Assessing the Delivery of Neutrophils to Tissues in Neutropenia

By D.G. Wright, A.I. Meierovics, and J.M. Foxley

Studies of neutrophil kinetics in neutropenic individuals, as well as clinical observations of variability in the occurrence of infection among patients with neutropenia, have suggested that blood neutrophil counts may not uniformly reflect the effective delivery of neutrophils to extravascular tissues where the cells perform their principal host defense functions. To evaluate this possibility we developed a sensitive, reproducible method of measuring the extravascular delivery of neutrophils to a normal mucosal site of neutrophil turnover. This method is based upon the quantification of neutrophils recoverable from saline mouth wash specimens. Twenty-five mL specimens, obtained in a controlled manner from neutropenic patients and normal subjects, were centrifuged and the sediments resuspended in 1.0 mL Hank’s buffer with 2 μg acridine orange, incubated at 37 °C for 15 minutes, and then examined in a hemocytometer chamber by fluorescence microscopy. Neutrophils could be clearly distinguished by their characteristic fluorescence and were counted. With this method as few as 1,500 neutrophils were detected reliably in mouth wash specimens. Mucosal neutrophil counts varied less than 10% with repeated sampling of individual subjects over 5-day periods and were consistently greater than 1.3 × 10⁶/specimen in non-neutropenic individuals. Although profound neutropenia was generally reflected by lower than normal oral mucosal neutrophil counts, these counts were significantly higher in individuals with chronic severe neutropenia (blood neutrophils <300/mm³) than in patients with acute neutropenia of comparable severity that had developed following chemotherapy. Also, in individuals recovering from profound neutropenia, neutrophils usually reappeared earlier in mouth wash specimens than in blood, and oral mucosal neutrophil counts attained recovery levels more rapidly than did blood counts. This phenomenon was particularly evident in an individual with cyclic neutropenia. Moreover, mucosal neutrophils could occasionally be detected in profoundly neutropenic patients when neutrophils were not present in blood samples. These findings indicate that mucosal neutrophil counts in individuals with neutropenia provide information about the delivery of neutrophils to tissues that may not be evident from blood neutrophil counts alone.

It is well known that neutropenia is associated with an increased risk of bacterial and fungal infections, particularly when blood neutrophil counts are less than 500/mm³. However, neutropenic individuals may vary considerably in their apparent susceptibility to infection. The incidence and severity of infections are especially variable among individuals with chronic neutropenias. The clinical courses of such individuals are often remarkably benign despite profound neutropenia, unlike the usual experience of patients with an equivalent degree of neutropenia that is of acute onset following myelotoxic chemotherapy or radiation.

Variability in the susceptibility to infection in neutropenia suggests that blood neutrophil counts may not uniformly reflect either the body’s supply of neutrophils or the delivery of neutrophils to extravascular tissue sites where the cells perform their principal host defense functions. Furthermore, studies of neutrophil kinetics in both neutropenic and normal individuals, using techniques dependent upon the measurement of radiolabeled neutrophils in the circulation, have shown that an acute or chronic redistribution of neutrophils from marrow storage to the intravascular space and/or between circulating and marginated pools may cause variations in blood neutrophil counts that do not reflect changes in the body’s total neutrophil supplies or the neutrophil turnover rate. It has been observed in particular that the proportion of neutrophils in the marginated pool tends to increase as blood neutrophil concentrations decrease both in normal subjects4 and in neutropenic patients. These observations also suggest that blood neutrophil counts by themselves may be misleading with respect to the actual availability of neutrophils to tissues in neutropenic individuals. Studies of the delivery of neutrophils to tissues using the Rebuck skin window technique have in general demonstrated an impairment of neutrophil delivery to tissues in profoundly neutropenic patients. However, this technique, which is time consuming and difficult to standardize, is unlikely to be able to detect differences in the delivery of neutrophils to tissues that may exist among individuals with similar degrees of neutropenia, nor is it readily applicable to the study of patients with profound neutropenia associated with myelosuppression and thrombocytopenia.

In order to examine variations in the delivery of neutrophils to extravascular tissues that may exist in neutropenia, we developed a method of quantifying neutrophils that can be washed from the oral mucosa. This method is a refined version of a technique that has been used for some time to provide a semiquantitative measure of periodontal disease severity in hematologically normal individuals. Using this method, we find that mucosal neutrophil counts provide information about the delivery of neutrophils to tissues that may not be apparent from blood neutrophil counts alone. We find, in particular, that the supply of neutrophils to tissues as reflected by mucosal neutrophil counts is frequently underestimated by blood neutrophil counts in patients with chronic neutropenias and during the early phases of recovery from acute neutropenic episodes.

MATERIALS AND METHODS

Study subjects. Twenty-seven healthy men and women between the ages of 22 and 58, with a normal oral and pharyngeal exam and with teeth in good repair, were studied to define a normal range for measurements of oral mucosal neutrophil counts. Mucosal neutrophil counts were also measured in two men and two women (ages 51 to 60) who were edentulous but otherwise in good health. These
healthy individuals were specifically without clinically evident oral mucosal disease or symptoms, and all had normal blood neutrophil counts (2000 to 6000/mm³) at the time of study. Twenty-six individuals with either acute or chronic neutropenia, who were patients at the Walter Reed Army Hospital, were also studied. Of these, eight had only mild to moderate degrees of neutropenia (between 500 and 1800/mm³) at the time of study and 17 had blood neutrophil counts in the severely neutropenic range (0 to 300/mm³). Acute neutropenias were in all cases the result of myelosuppressive chemotherapy given within seven days of the onset of neutropenia. All patients with chronic neutropenias were known to have had neutropenia for at least 2 months prior to study. Underlying conditions in these neutropenic subjects are summarized in Table 1.

Patients and healthy volunteers were intentionally selected such that the study populations would be free of moderate or severe oral mucosal disease. The oral mucosa was examined in potential study subjects and the status of mucosal tissues was estimated by defining a gingival index.9 The scoring criteria for determining this gingival index were as follows: a score of 0 = an absence of inflammation in gingival tissues; 1 = mild inflammation, characterized by reddening of the tissues without change in texture; 2 = moderate inflammation, characterized by moderate glazing, redness, edema, and hypertrophy of gingival tissues with or without bleeding on pressure from a probe; 3 = severe inflammation, characterized by marked redness and hypertrophy with a tendency toward spontaneous bleeding, with or without ulcerations. All of the normal subjects had a gingival index score of 0, and none of the neutropenic subjects included in this study (other than a patient with cyclic neutropenia) had a gingival index score greater than 1 at the times that mucosal neutrophil counts were measured. All study subjects gave informed consent for the examinations that were performed in accordance with a human use protocol approved at Walter Reed.

Recovery and quantification of neutrophils from the oral mucosa. Neutrophils present in the tissues of the oral mucosa were quantified by counting these cells in timed, saline mouth wash specimens. After an initial evaluation of several variations in technique discussed below, the following basic method for obtaining and examining specimens was used. Sterile, normal saline solution (0.9 g% used. Sterile, normal saline solution (0.9

After an initial evaluation of several variations in technique discussed below, the following basic method for obtaining and examining specimens was used. Sterile, normal saline solution (0.9 g%) was measured into 25 mL aliquots in spumus cups. Study subjects swirled these measured amounts of saline in their mouths for 30 seconds, timed by a stop watch, and then returned the specimen to the spumus cup. Duplicate specimens were obtained with each sampling, one specimen obtained immediately following the other. The specimens were then centrifuged at 200 g for 10 minutes (within one hour of sampling), the supernatants decanted and discarded, and the pellets resuspended with a pasteur pipette in 1.0 mL of Hank's balanced salt solution (without phenol red) which contained 2.0 µg/mL acridine orange (3,6-bis[Dimethylamino] acridium chloride hemi-[zinc chloride salt], Sigma, St. Louis, Mo). These sediment suspensions were then incubated for 15 minutes at 37 °C in a shaking water bath and again resuspended thoroughly with a pasteur pipette. A drop of each suspension was transferred to a hemocytometer chamber (American Optical, Buffalo, NY) and examined by fluorescence microscopy, using a 20 x, long focal length objective lens and 10 x ocular lenses.

Neutrophils were clearly distinguishable from extraneous cellular and microbial debris in the specimen by their characteristic orange granular fluorescence (Fig 1B). Rarely, budding yeast forms or epithelial cells showed some orange fluorescence but this was readily differentiated from that of neutrophils in the specimen. Monocytes were also observed to fluoresce after staining with acridine orange. However, the orange granular fluorescence and yellow-green nuclear fluorescence of monocytes is much less bright than that of neutrophils, and these cells are distinguishable both by cytofluoro-

graphy13 and by eye. In performing mucosal neutrophil counts, cells with equivocal fluorescence staining were not counted.

The area that the 20 x microscope field covered on the hemocytometer grid (final magnification = 200 x) was determined to be 0.672 mm², defining a volume of 0.672 mm³ per 10 fields. Total neutrophils seen in 10 separate microscope fields were counted, and the numbers converted to the neutrophil concentration in the mouth wash sediment suspension according to the following formula: Neutrophils in specimen = (number of cells/10 fields) x (1.49) x (10²). For convenience, mucosal neutrophil counts were expressed in general as cells/10 microscope fields. In serial studies with patients, saline mouth wash specimens were always obtained at mid-morning (about 10 AM).

For certain studies, up to six successive mouth wash specimens were obtained, one immediately after the other; replicate specimens were also obtained at 15-minute intervals. To assess the accuracy of this fluorescence staining method for counting neutrophils, the method was also applied to counting neutrophils present in leukocyte suspensions prepared by dextran sedimentation of normal venous blood, for which the numbers of neutrophils, monocytes, and lymphocytes had been calculated separately from standard chamber counts and differentials of Wright-Giemsa stained cytospins.

Peripheral blood neutrophil counts. For blood neutrophil counts, venous blood samples (5 mL) were collected into standard vacutainer tubes anticoagulated with EDTA (Vacutainer Systems, Rutherford, NJ). Blood smears stained by an automated method with Wright-Giemsa were prepared, and total white blood cell counts were determined by a Coulter particle counter (Coulter Electronics, Hialeah, Fla). Absolute neutrophil counts were calculated from total white blood cell counts and 200 cell leukocyte differentials done exclusively by one of two observers (DW or JF). In serial studies with patients and with normal volunteers, blood samples were routinely obtained shortly before the mouth wash specimen.

RESULTS

Recovery of oral mucosal neutrophils from healthy hematologically normal individuals. Neutrophils could be readily seen in Wright-Giemsa stained, cytospin preparations of oral saline rinses obtained from hematologically normal, healthy adults who were free of oral or dental disease (Fig 1A). However, in order to quantify these cells in a reproducible manner and with some degree of detection sensitivity, it was necessary to use a selective staining technique that would permit relatively small numbers of neutrophils to be clearly distinguished from a large and highly variable quantity of epithelial cells, cellular debris, and microbial flora. This was accomplished by exploiting the fluorescence staining properties of acridine orange, a stain upon which certain automated fluorescence activated leukocyte differential systems have been based.13 As is illustrated in Fig 1B, neutrophils that have taken up this stain fluoresce

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<th>Underlying Conditions</th>
<th>Acute Neutropenia</th>
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<tr>
<td>Acute myelogenous leukemia</td>
<td>4</td>
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<tr>
<td>Acute lymphocytic leukemia</td>
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<td>Feit’s Syndrome</td>
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<td>Metastatic carcinoma of breast</td>
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<td>Cyclic neutropenia</td>
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<td>Total</td>
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Table 1. Neutropenic Subjects: Underlying Conditions
Fig 1. Oral mucosal neutrophils. (A) Wright-Giemsa stained, cytospin preparation of a saline mouth rinse specimen from a hematologically normal volunteer with healthy oral mucosa (approximate magnification × 300). (B) Fluorescence microscopy of acridine orange stained suspension of mouth rinse sediment from healthy normal volunteer (approximate magnification × 300).
brightly and stand out clearly under fluorescence microscopy. Because of their characteristic orange granular fluorescence, nuclear morphology, and size, neutrophils could be recognized and counted, even if present in very low numbers. By combining this staining technique with a standard hemocytometer chamber, it was possible to measure neutrophil concentrations reproducibly down to a detection threshold of approximately 1,500 neutrophils in an individual 25 mL mouthwash specimen. This fluorescence staining method was found to detect neutrophils accurately in a mixed leukocyte preparation over a broad range of cell concentrations (Fig 2).

Counts from two successive mouthwash specimens, one obtained immediately after the other, were averaged. Unexpectedly, the neutrophil count in the second of these successive specimens was not much lower on average than that in the first specimen (2nd specimen count = 92 ± 7% of 1st specimen count, mean ± SEM, n = 32), although neutrophil counts dropped markedly in subsequent mouthwash specimens obtained immediately thereafter (Fig 3A). Moreover, the mean values for two successive counts showed less variability than either count alone when normal volunteers were studied repeatedly at 1-day intervals. Addition of EDTA (10 mg %) to the saline rinse solution increased the recovery of neutrophils slightly (by 5% to 15%) but did not provide more reproducible mucosal neutrophil counts than did normal saline alone, which was more readily applicable to studies of neutropenic patients.

![Figure 2](image-url) Neutrophil counts obtained by fluorescence microscopy of acridine orange stained suspensions of leukocytes (isolated from venous blood by dextran sedimentation) plotted against predicted counts. Samples for counting were prepared by multiple dilutions of a cell suspension with known neutrophil concentration (10^7/mL). This leukocyte suspension contained 75% neutrophils, 8% monocytes, and 17% lymphocytes. Numbers in parentheses represent neutrophils/10 microscope fields detected by fluorescence microscopy. Means ± SEM for 5 replicate determinations are shown.

![Figure 3](image-url) Repetitive oral mucosal neutrophil counts in normal individuals. (A) Mucosal neutrophil counts in successive mouthwash specimens, one taken immediately after the other. Results represent means ± SEM from 5 replicate studies. (B) Daily mucosal neutrophil counts in two healthy subjects whose blood neutrophil counts averaged 3.825 ± 219/mm³ (subject 1) and 2.670 ± 337 (subject 2).

Mouthwash samples from normal volunteers were found to contain up to 10.8 × 10^6 neutrophils and never less than 1.3 × 10^6/sample unless the individual was edentulous (see Fig 4A). Even in this group of study subjects with normal gingival indices and equivalent dentition, there was considerable variability in mucosal neutrophil counts from person to person, and only a very loose correlation between blood neutrophil counts and mucosal neutrophil counts (Fig 4A). However, there was much less variability in mucosal neutro-
phil counts obtained repeatedly from a single person on successive days. This variability was on average less than 10% for 6 normal subjects when studied on each of 5 successive days, or at intervals of greater than 2 hours. Representative results from these studies are shown in Fig 3B.

When mucosal neutrophil counts were obtained from normal subjects at 15-minute intervals (each count representing the average from two successive mouth wash specimens taken at each interval), it was found that the counts fell to a relatively stable level by the third set of samples. Pooled data from 5 normal subjects studied in this manner are shown in Fig 5. If one assumes that the reduced but stable level of mucosal neutrophil counts observed with successive mouth wash sampling represents a balance between the loss of cells that are washed from the mucosa and the arrival of new cells, then this level may be considered to reflect the rate at which neutrophils are delivered to the mucosal sites that are sampled.

Corticosteroids when taken daily have been shown to cause an impairment in the delivery of neutrophils to tissues, as measured by the Reckup skin window assay. This effect of corticosteroids could also be detected with mucosal neutrophil counts. In studies of healthy volunteers, it was found that mucosal neutrophil counts were significantly reduced 6 hours after a single dose of 40 mg prednisone (by 46 ± 12%, mean ± SEM from five replicate studies) even though blood neutrophil counts had more than doubled. As shown in Fig 5, the apparent rate of neutrophil delivery to the oral mucosa reflected by repeat counts at 15-minute intervals was also reduced by this corticosteroid.

Recovery of mucosal neutrophils from neutropenic individuals. Profound neutropenia (< 300/mm³) was in general associated with decreased mucosal neutrophil counts compared with normal controls (Figs 4 B, C, D). However, as with normal individuals, there was considerable variability in mucosal neutrophil counts among individuals with comparable degrees of neutropenia, particularly in the mild to moderately neutropenic range (500 to 1800/mm³). As shown in Figs 4 B, C, and D, the highest degree of correlation between blood neutrophil counts and mucosal neutrophil counts was observed in patients with acute neutropenia during the onset of neutropenia; such correlations were much looser in these same patients during recovery from neutropenia and were inapparent in data from patients with chronic neutropenias.

In patients with acute, severe neutropenia following myelosuppressive chemotherapy, neutrophils were commonly observed to reappear and return to a stable level earlier in the oral mucosa than in the blood. This phenomenon is illustrated in Fig 6A. An analogous phenomenon was very obvious in serial counts obtained from a young woman with cyclic neutropenia (Fig 7).

It was also observed that low but detectable numbers of neutrophils could be recovered occasionally from the oral mucosa in patients with no neutrophils evident in the blood on repeated sampling. An example of this is shown in Fig 6B, which presents data obtained from a patient who was observed for nine days to have no detectable neutrophils in the blood, and who then developed worsening clinical signs of...
21-year-old woman with cyclic neutropenia. Closed circles, solid lines = blood neutrophil counts; open circles, dashed lines = oral mucosal neutrophil counts.

Infection, despite broad spectrum antibiotics, prompting the institution of leukocyte transfusions. Of interest, it appeared that the effects of leukocyte transfusion could be detected in mucosal neutrophil counts in this patient. Although it was not possible to prove in this case that the rise in blood and mucosal neutrophil counts observed with leukocyte transfusions reflected the presence of donor cells, this was highly likely since blood neutrophil counts returned to zero after a day’s hiatus in leukocyte transfusions.

Patients with profound degrees of neutropenia (<300/mm³) that was chronic (ie, established for ≥ 2 months) were distinguishable as a group from patients with acute neutropenias of comparable degrees of severity by the oral mucosal neutrophil counts. As shown in Table 2, mucosal neutrophil counts from patients with profound chronic neutropenias (0 to 100/mm³ and 100 to 300/mm³) were significantly higher than those from patients with acute neutropenias of comparable severity. It was possible that differences in the status of the oral mucosa in these two groups of patients contributed to this observation; however, such differences were not evident from clinical examination of the oral mucosa.

The relevance of mucosal neutrophil counts to the clinical status of these markedly neutropenic patients was suggested by relationships that appeared to exist between the blood and mucosal neutrophil counts and the presence or absence of fever. None of the patients with chronic neutropenias, whose blood and mucosal neutrophil counts were summarized in Table 2, were febrile when studied. In contrast, most of the acutely neutropenic patients represented in Table 2 were febrile at some time during their nadirs of neutropenia.

As has been pointed out above, one of the distinctions between these groups of patients was that mucosal neutrophil counts were on average substantially higher with respect to blood neutrophil counts for patients with chronic, profound neutropenias than was the case for patients who were acutely neutropenic. Moreover, when the relationship of mucosal neutrophil counts to blood neutrophil counts was expressed as a ratio (mucosal neutrophils per 10 microscope fields, divided by blood neutrophils per mm³), it was found that this ratio was almost always > 1.0 for patients with chronic neutropenias, while it was frequently < 1.0 for the acutely neutropenic patients. Moreover, when individual patients were followed throughout episodes of myelosuppression following cytotoxic chemotherapy, it was observed that the highest degrees of fever tended to occur when the ratios of mucosal neutrophil counts to blood neutrophil counts were lowest, as exemplified by the data shown in Fig 6A.

Table 3 summarizes data from five patients who were studied repeatedly during chemotherapy-induced acute neutropenia. Mucosal and blood neutrophil counts were obtained from each of these patients on at least six separate days during their nadirs of neutropenia. These patients were febrile (≥ 38 °C) on 16 of 30 occasions (53%) when their neutrophil counts were studied; fever ≥ 38.5 °C was recorded on six occasions (20%). As might be expected, the highest fevers tended to occur more frequently with blood neutrophil counts of 0 to 100/mm³ than with counts of 100 to 300/mm³ (P = 0.10, Chi Square; P = 0.18, Fisher exact test). However, when these same data were examined with respect to the ratios of mucosal to blood neutrophil counts, it was found that fever (≥ 38.5 °C) was significantly more likely to be present when the mucosal/blood neutrophil ratio was ≤ 0.4 (the median value observed in these patients), both when all observations (blood neutrophils = 0 to 300/mm³) were analyzed (P < 0.01, Chi Square; P < 0.02, Fisher exact test) and when subgroups of observations (blood neutrophils = 0 to 100/mm³) were analyzed (P < 0.05, Fisher exact test).

**DISCUSSION**

It has long been recognized that neutrophils are normally present on mucosal surfaces, in saliva, and in tears. According to an old clinical adage, a patient’s awareness of a dried conjunctival exudate at the corner of the eye upon awakening is a very early sign of recovery from agranulocytosis. Furthermore, it has been suggested that the presence of neutrophils in conjunctival secretions, or in a swab of the nasal mucosa, might provide useful information for assessing the relative risk of infection in a patient with profound neutropenia. Animal studies have indicated that the delivery of neutrophils to mucosal tissues, particularly that of the gastrointestinal tract, accounts for a substantial proportion of daily neutrophil turnover, and it would appear likely that a constant turnover of neutrophils at such sites constitutes an important component of mucosal barriers against the invasion of resident microbial flora, for when production and turnover of neutrophils cease, sepsis with organisms from this normal flora quickly ensues.

For the studies described above, we developed a method for quantifying mucosal neutrophils recoverable in mouth wash specimens. We then used this method to determine if mucosal neutrophil counts might provide information that is different from or supplemental to that provided by blood neutrophil counts in neutropenic individuals. There are a number of reasons to suspect that the measurement of neutrophils that have been delivered to a normal tissue site of turnover in neutropenic individuals might differ from that of blood neutrophils, which are in transit to tissues. First, if neutropenia is associated with substantial shifts in the proportion of neutrophils in the circulating pool to that of the
Marginal neutrophils, as has been suggested by neutrophil kinetic studies to be the case in neutropenia, or if neutropenia is associated with a decrease in the transit time of neutrophils through the blood to tissues, then one can expect that blood neutrophil counts might underestimate the supplies of neutrophils available to tissues. Second, clinical observations of variability in the occurrence of infection among patients with acute and chronic neutropenias suggest that blood neutrophil counts may not uniformly reflect the effective delivery of neutrophils to extravascular tissues where the cells perform their principal host defense functions.

The results of our studies indicate that blood neutrophil counts do indeed underestimate the delivery of neutrophils to tissues in certain settings of profound neutropenia, particularly among individuals with chronic, steady-state neutropenias, and during early recovery from an episode of myelosuppression when neutrophil reserves and production are returning. An apparent discordance of blood neutrophil counts with the delivery of neutrophils to tissues was also detected by mucosal neutrophil counts in normal individuals who had taken corticosteroid. This finding is consistent with previous observations from studies using the Rebuck skin window technique.

As would be expected, mucosal neutrophil counts indicated in general that the delivery of neutrophils to tissues is impaired in profound neutropenia. Moreover, a direct relationship between blood neutrophil counts and mucosal neutrophil counts was found to be particularly strong during the development of neutropenia following myelosuppression. This relationship, however, was much less apparent during recovery from myelosuppression and among chronically neutropenic patients in whom neutrophil production is preserved albeit at a limited level. The finding that neutrophils reappeared earlier in mucosal counts than in blood counts during myelopoietic recovery is consistent with the diminished correlation between blood and mucosal neutrophil counts that was observed during this phase of acute neutropenia.

Approximations of the relative numbers of neutrophils in mouth rinse specimens have been used to grade the severity of periodontal disease. Nonetheless, the presence of neutrophils in saliva or on the oral mucosa is not per se an indication of mucosal pathology or oral infection, for these cells can be readily detected in healthy individuals with normal mucosal and gingival tissues and with normal dentition. The principal sites of the oral mucosa for neutrophil turnover are in alveolar crevices surrounding the teeth, and our finding of relatively low numbers of oral mucosal neutrophils in edentulous but otherwise healthy people is entirely consistent with the prior observations of others. Variations in the numbers of teeth were, however, unlikely to account for the variability in mucosal neutrophil counts observed among normal and neutropenic individuals, for all study subjects were selected for normal dentition and differed only with respect to the numbers of wisdom molars that were missing.

Counts of oral mucosal neutrophils have been studied

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<th>Table 2. Relationship Between Blood Neutrophil Counts and Oral Mucosal Neutrophil Counts in Patients With Severe Neutropenia</th>
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<tr>
<td><strong>Range of Blood Neutrophil Counts</strong></td>
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<td>(PMNs/mm³)</td>
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<td>0–100</td>
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*Observations from 9 patients with acute neutropenia following cytotoxic chemotherapy (3 AML, 2 ALL, 2 Non-Hodgkins lymphoma, 2 Ca-Breast). Data represent the lowest blood neutrophil counts observed in these patients (3 observations from each of 6 patients, 2 observations from 1 patient, and 1 observation from 2 patients). Mean gingival index scores at times of study for patients with blood neutrophil counts of 0–100/mm³ = 0.7, and for patients with blood neutrophil counts of 100–300/mm³ = 0.8.

†Observations from 8 patients with chronic neutropenia syndromes (2 Idiopathic, 4 Felty's, 2 CLL). Two observations are from each of 7 patients and 3 observations are from 1 patient. Mean gingival index scores at times of study for patients with blood neutrophil counts of 0–100/mm³ = 0.8, and for patients with blood neutrophil counts of 100–300/mm³ = 0.8.

‡Results expressed as mean values ± SEM (number of observations).

§Interrelationship between mucosal/blood neutrophil counts and the presence or absence of fever is significant by Fisher exact test (P < 0.05).

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<thead>
<tr>
<th>Table 3. Fever in Patients With Acute Neutropenia*</th>
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<td><strong>Fever ≥38 °C</strong></td>
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<td>M/B &gt; 0.4</td>
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<td>M/B ≤ 0.4</td>
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<td><strong>Fever ≥38.5 °C</strong></td>
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<td>M/B ≤ 0.4</td>
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<td>Total</td>
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*Observations from 5 patients during the nadir of neutropenia following cytotoxic chemotherapy; 6 observations from each patient.

†M/B = mucosal neutrophil count (PMNs/10 MF) divided by the blood neutrophil count (PMNs/mm³).

‡Percent of observations associated with fever (number with fever/total number).

§Interrelationship between mucosal/blood neutrophil counts and the presence or absence of fever is significant by Fisher exact test (P < 0.05).
previously as a means of assessing the effects of leukocyte transfusions; however, the techniques used in these prior studies were not of sufficient sensitivity to detect either endogenous or transfused neutrophils in profoundly neutropenic individuals except when clinically evident oral mucositis was present. The techniques developed for our present studies, on the other hand, proved to be sufficiently sensitive to detect the presence of mucosal neutrophils even, in some cases, when neutrophils were undetectable in the blood.

Differences in the status of the oral mucosa could account in part for differences in the numbers of oral mucosal neutrophils that were observed among patients with acute and chronic neutropenias, for it is well known that individuals with chronic neutropenias tend to develop recurrent stomatitis and periodontal disease, as was the case in the patient with cyclic neutropenia described in this report. However, if there were differences in the oral mucosa among our study subjects, they were not apparent by direct inspection alone. These subjects were intentionally selected to be free of gingival and dental disease in order to eliminate mucosal inflammation as an independent variable as much as possible.

In any case, our observations are of interest because individuals with chronic, established neutropenias, even when profound, tend to present a relatively benign clinical picture compared with patients who develop acute neutropenia following myelosuppressive chemotherapy. Patients with chronic neutropenias rarely develop sudden, overwhelming sepsis or septicemias, and this clinical distinction is consistent with a difference in the effective delivery of neutrophils to normal mucosal sites of neutrophil turnover.

It has been suggested that monocytes, which are often preserved in chronic neutropenias and may be increased in number, may assume some of the host defense functions of neutrophils, thereby accounting for the relatively mild host defense defect that is often apparent with chronic neutropenia. Monocytes could possibly have masqueraded as neutrophils in the mucosal counts performed on chronically neutropenic study subjects. However, it is unlikely that monocytes accounted for much, if any, of the mucosal neutrophil numbers detected in these patients since the fluorescence staining technique used distinguishes neutrophils from monocytes fairly efficiently.

The apparent relationship between the ratio of mucosal to blood neutrophil counts and the presence or absence of fever in profoundly neutropenic patients is intriguing. Certainly, it is attractive to suppose that a direct measure of the delivery of neutrophils to tissues might supplement blood neutrophil counts in predicting the extent of a host defense defect and the risk of infection in such patients. However, our findings must be interpreted cautiously in this regard, since these studies were not specifically designed to address the question of infection risk. Moreover, the neutropenic patients who were studied represent a selected population. Nonetheless, our findings do suggest that the quantitative measurement of mucosal neutrophils deserves further consideration and study as a clinically useful tool in neutropenic patients.

ACKNOWLEDGMENT

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REFERENCES

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