Effects of Surgery on Neutrophil Granulocyte Function

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The high incidence of postoperative infections raises the possibility of a reduced defense to infection during this period. For this reason, neutrophil function and enzyme activity were investigated after surgical trauma. The microbicidal ability of neutrophils was markedly impaired within 2 h of elective abdominal hysterectomy, but phagocytosis was unaffected. Loss of microbicidal activity was associated with loss of the lysosomal enzyme myeloperoxidase. It is suggested that these changes are due to activation and partial degranulation of circulating neutrophils. Defective microbial killing by individual cells should be compensated by an accompanying rise in the number of circulating neutrophils.

Phagocytosis and intracellular killing of microorganisms are important functions of neutrophil granulocytes, and impairment of these functions may allow the development of opportunistic infections. The best-known example of this is the inherited defect of neutrophil bactericidal capability which causes chronic granulomatous disease of children (21). It is also possible for there to be acquired and temporary defects of microbicidal ability, such as those that can follow burns (3) or severe trauma (2) or appear a complication of the infection itself (16, 22, 23). In these situations, defective neutrophil function may contribute to a fatal outcome of the disease. However, opportunistic infections are common after routine surgery, when they are an important cause of morbidity and mortality. Consequently, we have investigated the possibility that even the relatively minor trauma of elective abdominal hysterectomy impairs neutrophil function and so may contribute to the development of infections.

We tested the ability of neutrophil granulocytes from surgical patients to phagocytose and kill a species of *Candida* and related the results to the number of circulating neutrophils and to the levels of cellular enzymes. *Candida* was chosen as the test organism not because it is a common postoperative pathogen, but because it allows accurate measurement of both phagocytosis and microbial killing (6). Furthermore, the most important intracellular mechanism responsible for *Candida* killing is the peroxidase-hydrogen peroxide-halide system described by Klebanoff (14), which is known to be important in the killing of microorganisms, particularly *Candida* (14, 18).

**MATERIALS AND METHODS**

**Patients.** Studies were performed on 31 female patients (mean age, 44 years; range, 27 to 79 years) who were undergoing elective abdominal hysterectomy for dysfunctional uterine bleeding. All patients had normal hematological indices before operation, and none required transfusion during the period of investigation. Blood samples were taken before and at intervals after operation, and routine blood parameters, including the leukocyte count and differential count, were recorded. Neutrophils from 20 of these patients were studied at intervals until the neutrophilia, changes in function, and enzyme levels had returned to preoperative levels. A group of 11 patients was then studied immediately after returning to the ward from the operating theater, approximately 2 h after surgery.

**Leukocytes.** Peripheral blood leukocytes were prepared by a sterile procedure as follows: 20 ml of venous blood was mixed with 500 U of preservative-free heparin (Weddel Pharmaceuticals Ltd.) in a sterile plastic bottle, 5 ml of Plasmagel (Laboratoire Roger Bellon) was added, the mixture was drawn into a plastic syringe and then inverted at 45° in a 37°C incubator, and the erythrocytes were allowed to sediment for 45 min. The leukocyte-rich supernatant was expelled through a bent needle into a flat-bottomed cuvette, centrifuged at 150 × g for 5 min, and suspended in the plasma-Plasmagel mixture at a concentration of 9 × 10⁶ neutrophils/ml. The number of neutrophils was calculated from a total and differential count. Viability by trypan blue exclusion was greater than 95%.

**Organisms.** *Candida guilliermondii* was cultured overnight in glucose broth, gently sonicated to break up any clumps into individual cells, and suspended in cold tissue culture medium 199 at a concentration of 4 × 10⁷ organisms/ml. Methylene blue exclusion confirmed more than 99% viability. Viability was greater than 95% after 90 min of incubation at 37°C of the control tubes containing *C. guilliermondii* and the plasma-Plasmagel mixture but no leukocytes. This
organism was chosen because it remains in the yeast form, both in serum and within cells, and does not produce hyphae even at ratios of four or five organisms to one neutrophil (6).

Phagocytosis. Experiments were carried out in plastic tubes to which were added 0.25 ml of C. guilliermondii suspension and 0.5 ml of tissue culture medium 199. The tubes were equilibrated at 37°C for 5 min before addition of 0.25 ml of leukocyte suspension, gently mixed, and incubated without agitation. At 15 min the tubes were mixed again before a 0.1-ml portion was transferred into 1 ml of cold saline containing 6 mM ethylenediaminetetraacetic acid to stop further phagocytosis. Slides prepared with the cytocentrifuge and stained with Leishman stain showed dark blue C. guilliermondii and almost no degraded organisms or ghosts, which stain pink. The organisms within at least 200 neutrophils from each tube were counted, and the results of three replicates were averaged. Two separate measurements were made for phagocytosis: first, the proportion of neutrophils that had engulfed C. guilliermondii (percentage of phagocytosis), and second, the proportion of organisms that had been engulfed (percentage of uptake).

The suspension of C. guilliermondii contained 4 × 10⁶ organisms/ml and the leukocyte suspension contained 9 × 10⁶ neutrophils/ml, giving a ratio of 4.4:1 in the final mixture. This ratio is optimal for the measurement of both phagocytosis and killing of Candida (6). The final mixture contained approximately 20% autologous plasma.

Candida killing. After 15 min of incubation for the measurement of phagocytosis, the tubes were sealed with plastic stoppers and transferred to a rotating wheel on which they were inverted 24 times per min. Incubation at 37°C was continued for 75 min, making a total of 90 min. Samples removed during incubation confirmed that the remaining organisms were rapidly ingested, so that 15 min after starting to rotate the tubes all organisms had been engulfed and almost all neutrophils contained C. guilliermondii. After 90 min, the mixture was sonicated for 10 s to disrupt the neutrophils without affecting the organisms. Cytocentrifuge slides were prepared, stained with Leishman stain, and counted for both dark blue living organisms and pale pink degraded ones or ghosts. The percentage of pink dead organisms among the total C. guilliermondii is referred to as the percentage killed.

Neutrophil enzymes. Leukocytes were washed, suspended in saline, and disrupted by sonication in an ultrasonic disintegrator (Rapidis 50; Ultrasonics Ltd.) at setting 5 for 30 s, conditions that caused maximal enzyme release. Both alkaline phosphatase and myeloperoxidase were measured quantitatively in the total homogenate. Alkaline phosphatase was assayed by using p-nitrophenol phosphate as the substrate and noting the change in optical density at 405 nm (10). Myeloperoxidase was assayed by the rate of decomposition of hydrogen peroxide, using o-dianisidine as the hydrogen donor and noting the change in optical density at 460 nm (13). Both of these enzymes are present mainly in the granulocytes, and therefore neutrophils were not separated from other leukocytes. Enzyme activity was expressed as the change of optical density per minute per 10⁶ neutrophils; this value is referred to as units per 10⁶ neutrophils.

Statistics. Student t-tests were used to determine the significance of changes before and after surgery. Correlation coefficients were calculated for the significance of associations between two variables.

RESULTS

Figure 1 shows the results from the 20 patients who were followed for up to 10 days after surgery. Although phagocytic ability did not vary after the operation either in the number of neutrophils ingesting Candida or in the number of Candida ingested (not shown), the neutrophil microbicidal ability showed very marked differences, with a 25% reduction (P < 0.001) from the preoperative level within 1 day. On day 3, killing ability was still low, with a 24% reduction (P < 0.001), and this gradually recovered to preoperative levels by day 8. The pattern of change in neutrophil content of myeloperoxidase was very similar, although the fall was even more marked, with a 47% reduction (P < 0.001) from the preoperative level within 1 day, followed by recovery by day 8. This similarity was confirmed by a significant correlation between microbicidal activity and neutrophil myeloperoxidase content (r = 0.54; P < 0.001). Moreover, there was significant inverse correlation between neutrophil count and microbicidal activity (r = -0.75; P < 0.001) and between neutrophil count and myeloperoxidase content (r = -0.54; P < 0.001). By contrast, the change in neutrophil alkaline phosphatase was slower, reaching a peak by day 3 and returning to preoperative levels by day 6, and did not correlate with changes in microbicidal activity or the neutrophil leukocytosis.

The speed of these changes in enzymes was demonstrated by studying 11 patients within 2 h of operation (Table 1). The changes in microbicidal activity, neutrophil enzyme content, and neutrophil count previously followed over a period of days were already well marked.

DISCUSSION

The results show a marked influence of routine surgery on the ability of neutrophil granulocytes to kill a species of Candida (C. guilliermondii), although phagocytosis was not affected. Impaired Candida killing indicates a general microbicidal defect of neutrophils, for it involves the combination of myeloperoxidase, a halide, and hydrogen peroxide and is the same mechanism as is important in the killing of bacteria and viruses, although it is not the only microbicidal mechanism of the cells (4, 15, 17, 18). Whether the impaired microbicidal ability of circulating neutrophils contributes to devel-
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Inflamed tissues is more important than cells in the circulation, and we do not know their microbicidal ability.

The immediate neutrophilia accompanied by a rise in alkaline phosphatase suggests that young cells are released into the circulation from the marginated and marrow reserve pools. There is a continuing rise in neutrophil alkaline phosphatase, reaching a peak 3 days after surgery and subsiding to preoperative levels by day 8. The fact that the highest levels of this enzyme occur in the youngest circulating cells (24) suggests that after hysterectomy the mobilization of reserve pools is followed by increased production and release of neutrophils from the bone marrow which begins to subside after 3 to 4 days. Previous measurements of granulocyte progenitor cells in the circulation after abdominal hysterectomy support this interpretation (20).

Loss of microbicidal ability of individual neutrophils may be due, at least in part, to loss of myeloperoxidase, since the pattern of change in microbial killing correlated well with changes in this enzyme, although there are other candidal mechanisms within human neutrophils which do not involve this enzyme (17). Myeloperoxidase levels within normal neutrophils fall during ingestion and killing of Candida in vitro due to the release of lyosomal enzymes contained within the primary granules of the cells (6). Similarly, degranulation is the probable mechanism for enzyme loss in vivo, since increased serum levels of lysozyme and myeloperoxidase have been detected after burns and during infections, when neutrophil microbicidal ability is impaired (1, 10). The usual stimulus for degranulation is phagocytosis of opsonized particles or immune complexes (11), but such particles are unlikely to be circulating within 2 h of elective abdominal hysterectomy. An alternative mechanism might be the soluble complement component C5a, which is a nonphagocytosed stimulus of neutrophils that could be released during surgery by interaction of complement with traumatized and dead tissue (8, 12). The anesthetic is unlikely to be important, since a neutrophil leukocytosis does not follow examinations under anesthetic when there is no tissue trauma. The fall in myeloperoxidase accompanies the neutrophil leukocytosis, and the two are so closely associated that they would seem to be responding to the same stimulus. Whatever the stimulus is, loss of myeloperoxidase and impaired microbicidal ability occur very quickly after surgery and probably indicate that the circulating neutrophils have already discharged some of their granules and are partially exhausted (7).

**Fig. 1.** Changes in leukocyte numbers, microbicidal ability, and neutrophil enzyme content after elective abdominal hysterectomy. Results are shown as means ± standard error. TLC, Total leukocyte count; PMN, polymorphonuclear neutrophils.
The changes in neutrophil granulocyte numbers and function after surgery are very similar to those found during infections (16, 22, 23). Impaired bactericidal activity during infection has been related to the appearance of immature cells in the circulation, with the suggestion that infection interferes with the normal production of enzymes, including myeloperoxidase, in the maturing granulocytes of the bone marrow (9, 16, 23). However, changes occur too quickly after surgery for production of defective new cells to be a likely mechanism, for passage of cells from the myelocyte stage in the marrow to band and polymorphonuclear forms in the circulation requires at least 8 h, even during a neutrophil leukocytosis (5). Myeloperoxidase synthesis is complete by the myelocyte stage of maturation (4), and immature granulocytes separated by density centrifugation contain the same amount of this enzyme as mature cells (19). Consequently, there is no reason to expect young neutrophils to lack this enzyme. It is more likely that both surgical trauma and infection cause degranulation of already mature cells and that this is the mechanism for loss of myeloperoxidase and microbicidal activity.

ACKNOWLEDGMENTS

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LITERATURE CITED


Table 1. Neutrophil granulocyte numbers, Candida killing, and neutrophil enzymes before and 2 h after operation

<table>
<thead>
<tr>
<th>Time</th>
<th>Neutrophil granulocytes (10^9/ml)</th>
<th>Total leucocyte count (10^9/ml)</th>
<th>% Candida killed</th>
<th>Myeloperoxidase (U/10^9PMN)</th>
<th>Alkaline phosphatase (U/10^9PMN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperation</td>
<td>3.7 ± 0.4</td>
<td>6.0 ± 0.4</td>
<td>53.3 ± 2.1</td>
<td>63.0 ± 3.3</td>
<td>31.9 ± 2.0</td>
</tr>
<tr>
<td>Postoperation</td>
<td>10.8 ± 1.0</td>
<td>13.2 ± 1.1</td>
<td>43.4 ± 1.8</td>
<td>48.2 ± 5.1</td>
<td>44.5 ± 3.6</td>
</tr>
<tr>
<td>Significance (P)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
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* Results are expressed as means ± standard error. PMN, Polymorphonuclear neutrophils.