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Age-Dependent Susceptibility of Neonatal Rats to Group B Streptococcal Type III Infection: Correlation of Severity of Infection and Response of Myeloid Pools

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A distinct age-dependent susceptibility to group B streptococcus type III (GBS) was demonstrated, utilizing a neonatal rat model. The most dramatic changes in susceptibility occurred within the first 7 days of postnatal life. To further investigate this susceptibility, experiments were performed utilizing two age groups of rats: (i) animals within the first 24 h of life (NB) and (ii) 7-day-old animals (7d). The infective dosage used was 10^4 GBS per g of body weight, a dose lethal to 100% of NB but only to 15% of 7d. The responses of the myeloid cells in the peripheral blood, spleen, and bone marrow were evaluated at intervals during the first 24 h post-GBS infection. The susceptibility of the NB to GBS appeared to be associated with a number of events, including smaller base-line levels of myeloid elements particularly in the bone marrow, a lag of at least 2 h in their initial response to infection, and an inability to maintain the myeloid pools. The band form of neutrophils appeared to be the predominant cell type in both total number and rapidity of response to infection. Moreover, an initial depletion of this band form was seen in both groups, which returned to base-line levels with recovery in 7d but persisted until death in NB animals. Similarly, shifts in numbers of peripheral nucleated erythrocytes appeared to reflect changes in the myeloid storage pools, with numbers of nucleated erythrocytes significantly decreasing in 7d animals with recovery in contrast to persistence in NB until death. Therefore, shifts in these cells in peripheral blood during infection appear to reflect the state of myeloid storage pools which parallel disease outcome.

Bacterial infection in the human newborn infant, particularly those caused by group B beta-hemolytic streptococci (GBS), remains a continuing clinical problem. Age-dependent susceptibility to such infection is well established, and several investigations have implicated immaturity of the inflammatory response as a major determinant in the outcome of neonatal infection.

The results of studies of phagocytic cell function indicate important differences between neonatal and adult inflammatory responses. These have included studies of chemotaxis (4, 14, 20, 24; M. E. Miller and A. T. W. Cheung, Annu. Meet. Am. Pediatr. Soc. and Soc. Pediatr. Res., 1980, abstr. no. 302), opsonins (12, 13, 17, 23), microbicidal capacity (2, 15, 21, 31, 33), and metabolic activity of phagocytic cells (3, 22, 26; L. S. Nerurkar, B. J. Zeligs, and J. A. Bellanti, Fed. Proc. 35:716, 1976). Because of the clinical association between neutropenia and fatality resulting from overwhelming sepsis (7, 32), recent attention has focused on the initiation and maintenance of the inflammatory response, with a particular emphasis on the size of and mobility of phagocytic cell pools (8, 29).

Fischer et al. (10) have recently described an animal model for the study of GBS infection. In this system, induction of bacteremia, pneumonia, meningitis, and death can be achieved closely paralleling the responses to GBS infection in the human newborn. We have adapted this model to examine certain in vivo parameters of the peripheral blood and storage pool phagocytic cell responses after GBS infection. The results of the present studies indicate that the newborn host has both deficient myeloid storage pools and mobilization capacity which collectively compromise the initial nonspecific immune response and contribute to the age-dependent susceptibility of the young host to infection.

MATERIALS AND METHODS

Animals. Time-pregnant Wistar rats were obtained from Charles River Breeding Laboratories, Wilmington, Mass. The pregnant females were transported in

the second week of pregnancy, housed separately in polycarbonate cages with hardwood litter, and given standard food and water ad libitum. The animals were carefully observed, two to three times per day, at the end of the gestational period to determine time of litter delivery. Suckling rats of both sexes in groups of 6 to 12 animals were used at each of the following ages: within the first 24 h after birth (NB); 2, 3 to 5, 7, 10, and 14 days after birth; and 60 or more days after birth (adult).

Bacterial cultures. The strain of GBS SS620 (type III) used in these studies was kindly provided by Richard Facklam, Centers for Disease Control, Atlanta, Ga. GBS type III was selected for these studies because it is responsible for approximately 30% of the GBS infections in neonates less than 5 days of age and for virtually all late-onset GBS infections in those older than 7 days to approximately 3 weeks (30). The bacteria were isolated on blood agar plates, and a stock preparation was stored at 4°C. Before each experiment, a fresh blood agar plate was prepared from the stock preparation, incubated for 18 h at 37°C, and then subcultured into Todd-Hewitt broth for 2.5 h at 37°C. Organisms were then washed and resuspended in Gey's buffer to a concentration of 3×10^8 colony-forming units/ml determined by optical density. This stock culture was held at room temperature and diluted immediately before use for each experimental group. Colony-forming units were determined on dilutions immediately after animal injections to monitor the exact numbers of organism used.

Method of experimental infection. A modification of the method of Fischer et al. (10) was used to infect suckling rats with GBS. The mother rats were removed from their litters approximately 1 h before the time of infection. The suckling rats from several litters were pooled, weighed, and divided into groups equal in weight variation. The mothers were then returned to the litters, and a period of approximately 1 h for maternal bonding and suckling was permitted.

Suckling rats were injected subcutaneously immediately cephalad to the tail with either GBS suspension (experimental group) or Gey's buffer (controls). To decrease the possibility of physical trauma, the volumes injected were 0.006 ml/g of body weight in rats ≤10 days old and 0.01 and 0.02 ml/g of body weight in 14-day-old and adult rats, respectively. Litters were carefully observed for a period of 48 h after injection, which was the period of maximal mortality, after which they were examined twice daily. Groups in which maternal hostility or neglect occurred were discarded.

Determination of lethal dosage of GBS. Groups of 10 to 20 suckling rats ranging in age from 1 to 14 days were injected with either Gey's buffer or various dosages of GBS per gram of body weight as described previously; a total of 12 adult rats were similarly injected. Each litter consisted of infected and control rats, and in no experiment did the control animals show signs of infection or lethality. Groups were observed for clinical signs of infection and followed for at least 1 month. Time-dosage survival curves were obtained by using standard methods of estimating 50% endpoints (27).

Cellular kinetic responses of NB and 7d rats to GBS infection. Groups of 6 to 10 NB and 7-day-old (7d) rats were injected with either Gey's buffer or 10^4 GBS/g of body weight, a dosage shown to uniformly cause 100% mortality in NB but only 15% mortality in 7d animals. This was a total dose of approximately 5×10^4 GBS per NB and approximately 1.5×10^5 GBS per 7d. At 2, 4, 8, 12, 18, and 24 h after injection, control and infected groups at each age group were sacrificed by decapitation. Blood for leukocyte counts, peripheral smears, and bacterial culture was drawn immediately. Specimens of femur, spleen, and liver were removed and cultured for bacteria, and smears were prepared for leukocyte differentials.

Identification and enumeration of cell types. Leukocyte counts were performed with a hemocytometer. Differentials were carried out by counting 100 to 200 leukocytes on peripheral blood and tissue smears which had been methanol fixed and stained with Giemsa. The various myeloid cells, i.e., polymorphonuclear leukocytes (PMN), bands, metamyelocytes, and myelocytes, in peripheral blood, spleen, and bone marrow smears were identified according to the nuclear morphology outlined by Chervenick et al. (5). The number of myeloid cells was determined on spleen and bone marrow smears by counting the number of cells of the myeloid series and other nucleated cells in 10 high-power fields (×400 magnification), using a gridded eyepiece.

RESULTS

Lethal dosage of GBS in rats of various postnatal ages. It can be seen in Fig. 1 that a progressive increase in the dosage was required to kill 50% of the animals and that this corresponded to increasing animal age. Striking logarithmic increases in the 50% lethal dosage occurred during the first postnatal week, i.e. 10^2 to 10^5 GBS/g. Between days 7 and 14 of age, a further log increase occurred, and in the adults 2 more log increases in the dosage were observed (5×10^7 GBS/g). Thus, the most rapid changes occurred within the first 7 days of age.

Animals infected with lethal dosages of GBS displayed specific patterns of illness. Lethargy and anorexia developed between 6 and 12 h after infection, associated with local erythema at injection site and labored noisy respirations by 12 to 18 h, followed by death usually within 48 h. Death rarely occurred in animals after 72 h postinfection. Blood and tissue cultures for GBS were uniformly positive in lethally infected animals 12 h after infection. Positive bacterial cultures persisting beyond 18 h correlated with irreversible infection.

Kinetics of inflammatory cell responses of NB and 7d rats to GBS infection. Based upon lethal dosage experiments, it was determined that equal dosages of 10^4 GBS per g of body weight killed 100% NB but only 15% of 7d. To examine this age-dependent variation in susceptibility to GBS, experiments were performed to compare and contrast the kinetics of the inflammatory cell responses of NB and 7d rats challenged with this dosage.

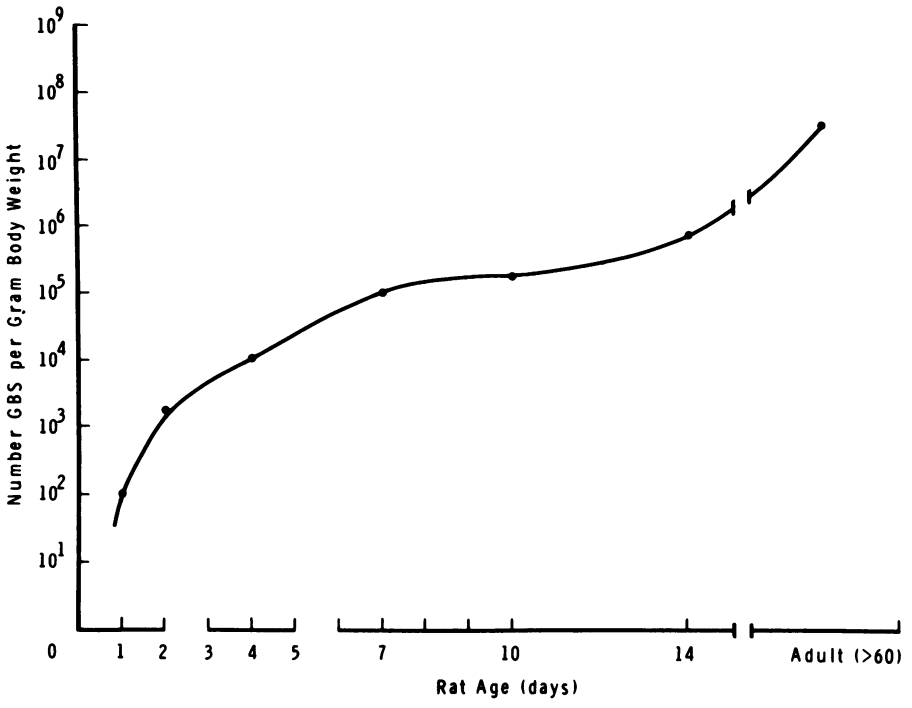

FIG. 1. Number of GBS per gram of body weight of rats of various ages required to kill 50% of animals (LD_{50}) after a subcutaneous injection. LD_{50} was calculated from 10 to 20 animals in suckling rat groups and from 6 animals in adult groups.

Peripheral leukocyte counts and differentials of NB and 7d rats at various time periods after GBS challenge are shown in Table 1. It can be seen that leukocytes in unchallenged NB, i.e., base-line value, was only 43% of 7d leukocytes. After infection, a marked leukopenia developed, which occurred in 2 h in 7d compared with 4 h in NB. This leukopenia persisted through 24 h in NB, 50% of baseline, but a rebound occurred in 7d, returning to 77% of baseline by 24 h. The differentials show a pattern similar to leukocytes with an initial decrease in percentage of PMN and bands in both NB and 7d which persists in NB but returned to baseline in 7d. The base-line percentage of cell types in the NB show similar proportions of PMN and bands compared with the percentage of these in the 7d, where there are three times more bands than PMN. The initial drop in percentage of PMN occurred in both NB and 7d 2 h post-GBS challenge, with a concomitant drop in the percentage of bands in the 7d only. In contrast, the NB showed the most significant ($P < 0.01$) drop in both PMN and bands at 4 h. Thus, the decrease in percentage of myeloid cell types in the NB persisted through 24 h after GBS infection, in contrast to initial shifts in these leukocyte components with a return to baseline in 7d during this period.

Figure 2 illustrates responses of total myeloid cells and individual cell types in the peripheral blood in NB and 7d animals. Striking differences were observed in the total myeloid cell populations in response to GBS in the two age groups. Total numbers of base-line peripheral blood myeloid cells in the 7d animals were significantly ($P < 0.01$) greater than in the NB. A significant ($P < 0.01$) decrease of 85% was observed 24 h after GBS challenge in the number of these cells in the NB compared with a return to baseline in 7d. A distinct difference was observed in the time required for the NB to exhibit a peripheral blood myeloid response to GBS, in contrast to the response seen in 7d animals, in which a prompt decrease of 67% in circulatory myeloid cells occurred by 2 h. But at 4 h post-GBS, a significant ($P < 0.01$) decrease in the NB cells had occurred. By 8 h an increase in the numbers of myeloid cells occurred in both age groups, after which the NB exhibited an irreversible depletion of myeloid cells compared with a return to baseline in the 7d. The base-line values of the various cell types are strikingly different in the NB compared with the 7d. Further, although NB show comparable numbers of PMN and bands, in the 7d animals the predominant cell type is the band form. In addition, there are 10 times more metamyelocytes-myelocytes in NB than in 7d. After GBS challenge there was a striking de-

TABLE 1. Total numbers and percentages of peripheral blood leukocytes from newborn and 7-day-old rats at various times after GBS infection

Rats	Hours after infection	Cell type[a]				
		WBC[b]	PMN[c]	Band[c]	Meta[c]	Mono[c]
Newborn	0[d]	3,157 ± 226	20 ± 2	19 ± 2	8 ± 1	54 ± 2
	2	4,571 ± 370	9 ± 3	25 ± 3	9 ± 1	57 ± 4
	4	1,892 ± 300	1 ± 1	8 ± 2	7 ± 3	84 ± 2
	8	2,628 ± 416	5 ± 1	25 ± 3	9 ± 1	64 ± 4
	12	1,049 ± 111	5 ± 1	14 ± 2	13 ± 2	68 ± 4
	18	1,419 ± 312	3 ± 1	14 ± 3	6 ± 1	77 ± 5
	24	1,656 ± 192	4 ± 1	11 ± 2	0	85 ± 2
7 day old	0[d]	7,521 ± 762	7 ± 1	29 ± 2	0.4 ± 0.3	63 ± 3
	2	4,691 ± 355	4 ± 1	12 ± 2	19 ± 3	81 ± 2
	4	4,060 ± 265	2 ± 1	12 ± 2	2 ± 1	84 ± 2
	8	4,562 ± 651	3 ± 1	16 ± 3	11 ± 2	70 ± 4
	12	3,168 ± 435	3 ± 1	18 ± 3	21 ± 2	68 ± 3
	18	4,007 ± 385	7 ± 2	16 ± 2	6 ± 1	71 ± 3
	24	5,788 ± 610	6 ± 1	33 ± 3	1 ± 0	60 ± 3

[a] Cell types were determined on blood smears from 6 to 12 rats at each time period by use of a hemocytometer. WBC, total leukocytes; PMN, segmented neutrophils; Band, band neutrophils; Meta, metamyelocytes; and Mono, all mononuclear (lymphoid and monocytic) leukocytes.

[b] Values are the mean number of cells per cubic millimeter ± standard error.

[c] Values are the mean percentages of cell type ± standard error.

[d] Healthy, unchallenged control rats.

crease in all cell types in the NB 24 h post-GBS challenge compared with a return to base-line values in 7d. Again, by 2 h post-GBS the 7d showed significant ($P < 0.01$) decreases in number of both PMN and bands, with an increase in the metamyelocyte-myelocyte numbers. This contrasts with a 1.6-fold increase in bands in NB at this time, with striking decreases in PMN and bands occurring only after 4 h post-GBS.

The peripheral blood myeloid responses were reflected in concomitant spleen cell responses. Figure 3 shows the number of myeloid cells as well as cell types in the spleens of NB and 7d animals after GBS infection. Again, the base-line numbers of myeloid cells was significantly greater ($P < 0.05$) in 7d animals than in NB. Further, the number of splenic myeloid cells fell significantly ($P < 0.01$) in the NB compared with a 2.4-fold increase in 7d 24 h after GBS challenge. In contrast to the early decreases seen in the number of myeloid components in peripheral blood of 7d rats at 2 h post-GBS, an increase occurred in the spleen, with only a slight decrease in myeloid cells occurring at 4 h in both NB and 7d of 32 and 38% of base-line values, respectively. Again, as in the peripheral compartment at 8 h post-GBS infection, there is a rebound to base-line values in both age groups after which the NB spleens show an irreversible decrease in the numbers of myeloid elements. In contrast, the 7d animals exhibit a 2.4-fold increase in cell numbers and a 3-fold expansion in splenic myeloid elements by 24 h post-GBS.

The base-line values of the various myeloid components in the spleen show that the major NB cell type is the band, whereas the myelocyte is the predominant cell in 7d. With GBS infection all cell types show significant decreases in the NB in 24 h, whereas the 7d rats show a 2-fold increase in PMN and a 14-fold increase in bands with only small decreases in the other cells. The percentage of each type in the NB as in the peripheral compartment shows some maintenance of cell type-to-cell type ratio even when there is severe depletion of their numbers. In the 7d animals there is a dramatic shift in the percentage of less differentiated myeloid elements, i.e., myelocytes and metamyelocytes to more mature bands.

Figure 4 illustrates the numbers of myeloid cell types within the bone marrow compartment after GBS infection. The base-line values of the number of myeloid cell types of the NB are significantly less ($P < 0.01$) than those of the 7d rats, with the band as the major cell type in both age groups followed by the myelocyte. In the NB, after GBS infection, the bone marrow becomes hypocellular and is depleted of myeloid elements, with the remaining cells poorly differentiated into morphologically distinct cell types of either erythroid or leukocytic series. Conversely, the 7d bone marrow shows a pattern of response similar to that seen in the spleen and peripheral compartments, with either an increase or return to base-line values of cell numbers by 24 h post-GBS infection in all cell types

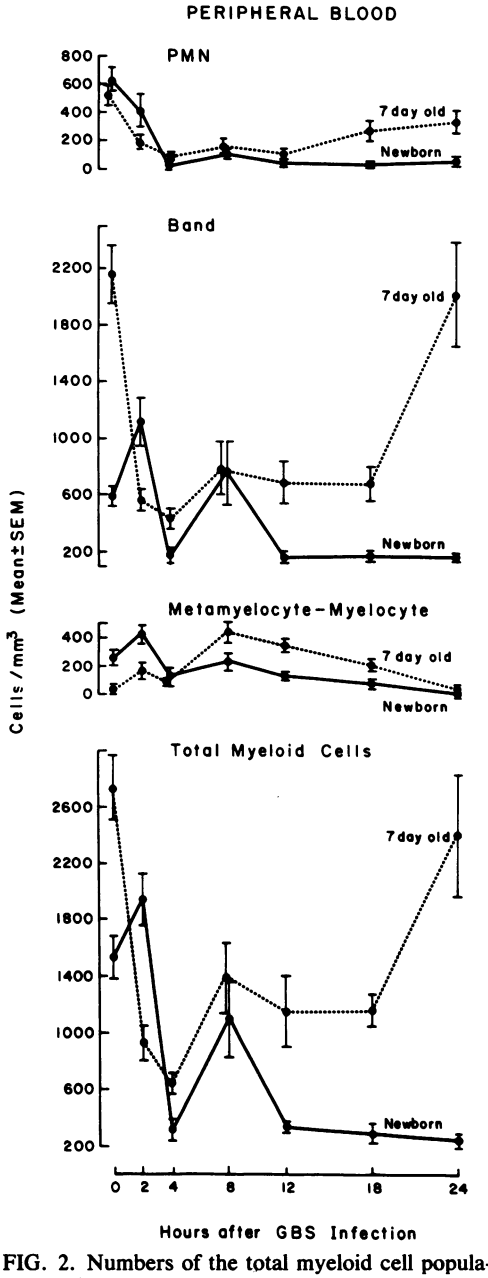

FIG. 2. Numbers of the total myeloid cell population as well as each type, e.g., metamyelocyte-myelocyte, band, and PMN, in the peripheral blood of NB and 7d rats at various hours after GBS infection. Results were obtained from 6 to 12 animals per time period in each age group and are expressed as the mean number of cells per cubic millimeter ± standard error.

except metamyelocytes. This suggests that the older animals have the ability to maintain myeloid cell numbers during infection compared with the severe cell depletion seen in the NB.

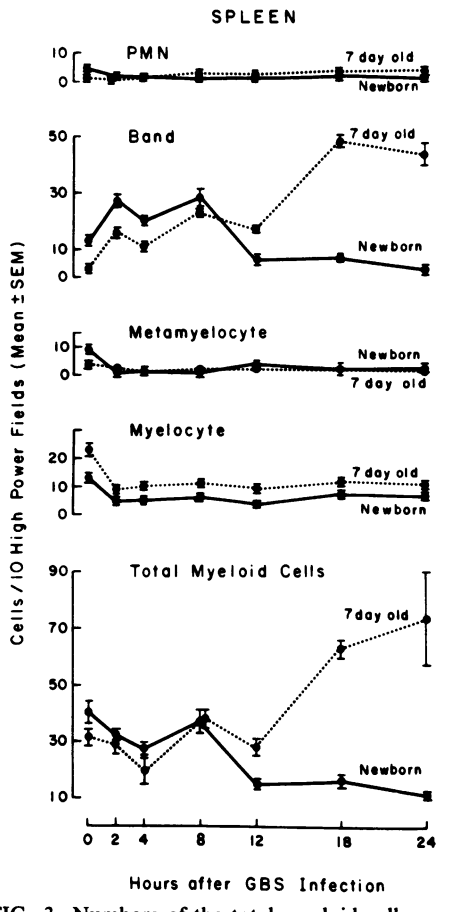

FIG. 3. Numbers of the total myeloid cell population as well as each type, e.g., metamyelocyte, myelocyte, band, and PMN, in the spleen of NB and 7d rats at various hours after GBS infection. Results were obtained from 6 to 12 animals per time period in each age group and are expressed as the mean number of cells per 10 high-power fields ± standard error.

Collectively, these results suggest that the baseline values of myeloid cells in these three storage pools as well as their ability to replenish and maintain these levels are greater in 7d than in NB rats. In addition, it appears that the band stage of differentiation is the major cellular component in both age groups.

In addition to the responses seen in myeloid cells during GBS infection, changes were observed also in the numbers of nucleated erythrocytes in the peripheral blood (Fig. 5). The baseline values in the 7d animals was higher than in the NB and, with infection, decreased significantly ($P < 0.01$) within 24 h in contrast to NB, in which the elevation was maintained. This suggests that the cell storage compartment, when depleted of myeloid elements in the newborn during infection, increases its output of

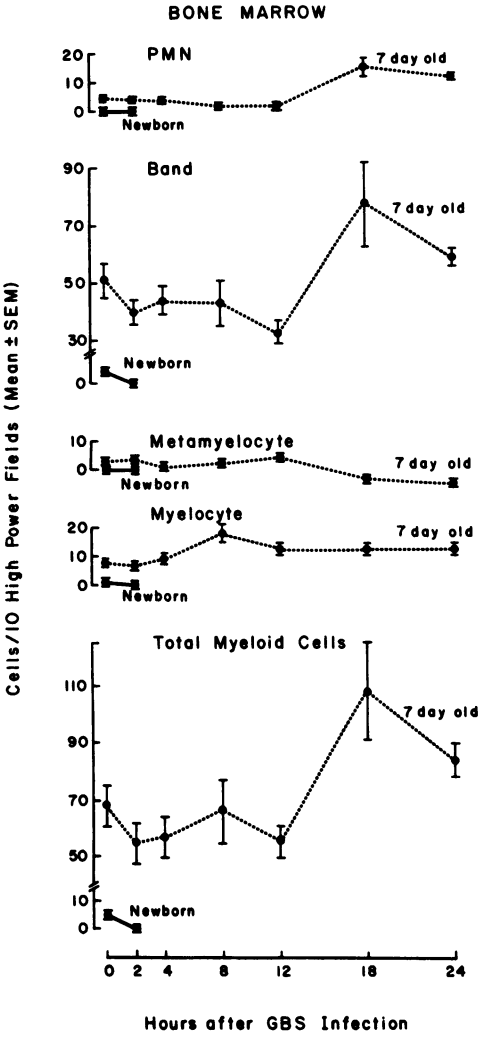

FIG. 4. Numbers of the total myeloid cell population as well as each type, e.g., metamyelocyte, myelocyte, band, and PMN, in the bone marrow of NB and 7d rats at various hours after GBS infection. Bone marrow from NB after GBS became hypocellular, and most elements were not differentiated into distinct cell types of either erythroid or myeloid series, which precluded enumeration. Results were obtained from 6 to 12 animals per time period in each age group and are expressed as the mean number of cells per 10 high-power fields ± standard error.

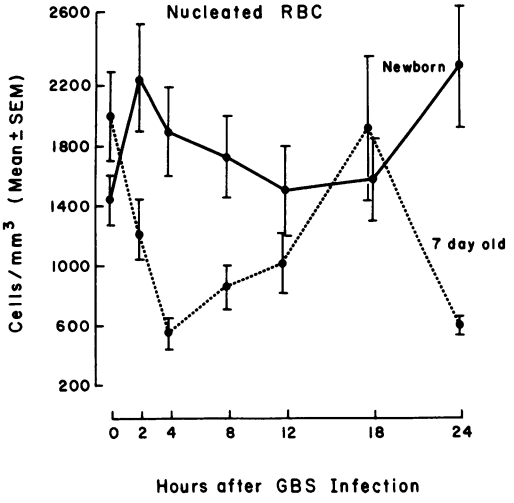

FIG. 5. Numbers of nucleated erythrocytes (RBC) in peripheral blood of NB and 7d rats at various hours after GBS infection. Results were obtained from 6 to 12 animals per time period in each age group and are expressed as the mean number of cells per cubic millimeter ± standard error.

immature erythroid cells compared with the 7d, in which there is an increase in the output of myeloid cells concomitant with a decrease in nucleated erythrocytes.

Finally, the response of NB versus 7d rats to the trauma of injection with sterile buffer versus infection with GBS was evaluated. The numbers of peripheral blood neutrophils at various times after injection appear in Fig. 6. The response of the NB to control buffer injections showed patterns similar to those of the infected groups but they were delayed about 4 h and the changes in cell numbers with buffer injection were less dramatic, fluctuating around the base-line (zero time) values only, with no dramatic decrease or animal death by 24 h postinjection. In contrast, the responses of the 7d rats' cells to either GBS infection or control buffer injection were almost identical, with a slightly larger decrease in neutrophil numbers in the infected group. These results suggest that in both NB and 7d animals there is a response in neutrophil numbers to injection of control buffer which is similar but less profound than that to GBS infection. This response was seen in all myeloid compartments. In addition, there appears to be a delay in the response of the NB controls: nadir at 8 versus 4 h in the infected group. Further, both NB and 7d control buffer groups returned to base-line values by 24 h postinjection, values which were similar to those found in normal uninjected animals of equivalent ages, i.e., 2 and 8 days old (data not presented).

DISCUSSION

The results of the present studies indicate that a temporal increase in resistance of the neonatal rat to GBS type III infection occurs during the first 2 weeks of life, with the most dramatic changes occurring during the first 7 days. Similar results have been reported by Ferrieri et al. (9) utilizing another animal model. Moreover,

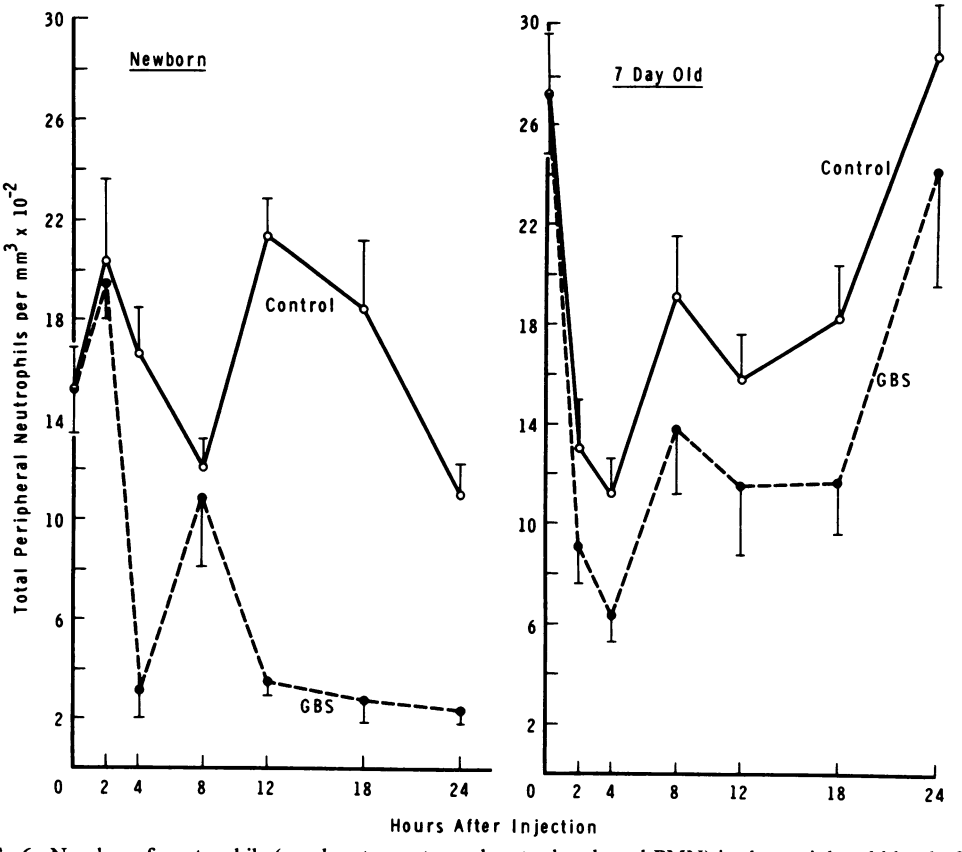

FIG. 6. Number of neutrophils (myelocyte, metamyelocyte, band, and PMN) in the peripheral blood of NB and 7d rats at various hours after injection of either control buffer (———) or GBS (- - - -). Results were obtained from 6 to 12 animals per time period in each group and are expressed as the mean number of cells per cubic millimeter ± standard error.

this age-dependent susceptibility to GBS infection parallels closely events seen in the human newborn infant (1, 18, 25, 30), in which the frequency of infection as well as severity of disease appear to be a function of the infant's chronological age.

In attempting to determine the factors involved in this susceptibility, elements of both specific and nonspecific immunity appear to be important. Specific antibody has been shown to offer significant protection in the newborn host (9, 10, 19, 28). Because of the immaturity of the specific neonatal immune response and the inability of the infant to produce significant amounts of antibody, and because the relative contribution of maternal antibody appeared to play little role since the mothers were carefully protected against exposure to GBS and because of the known absence of naturally occurring serum antibody in this model, the elements of nonspecific immunity, i.e., phagocytic cell function and complement, appear to play a more critical role in this model. The main phagocytic cellular components consist of cells of the myeloid series (i.e., peripheral blood neutrophils) and mononuclear phagocytes (i.e., monocytes and macrophages).

The results of the present studies suggest that a distinct correlation occurs between the myeloid cell responses and severity of GBS infection. This is indicated by a close correlation of cellular responses and mortality in 1-day-old and 7d rats to similar infective doses of GBS.

A distinct immaturity of the initial inflammatory response of the newborn has been suggested by the observations of Fischer et al. (11) and Ferrieri et al (9), in which they reported an absence of inflammatory cells at the site of GBS infection in NB rats compared with normal leukocyte infiltration in older infected animals. The results of the present studies confirm and extend these observations and suggest that this age-dependent variation in inflammatory response correlates with the known age-dependent susceptibility to GBS infection.

Several new factors may contribute signifi-

cantly to these variations in the inflammatory response. The first of these may be the quantitative difference in myeloid elements since, in the present study, both the number of neutrophils in the peripheral circulation and the myeloid pools in spleen and bone marrow were found to be less in the 1-day-old than in the 7d rats. In addition, after infection these pools were rapidly depleted in the 1 day olds whereas the 7d animals appear to recover after their initial responses and could maintain their myeloid pool size thereafter. The 1-day-old animals exhibited a distinct lag in their initial mobilization of myeloid cells followed by a dramatic decrease in their myeloid storage pools which continued until the death of the animals, 24 h after infection. This was in marked contrast to the responses seen in 7d animals, in which a rapid cellular mobilization occurred within 2 hours after infection. The complete clearance of circulating organisms 8 h after infection and replenishment and maintenance of myeloid storage pools within 24 h were events which correlated with animal recovery. Further, studies to evaluate the role of the liver as a myeloid compartment revealed that in both control and infected NB and 7d rats there were myeloid cells present there. The numbers were smaller in the 7d animals than in the NB and less in both age groups than those in their corresponding spleens. Further, the responses to infection were less dramatic than those seen in the spleen (unpublished data). This suggests that the liver does contribute to the myeloid response but to a lesser extent than the other compartments.

This variation in the rapidity of response to infection in the two age groups may be extremely relevant to their final outcomes since a delay in cell mobilization will allow the infecting microorganism to replicate. The observed lag in the mobilization of cells from the NB may, in part, reflect a reduced chemotactic activity since this function appears to mature with both animal maturation and cell differentiation (4). In addition, the variations in the ability of the two age groups to eliminate the organisms may result in part from differences in maturity of bactericidal activity of their phagocytic cells (2, 15, 21, 31, 33) which has been shown to increase with postnatal maturation. This may play a role in GBS sepsis since Becker et al. (2) have recently reported deficiencies in bactericidal activity of neutrophils from cord blood to GBS type III but not to all other GBS tested.

In support of these findings are the known observations of Christensen et al. (7, 8), who showed significant changes in the circulating and storage neutrophils in both clinical and experimental GBS sepsis. Further, these investigators reported that neonatal rats infected with GBS sustained a more severe neutropenia with an increase of circulating immature neutrophils. In these animals, the marrow, spleen, and hepatic neutrophil storage compartments revealed a diminution of postmitotic myelocytic series, but little change in proliferative stages. Further, Christensen and Rothstein (6) have reported that NB rats have a significantly smaller neutrophil storage pool than do 1-month-old animals. Moreover, although NB rats can release a significant percentage of these cells in response to a stimulus, only a small number of cells arrive at the source of stimulation compared with 1-month-old animals. Further, Christensen et al. have recently reported not only marked depletion of neutrophil storage pools in GBS infection of NB rats, but also a diminution of proliferative neutrophils and stem cells compared with increases in these in the adult (R. D. Christensen, J. L. MacFarlane, N. L. Taylor, H. R. Hill, and G. Rothstein, Pediatr. Res., in press). These results are concordant with the data of Manroe et al. which show a severe neutropenia during GBS infection in NB (16).

Further, it was observed in this study that the band form of neutrophils appears to be the predominant cell stage in both quantity, i.e., in the storage pools, and response, i.e., in shifts in peripheral circulation. In addition, we also found this to be true in the storage pools of animals throughout adulthood. (data not shown). Similarly, shifts in the numbers of circulating nucleated erythrocytes also appears to reflect the state of storage pools, with the numbers significantly decreasing in the 7d animals with recovery versus persistence or even increase in NB with depletion of myeloid elements. Therefore, shifts in these populations in the peripheral blood appears to reflect events in the storage pools and may have clinical significance.

In summary, these studies provide additional data concerning the heterogeneity of the age-dependent susceptibility of the young host to GBS infection. First, the numbers of myeloid cells in the peripheral circulation as well as the size of the myeloid storage pools appear to be a critical event. Further, the rapidity of mobilization of these cells as well as ability to maintain the myeloid pools may be the decisive factor in the outcome of the disease. Finally, the outcome of these host-microbial interactions are related to the functional maturation of the cells which arrive at the infective site.

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