Periodic Variation in the Antibacterial Function of Human Neutrophils and Its Relationship to Sepsis

J. Wesley Alexander, M.D., Sc.D., Renzo Dionigi, M.D.,*
Jonathan L. Meakins, M.D.

From the Department of Surgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45229

Phagocytic cells have been known to play an important role in resistance to bacterial infection since the early studies of Metchnikoff,[7] but congenital and acquired abnormalities of their antibacterial functions have been documented only recently.[1, 2, 4, 5, 9] Neutrophils are the most important phagocytic cells to enter developing inflammatory foci, and their functional integrity is responsible for the early destruction of bacteria at sites of potential infection.[6] It has recently been shown that the onset of life threatening sepsis in a variety of surgical diseases was preceded by the development of a diminished ability of the patient's neutrophils to kill ingested bacteria.[1, 4] In those studies, abnormalities of neutrophil function appeared to occur at periodic intervals in the few patients tested for long periods of time. Because of the exceptional biological importance of such an observation, the constancy of neutrophil function was studied for extended periods in both normal persons and patients known to be subject to the development of sepsis.

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* Present address: Instituto Di Semeiotica Chirurgica, Università Di Pavia, Italy.

Materials and Methods

A test of neutrophil function, described in detail previously,[1, 3] was used to study the ability of circulating neutrophils to ingest and kill *Staphylococcus aureus 502A*. In principle, approximately 5×10^6 isolated and washed neutrophils from each subject were continuously mixed with $1\text{–}2 \times 10^6$ *Staphylococcus aureus* at 37° C. in a tissue culture medium containing a 10% concentration of pooled human serum as a source of opsonins. The number of viable bacteria remaining in each test after 4 hours of neutrophil-bacterial interaction was determined by a standard pour plate technic. Gentle handling and accurate quantitation of the cells are important features of the test.

Results of the neutrophil function test were expressed in terms of a neutrophil-bactericidal index (NB index): the ratio between the bacteria *not killed* by a subject's neutrophils and the bacteria *not killed* by a normal control's neutrophils. If, for example, the NB index was 10, ten times

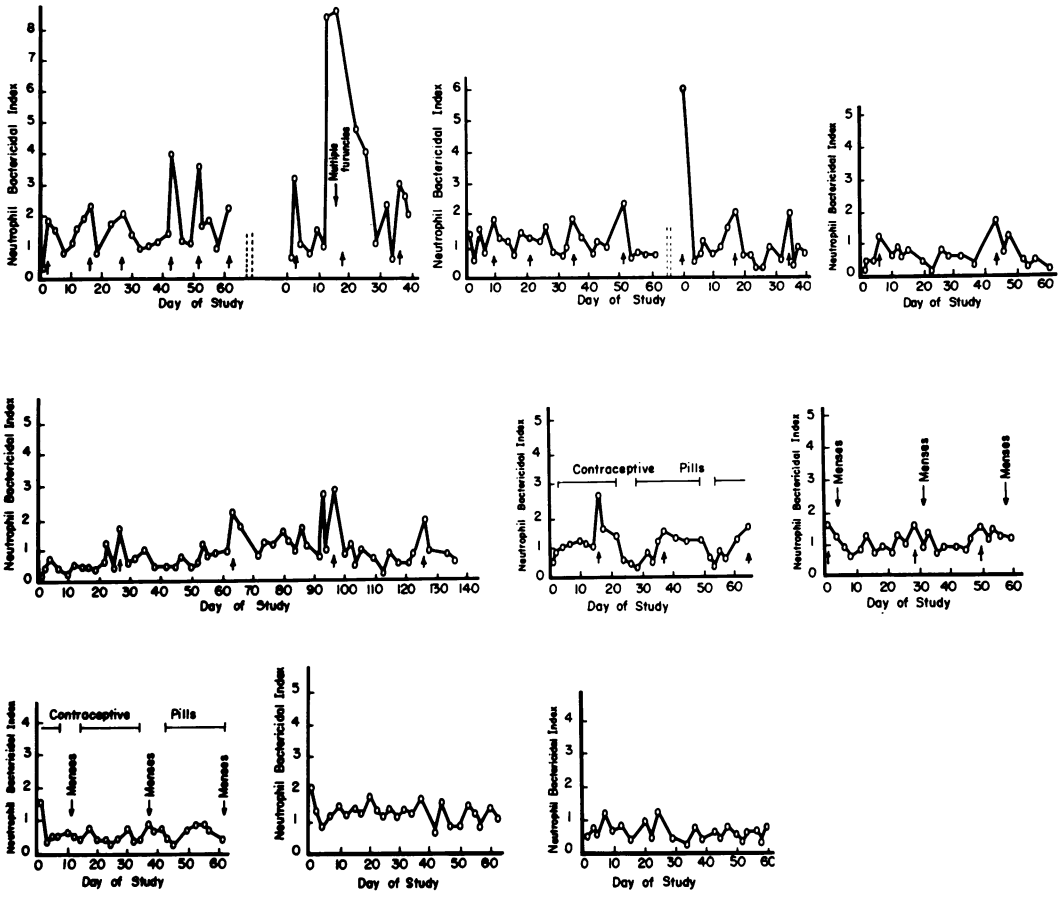

FIG. 1. Neutrophil function tests in normal volunteers. Two of the four women were taking contraceptive pills, and two were amenorrheic. In this and the following figures, the arrows at the bottom of the charts indicate the periods of relatively poor neutrophil function (elevated NB index). The first two subjects were also studied at another time. A (upper row, left) Male, aged 35, only normal subjects with documented infection during the period of study. B (upper row, center) Male, age 30; C (upper row, right) Male, age 29; D (middle row, left) Female, age 35; E (middle row, center) Female, age 26; F (middle row, right) Female, age 32; G (lower row, left) Female, age 32; H (lower row, center) Male, age 26; I (lower row, left) Male, age 27.

more bacteria remained alive in the subject's test than in the normal control's test after the period of incubation. NB indexes between 2–4 were considered to represent mild abnormalities; NB indexes between 4–8, moderate abnormalities; and NB indexes greater than 8, severe abnormalities.[1]

Circulating neutrophil counts and ratios of segmented to non-segmented neutrophils were determined for each of the subjects in an attempt to correlate these with changes in the ability of neutrophils to kill bacteria.

Normal Volunteers

Nine adult human volunteers, ages 21 to 36, were studied simultaneously each Monday, Wednesday, and Friday for at least 60 days. The number of bacteria not killed during the bacteria-phagocyte interaction was determined, and a "control" value was obtained by taking an average of the bac-

terial counts for the median six subjects. These counts seldom varied between highest and lowest to an extent greater than a ratio of 1.5:1. The neutrophil-bactericidal index (NB index) was then calculated for each individual by dividing the "control" number into the number of viable bacteria which remained in the subject's test. In this instance, therefore, the NB index provided a comparison of the killing capacity of neutrophils from a given subject with an average of the killing capacities of neutrophils from several controls. Two of the subjects (Fig. 1A & B) were studied in a similar manner for an additional 40 days at another time, and these results are included. One person (Fig. 1D) was studied for a longer period of time because of a proven diagnosis of chronic L-form septicemia with recurrent thrombophlebitis.

Patients Receiving Immunosuppressive Drugs

Eleven patients who had renal transplants and one patient with systemic lupus erythematosus were studied thrice weekly for as long as 6 months. Each patient received azathioprine and prednisone for immunosuppressive therapy. Neutrophils from one to three normal persons were used for controls, and NB indexes were calculated for each measurement of neutrophil function.

TABLE 1. *Distribution of Values for the NB Index in the Three Groups of Subjects*

Neutrophil-Bactericidal Index	Number of Determinations: Normal Volunteers	Patients Receiving Immunosuppressive Drugs	Burn Patients
0–2	265 (92.7%)	214 (61.7%)	53 (39.3%)
2–4	15 (5.2%)	79 (22.7%)	44 (32.6%)
>4	6 (2.1%)	54 (15.6%)*	38 (28.1%)**
Total	286	347	135

* Significantly different from normal volunteers ($X^2 = 33.1$, $p < 0.001$).

** Significantly different from normals ($X^2 = 66.5$, $p < 0.001$) and patients receiving immunosuppression ($X^2 = 24.8$, $p < 0.001$.).

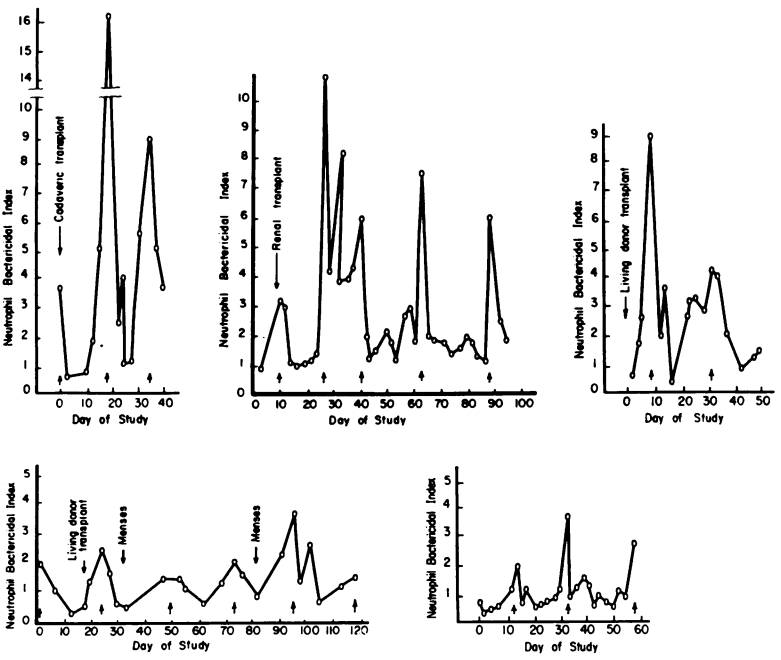

FIG. 2. Neutrophil function tests in patients receiving immunosuppressive drugs. All had renal transplants except the last patient who was being treated for systemic lupus erythematosus. A (upper left) Male, age 46; B (upper center) Male, age 28; C (upper right) Male, age 25; D (lower left) Female, age 17; E (lower right) Female, age 38.

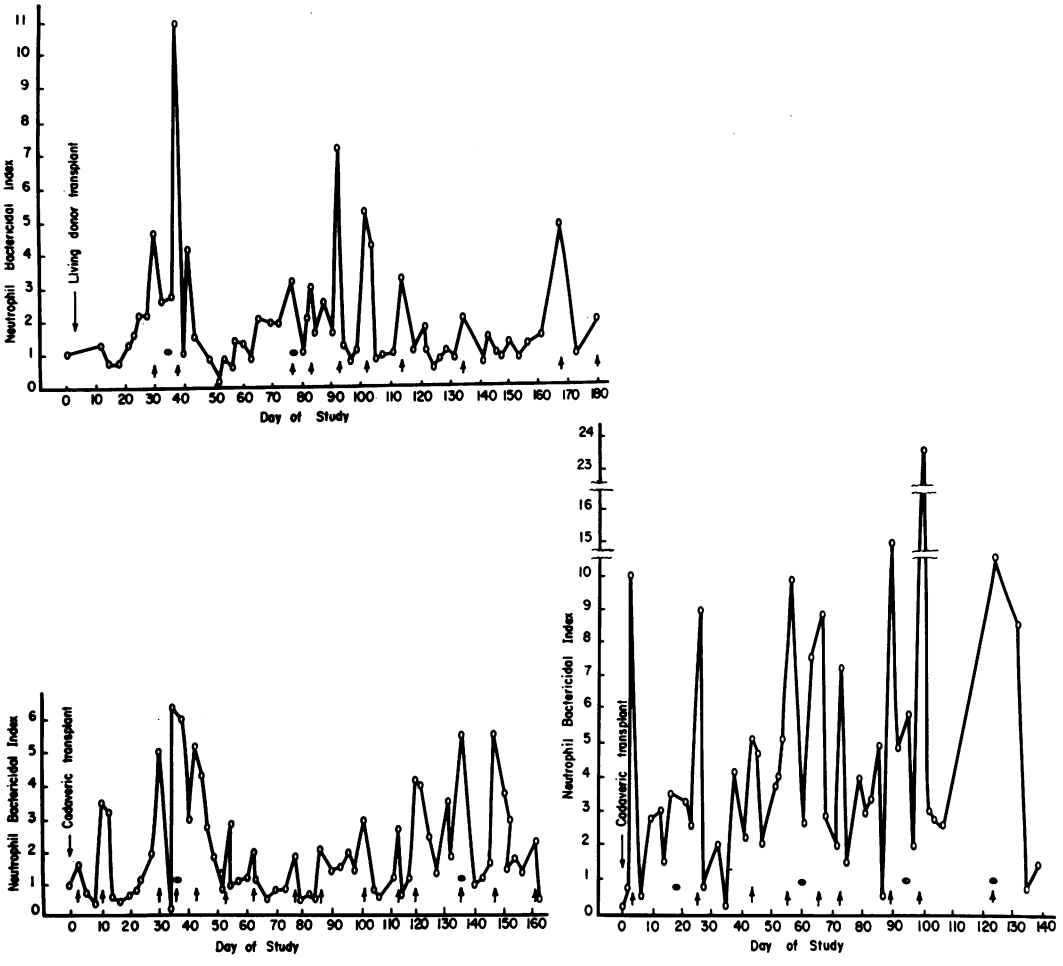

FIG. 3. Tests on three patients receiving immunosuppressives following renal transplantation. These patients were thought to have evidence of two cycles of different periodicity. ⓘ indicates intervals of the longer cycle. A (upper) Male, age 20; B (lower left) Male, age 48; C (lower right) Male, age 28.

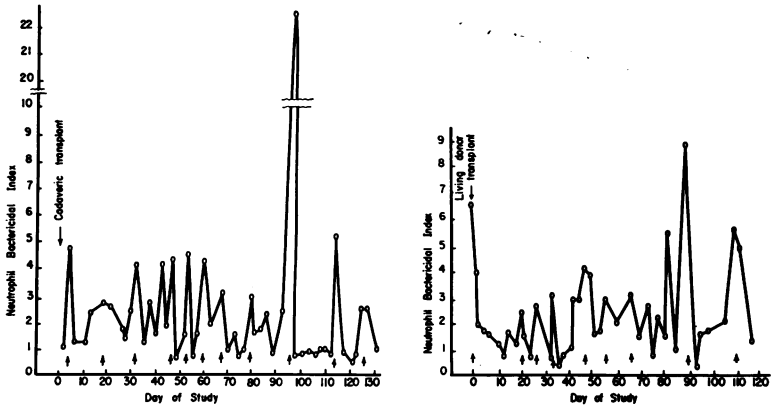

FIG. 4. Serial tests on two patients following transplantation. The significance of the irregular fluctuations is not known. A (left) Female, age 35, B (right) Male, age 36.

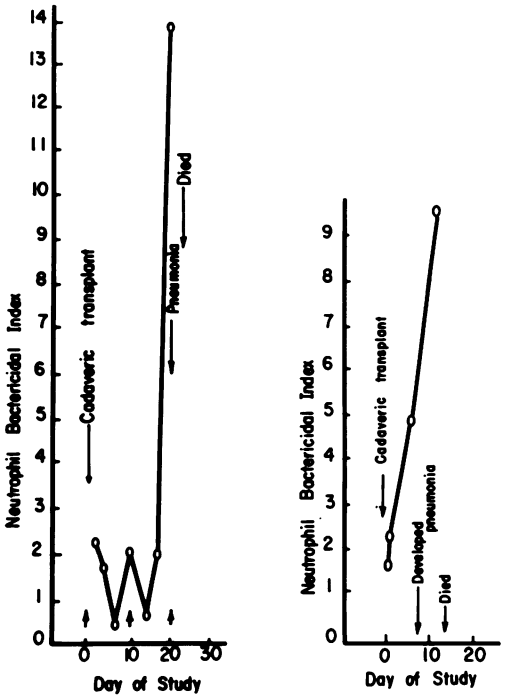

FIG. 5. Serial neutrophil function tests on two patients who developed fatal sepsis following cadaveric renal transplantation. In both, the onset of sepsis was related to an abnormality of neutrophil function. A (left) Female, age 50, B (right) Male, age 52.

Burn Patients

Neutrophil function tests were similarly done on eight patients with thermal injuries involving 46% to 60% total body surface area for periods ranging from 20 days to 80 days.

Results

A correlation between changes in neutrophil function, circulating neutrophil counts, and segmented to non-segmented neutrophil ratios could not be demonstrated in this study. However, significant observations were made concerning periodic variations in the antibacterial function of human neutrophils.

Normal Volunteers

Two hundred eighty-six measurements of neutrophil function were done in this group of volunteers (Table 1). Over 92% of the values for the NB index were less than two. Six measurements (2.1%) were above four, and five of these were in one subject (Fig. 1A). At the time of the poorest function of this subject's neutrophils, he spontaneously developed multiple furuncles. No other infections occurred in this group. Six of the nine subjects (Fig. 1A–F) showed evidence of a regularly occurring variation of neutrophil function. Depending upon the subject, the intervals between episodes of relatively poor function ranged from 12 to 40 days. In three subjects (Fig. 1G–I), a pattern of cycling could not be demonstrated.

Patients Receiving Immunosuppressive Drugs

Three hundred forty-seven measurements of neutrophil function were done in these 12 patients (Table 1). Compared to the normal subjects, only 61.7% of the determinations for the NB index were less than two, and 15.6% of the determinations were greater than four ($P < 0.001$).

In five patients, there was good evidence of a regularly occurring abnormality of neutrophil function (Fig. 2). The intervals between the relative abnormalities varied from 14 to 24 days, again being somewhat constant for the individual patient. The pattern was different, however, in another five patients. Three of these seemed to have two basic cycles which were superimposed (Fig. 3). In the shorter cycle, the periodic abnormality of neutrophil function often occurred as frequently as 10 days or less. Two patients (Fig. 4) had evidence of cycling part of the time, but there were also periods of irregular fluctuations in neutrophil function which could not be explained. The remaining two patients (Fig. 5) were followed for an insufficient period to establish a cyclic pattern. However,

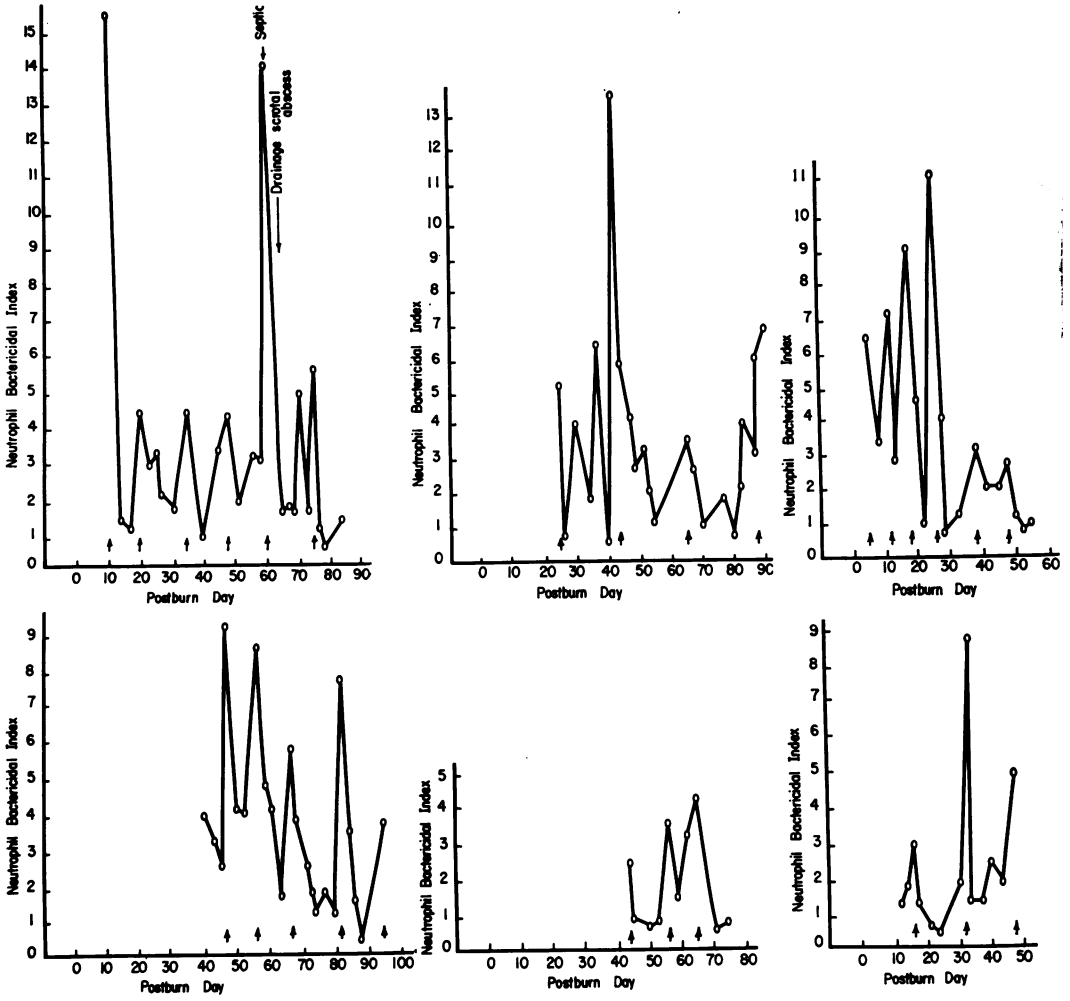

FIG. 6. Serial neutrophil function tests in six burn patients who survived. A (upper left) Male, age 11. Burn: 50% total, 45% 3°; B (upper center) Female, age 3. Burn 50% total, all 3°; C (upper right) Female, age 13. Burn: 46% total, 42% 3°; D (lower left) Female, age 40. Burn: 50% total, 46% 3°; E (lower center) Male, age 13. Burn: 46% total, 3% 3°; F (lower right) Male, age 4. Burn: 56% total, 54% 3°.

associated with a severe (NB index > 8) or moderately severe (NB index 4–8) abnormality of neutrophil function, both developed extensive pneumonias which resulted in death from sepsis.

Burn Patients

Basic neutrophil function was found to be distinctly abnormal in this group, with 28.1% of the 135 determinations for NB index greater than four, a highly significant difference from the preceding groups (Table 1). A basic pattern of regularly occurring abnormalities could be established in each of these patients (Figs. 6 & 7), but the length of time between the periodic abnormalities tended to be shorter than in the previous patients. Three of the patients became septic, associated with the presence of abnormal neutrophil function (high NB index). Two of these patients died from sepsis (Fig. 7), and the third recovered (Fig. 6A).

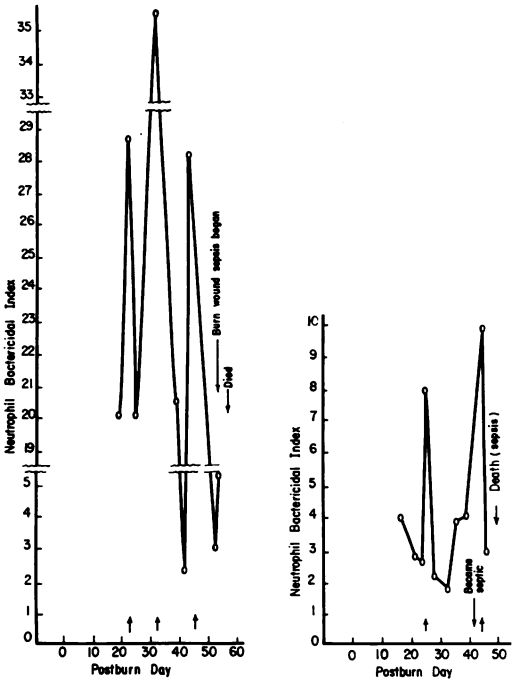

FIG. 7. Neutrophil function tests in two burn patients who died with classical burn wound sepsis. A (left) Female, age 7. Burn: 57% total 52% 3°, B (right) Female, age 10. Burn: 54% total, 49% 3°.

Discussion

The results of this study demonstrate that the ability of human neutrophils to kill bacteria is not constant. A relative abnormality occurs in the majority of tested persons at periodic intervals which vary individually between 10 and 40 days, usually between 14 and 24 days. Although the interval for the cycle of neutrophil function in this study was similar to the interval described by Morley [8] for a cyclic variation in the neutrophil counts of healthy individuals, a correlation between the number of circulating neutrophils and their antibacterial function could not be demonstrated.

Our results suggest that the periodic abnormality of neutrophil function might be related to the menstrual cycle of women. However, men and children also have this basic cycle, and preliminary endocrine ablation experiments in experimental animals have not shed light upon the regulatory mechanism of neutrophil function. Dogs in which the gonads and adrenal glands have been surgically extirpated continue to have a cyclic variation in neutrophil function.[4] The relative abnormality encountered during these studies was a diminished ability to kill ingested bacteria rather than an inability to ingest them,[1, 4] indicating the presence of a mechanism which controls either degranulation or the synthesis of important antibacterial substances within the neurophilic granules. The variability in the baseline of neutrophil function with immunosuppressive therapy and burn injury and the possibility of a dual cycle suggest that there are mutiple factors which influence the basic antibacterial function of human neutrophils.

The development of bacterial infection in humans depends upon the interaction of a large number of variables, but it is increasingly evident that the antibacterial function of neutrophils is one variable which has great clinical significance. Six of the 29 subjects in the present study developed infections during the course of observation, and each of these infections was associated with an NB index of 5 or more. Both transplant patients and two of the three burn patients who developed sepsis died as a result of the complications. The sixth subject with acute bacterial infection, a normal person, developed numerous furuncles at the time of an exceptionally high NB index. While the development of a high NB index was often not accompanied by sepsis, the risk of sepsis correlated well with the degree of abnormality, demonstrated by this test, and in this and previously reported studies,[1, 4] the development of bacterial sepsis has always been associated with a high NB index. It seems probable that abnormalities of neutrophil function contribute significantly to the development of

many unexpected infections in humans. Clearly, further investigation of the processes involving phagocyte-bacterial interactions can lead only to improved patient care.

Summary

Serial measurements were made of the antibacterial function of neutrophils from 29 human subjects for periods as long as 6 months. In the majority, there was clear evidence of a periodic variation in the ability of their neutrophils to kill bacteria. The relative abnormality of neutrophil function tended to occur at regular intervals, usually between 14 and 24 days. Burn injury and the administration of immunosuppressive drugs adversely affected the antibacterial function of neutrophils but did not abolish the basic cycle. The clinical importance of continued investigation of this cyclic function is emphasized by the observation that invasive sepsis appears to occur predominately at those times when neutropil function is relatively poor.

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