ± 3%, 80 ± 2% and 83 ± 2% in the control), and was dramatically corrected after levamisole (55 ± 1%, 70 ± 2% and 81 ± 2%).

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