Circulating and Storage Neutrophil Changes in Experimental Type II Group B Streptococcal Sepsis

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Summary

The availability of neutrophils is an important factor in host resistance to bacterial infection. Therefore, circulating and storage neutrophil quantification was carried out on groups of neonatal rats intranasally inoculated with type II group B streptococci. The dose of type II group B streptococci used produced 56% mortality in 48 neonatal rats with death being due to pneumonia and sepsis. Neutropenia ($1300 \pm 150/\text{mm}^3$ versus $2300 \pm 170/\text{mm}^3$; mean $\pm$ S.D.; $P < 0.01$) and an elevation in band/polymorphonuclear ratio (0.98 ± 0.04 versus 0.30 ± 0.04 ; $P < 0.01$) were observed in infected neonatal rats 24 hr following inoculation. Femoral marrow as well as splenic and hepatic neutrophil storage compartment quantification revealed diminution of postmitotic (polymorphonuclear, band, and metamyelocyte) neutrophils in the infected group ($P < 0.01$) with sparing of the proliferative neutrophils (myeloblasts, promyelocytes, and myelocytes). Repletion of the myeloid but not the splenic or hepatic neutrophil storage compartments with normalization of the neutrophil count and band/polymorphonuclear ratio occurred in animals surviving 72 hr. These studies establish that neutropenia and neutrophil storage pool depletion are prominent features of experimental type II group B streptococci infection in neonates.

Speculation

Although neutrophil storage pool depletion is rare in infected adult animals or humans, its presence can be correlated with an increased mortality. It appears that neutrophil storage pool depletion is a prominent feature of experimental neonatal type II group B streptococci infection. The resulting deficiency of phagocytic cell supply probably represents diminution in the already compromised host defense mechanism of the neonate. Thus, it is postulated that neutrophil storage pool depletion represents a pathophysiologic factor contributing to the high mortality rate observed in neonatal type II group B streptococci infection.

We recently have observed that pronounced depletion of the neutrophil stores in bone marrow aspirates of infected human neonates correlated with a fatal outcome (2). This finding suggested that neutrophil storage pool (NSP) exhaustion, with attendant diminution in neutrophil supply to infected tissues may, in some instances, play a role in the neonate's unusual susceptibility to overwhelming septicemia (11). This hypothesis has led us to explore, in a quantitative fashion, the circulating and storage neutrophil changes in experimental neonatal sepsis.

Because we had observed NSP depletion in human neonates with type II group B streptococci (GBS) sepsis, we elected to simulate human GBS disease in an animal model and to observe the accompanying neutrophil changes. We therefore developed an animal system in which rats in the first 12 hr of life underwent inhalation inoculation with GBS. These animals subsequently

developed the early mortality and pathologic findings describe in the early onset form of human GBS disease (4, 8). We have used this model to define the circulating and storage neutrophil changes which occur with experimental GBS infection.

MATERIALS AND METHODS

PREPARATION OF GROUP B STREPTOCOCCI

GBS from an infected infant was identified by the precipitin method using rabbit antisera (7) and was grown overnight at 37°C in Todd-Hewitt broth. Aliquots were frozen at -70°C . Prior to administration to the animals, an aliquot was thawed, inoculated into fresh Todd-Hewitt broth, and grown to logarithmic phase (4 to 5 hr). To detect possible bacterial contamination, an inoculum was plated onto a sheep blood agar plate. Bacteria were then sedimented by centrifugation, and the concentrated organism were washed three times in sterile phosphate-buffered saline. A standard suspension was prepared by diluting the concentrated washed organisms in phosphate-buffered saline to an optical density of 0.9 at 620 nm (14). These suspensions contained approximately 5×10^6 colony-forming units/5 μl .

ANIMAL INOCULATION

Sprague Dawley rats (15) in the first 12 hr of life were used. Bacterial suspensions in 5 μl were instilled into the anterior nares with a glass syringe (16). The animals' mouths were held shut until the inoculum had been aspirated. Control animals received 5 μl of sterile PBS intranasally. Rats were then placed 10 to 12 cage with the mothers. Twelve-wk-old rats anesthetized with methoxyfluorane (17) received 1×10^6 organisms/g body wt in one μl phosphate-buffered saline per g body wt (the same dose which produced 50% mortality in neonatal rats).

CULTURES OF INOCULATED ANIMALS

To detect systemic infection with group B streptococci, culture were performed on blood, lung, spleen, kidney, liver, and brain of animals inoculated with group B streptococci and also on control animals inoculated with PBS. Blood samples were inoculated directly onto sheep blood agar plates and into Todd-Hewitt broth. Organ specimens were homogenized with 1 ml Todd-Hewitt broth plated directly onto sheep blood agar plates, and also grown in Todd-Hewitt broth overnight.

QUANTITATIVE STUDIES OF CIRCULATING AND STORAGE NEUTROPHILS

Nucleated blood cell counts were performed electronically (18) in triplicate, and the average was determined. Cover slip blood smears were stained with Wright's stain and subjected to a 200-cell differential count. The mature NSP [all polymorphonuclear:

PMN's), bands, and metamyelocytes] were measured from the liver, spleen, and femoral marrow in the following manner. The liver and spleen were removed and separately sieved in 1-cc flasks balanced salt solution through a steel mesh gauze with 700 pores/cm². Then the suspensions were counted electronically in triplicate, and the average was recorded. Wright's stained smears of the suspensions were prepared and subjected to a 500- to 1000-cell differential count.

To obtain bone marrow specimens, the femurs were removed, and a 25 gauge needle was inserted into the femoral head through which 1.0 ml Hanks' balanced salt solution was flushed with a tuberculin syringe (1). The resulting cell suspensions were counted electronically, and a 1000-cell differential count was performed.

RESULTS

ANIMAL INOCULATION MORTALITY STUDIES

Forty-eight newborn rats weighing 4.5 to 5.5 g experienced 56% mortality after intranasally receiving 1×10^6 GBS organisms per body wt. Eighty-five percent of all deaths occurred between 24 and 48 hr after inoculation with an occasional animal dying as late as 1 wk postinoculum. Postmortem studies included bacterial cultures of the brain, lungs, liver, spleen, kidneys, and blood which were all positive for GBS. Hematoxylin- and eosin-stained post-mortem tissue sections confirmed the presence of pneumonia and

Fig. 1. a, blood neutrophil count in groups of infected and control rats 24 hr following inoculation and in survivors 48 and 72 hr following inoculation. Bracket, S.D.; b, band/PMN ratio in groups of infected and control rats following inoculation. Bracket, S.D.

Fig. 2. a, mature neutrophils in the femur in groups of infected and control rats 24, 48, and 72 hr following inoculation; b, mature neutrophils in the spleen in both groups following inoculation; c, mature neutrophils in the liver in both groups following inoculation. Bracket, S.D.

demonstrated areas of pulmonary macro- and microhemorrhage. Hepatic and splenic congestion were seen, but no pathological changes were observed in the brain or meninges. In 10 twelve-wk-old rats, the same dose of organisms per g body wt produced no positive blood cultures, no mortality, and no recognizable morbidity. In 28 neonatal rats receiving a much lower intranasal inoculation of 2×10^1 GBS, 2 deaths (7% mortality) were observed. Opsonic antibody specific for GBS were not found in any of the sera of either the 12-wk-old or the neonatal rats (3).

QUANTITATIVE STUDIES OF CIRCULATING AND STORAGE NEUTROPHILS

Twenty-four hr following intranasal GBS inoculation (a time when no mortality had yet occurred), neutropenia (Fig. 1a) and an elevated band/PMN ratio (Fig. 1b) were detected ($P < 0.01$). NSP assessment showed a reduction of the mature neutrophils (PMN, bands, and metamyelocytes) in femoral splenic and hepatic storage compartments ($P < 0.01$) but no reduction in the proliferative (myeloblast, promyelocyte, and myelocyte) neutrophil pool (Fig. 2).

Between 24 and 48 hr following inoculation, 85% of the GBS recipients died. Those surviving to 48 hr were seen to have neutrophilia (Fig. 1a) with band/PMN ratios and NSP measurements which did not differ from controls. Those animals surviving 72 hr showed no difference in neutrophil count, PMN ratio, or splenic or hepatic NSP size. They did, however, have an elevation of storage neutrophils in the femur (Fig. 2) compared to controls.

DISCUSSION

A decrease in the number of circulating neutrophils has previously been cited as a poor prognostic factor in neonatal bacterial infection (5, 13). In the present study, we have attempted to determine whether bacterial infection in neonatal rats is associated with neutropenia and whether this neutropenia was associated with a decrease in the number of neutrophils stored in the marrow, spleen or liver. Animals were inoculated intranasally with GBS to utilize a common human pathogen and to simulate a likely portal of entry in human GBS infection (4). Clinical infection with GBS did ensue, and postmortem study of the animals who died revealed GBS infection including pneumonia and sepsis. Interestingly, adult animals resisted GBS infection in a manner similar to that of adult humans (3). In infected neonatal rats, the mean blood neutrophil count was only 54% of the control value ($P < 0.01$), and coincident with this neutropenia, a reduction in the NSP was observed with similar depletion of marrow, liver, and splenic sites.

The process leading to exhaustion of the marrow NSP appeared to spare the neutrophils capable of proliferation, inasmuch as the number of myeloblasts, promyelocytes, and myelocytes was unaltered by GBS infection. It might be postulated, therefore, that the exhaustion of mature neutrophil supply occurred as a result of extensive mobilization of stored neutrophils from the marrow. Alternatively, a selective intramyeloid destruction of mature neutrophils may have occurred. In either case, the result would be the same, an inadequate supply of neutrophils available for mobilization and delivery to infected sites. In the neonatal rat (and in the premature human as well), neutrophil stores exist both in the marrow cavities and in the liver and spleen (12). In the present model, we demonstrate that all sites of neutrophil stores are available for mobilization in infection because depletion of bone marrow, liver, and splenic fractions occurred in a similar fashion. Also of note is that repletion of the bone marrow neutrophil stores took place, although repletion of the liver and splenic pools did not. This observation is in keeping with the limitation of myelopoiesis to the bone marrow in developing fetuses and neonates (6), as well as with our own noninfected rats during the first three days of life. It is of note that in the present model of bacterial infection, neutropenia reflected a depletion of neutrophil stores. Such depletion may be of importance in reducing host resistance to bacterial invasion because in this state, inadequate numbers of neutrophils are available for mobilization to infected tissues. Indeed, the temporal relationship of the NSP depletion, neutropenia, and mortality suggest that NSP depletion contributed to the mortality. However, this hypothesis remains to be tested directly. We have also observed NSP depletion during bacterial infection in human neonates, both at autopsy and in four living neonates who subsequently died of bacterial infection (2). Other experimental animal evidence of NSP exhaustion leading to neutropenia and death can be found in the studies of Marsh *et al.* (9), who correlated mortality with NSP exhaustion in adult dogs

infected with pneumococci. In adult humans with systemic bacterial infection, however, neutropenia secondary to NSP exhaustion is distinctly uncommon. It is occasionally seen in compromised hosts such as chronic alcoholics and in this context regarded as a grave prognostic indicator (10). In certain respect the human neonates seem to be a compromised host. They are unusually susceptible to bacterial infection, and often great difficulty is encountered attempting to sterilize a bacterial process despite adequate antibiotic treatment (11). Perhaps contributing to this condition is the phenomenon which we observed in the infected neonatal rats, diminution in the mature neutrophil stores in the marrow, spleen, and liver, with accompanying neutropenia and subsequently diminished neutrophil delivery to the infected tissues.

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