Experimental Infection of the Skin in the Hamster Simulating Human Impetigo

IV. Cellular Responses after Streptococcal and Staphylococcal Infections

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Various cellular responses to skin infections in an experimental animal model were explored. Total leukocyte counts varied after group A streptococcal infections, but a depression was commonly seen after M type 12 impetigo. Staphylococcus aureus infections resulted in moderate leukocytosis. A marked neutrophilia was universal with streptococcal or staphylococcal disease. A positive nitroblue tetrazolium (NBT) response appeared 24 hr after infection, reached a peak in 48 hr, and then declined. This occurred in the absence of extensive cellulitis or bacteremia. An increase in the percentage and absolute number of NBT-positive neutrophils occurred. M type 57 streptococcus produced a more strongly positive NBT test than did M type 12. Cell-free filtrates of a broth culture of M type 57 streptococcus produced NBT responses in hamsters comparable to the responses seen after injection of live organisms. These studies indicate the usefulness of this animal model to study various parameters of the NBT test.

The introduction of the nitroblue tetrazolium (NBT) test by Park and associates (8) has proved to be a very useful procedure for differentiating bacterial infections from nonbacterial disorders accompanied by leukocytosis. The percentage and absolute number of polymorphonuclear leukocytes (PMN) that reduce the pale yellow NBT to blue-black formazan deposits are increased in bacterial infections in children (4, 5, 8, 10, 11). Similarly, Matula and Paterson (6) demonstrated elevated NBT responses in adults with bacterial infections. A rapid return to normal values (less than 10%) occurs after initiation of effective therapy (6).

The mechanism underlying this test is not known. Systemic infections are thought to be more likely associated with a positive NBT test than localized infections (7). However, certain extracellular products, most notably endotoxin, can induce an increased reduction of NBT dye when added to whole blood (6, 9). The availability of an experimental model to study the dynamics of this test would greatly enhance our understanding of the mechanism involved.

The Syrian hamster has been shown to be a suitable model in investigating several parameters of experimental impetigo (1-3). The disease in the hamster resembles human impetigo in gross appearance of the lesions, in progression of the various stages, and in the histopathology of the process (2). The effect of various therapeutic regimens (1) and the interaction between staphylococci and group A streptococci (3) were also studied in this model. The present communication documents the usefulness of this model further to study cellular responses after skin infection. It demonstrates the suitability of the Syrian hamster to study the NBT reduction in vivo.

MATERIALS AND METHODS

Bacterial strains. All strains listed below have been utilized previously in the hamster model (1-3).

Two strains of group A beta-hemolytic streptococci were used. An M type 57 strain, PF 1643, was isolated from a skin lesion of a patient with nephritis. Strain 70-711 is an M type 12 recovered from a blood culture of a patient with septicemia. Both strains were grown in Todd Hewitt broth (Difco) for 6 hr at 37 C, and portions were then stored at -65 C until needed. Prior to animal inoculation, specimens were thawed, reinoculated into fresh broth, and grown 4 to 6 hr at 37 C.

The Staphylococcus aureus strain used, RL 3809, is a phage type 81 and was originally isolated from a skin lesion. The strain was grown in tryptic soy broth (Difco) and handled as above for the streptococcal strains.

Animal inoculation. The procedure for producing
lesions was as described previously (2). Each animal was infected at four sites, using 0.1 ml of a broth culture of the various organisms at each site.

Cardiac puncture was done on the animals to obtain blood for the various tests. Cultures were routinely done by adding approximately 0.5 ml of blood to 5 ml of Todd Hewitt broth. The NBT test was done as previously described by Park et al. (8). Total white blood cell (WBC) counts and differential counts were done on all blood specimens.

RESULTS

The total WBC count and differential counts were determined on 18 uninfected hamsters to establish a norm. The first panel in Fig. 1 is a scattergram of normal values for these control animals. Each circle represents an animal, and the horizontal bar is the mean for the group. The range of normal WBC count is 4,100 to 11,100/mm³ with a mean of 7,400. PMN cells represent approximately 40% of the total WBC, and the remaining cells are almost exclusively lymphocytes. Eosinophils were rarely encountered, and monocytes accounted for 1 to 2% of the total WBC. These findings are in agreement with the observations of Stewart et al. (12).

The responses to infections with two group A streptococcal strains and a staphylococcal strain are also shown in Fig. 1. The scattergrams represent data obtained 48 hr after initiation of the infection; however, similar results were also noted when animals were bled at 24 or 72 hr after infection. With M type 12 infection (second panel) 8 of 12 animals exhibited leukopenia (WBC < 4,000/mm³), 2 had normal counts, and 2 showed only slight leukocytosis. The frequent leukopenia was noted in subsequent experiments using this streptococcal type as the infecting agent. Infection with M type 57 streptococcus resulted in normal total WBC counts in the majority of the animals (9 of 13), with only four animals exhibiting leukopenia and none demonstrating leukocytosis. Of 12 animals infected with S. aureus, the WBC counts were normal in all but one that exhibited leukocytosis. Leukopenia was not present in any of the animals infected with this staphylococcal strain.

In contrast to this wide variation in WBC responses, neutrophilia was marked and universal in all instances after streptococcal or staphylococcal infections (panels 2, 3, 4 of Fig. 1). An increase in the percentage as well as in the absolute counts of PMN occurred. The mean absolute PMN counts for the various groups were: normal controls, 3,000/mm³; M type 12 streptococcal infections, 4,640/mm³; M type 57 streptococcal infections, 5,400/mm³; and staphylococcal infections, 8,100/mm³. Immature forms comprised approximately 20% of the PMN cells.

To determine the level of NBT reduction in normal hamsters and its response to infection, 14 animals were bled and then infected with M type 12 streptococcus. Daily bleedings were performed on the same animals for the next 4 days. The results are illustrated in Fig. 2. Prior to infection, all animals had NBT reduction levels of 4% or less, with eight of the animals showing responses of 1% or less. Positive NBT responses (levels of 5% or greater) are seen by

![Diagram showing NBT reduction over time](http://iai.asm.org/)}
24 hr postinfection in half of the animals. By 48 hr, 11 of 12 animals had positive responses, with one animal showing a marked level (28%) of NBT reduction. A decline in the NBT positivity occurs by 72 hr with return to almost normal values by 96 hr. Blood cultures done at all times after infection were sterile.

Repeated bleedings of the same animals resulted in a high rate of mortality. Since the most pronounced NBT responses occurred 48 hr after infection for M type 12 streptococci, this interval was selected to bleed animals in all subsequent experiments.

In Fig. 3 is a comparison of the NBT responses in four groups of animals. The 23 uninfected controls represented in the first panel are a separate group from the controls represented in Fig. 2. All but two animals in the control group in Fig. 3 had NBT levels of 4% or less. In contrast, NBT responses were detectable in almost all of the infected animals 48 hr after inoculation. Of the 41 animals in the groups infected with the three organisms, 37 (90%) had NBT responses greater than 5%. Only four animals showed no responses. Combination of controls in Fig. 2 and Fig. 3 indicates that 35 of the 37 animals showed NBT responses under 5%, with only two showing an 8% response. The selection of the 5% value, therefore, seems to be a reasonable level to separate normal from elevated responses in this animal.

Comparison of the three organisms (Fig. 3) shows certain differences in their capacity to elicit an NBT response. The mean NBT level after M type 12 streptococcal infection was 9.5%, compared to 14.5% after infection with M type 57 streptococci. S. aureus infections resulted in the most pronounced responses (mean 17.5%). Blood cultures in all the infected animals were negative.

The absolute numbers of NBT-positive cells for the above animals were determined when possible and are shown in Fig. 4. Normal animals had absolute numbers of 250/mm³ or less in 22 of 23 animals, with a mean of 80. Animals infected with M type 12 streptococci showed levels greater than 250 in 7 of 12 hamsters, with a mean of 375. Absolute numbers after M type 57 streptococcal infections were above 250 in 9 of 12 animals, and the mean was 947. S. aureus infections elicited the most marked increase. All but one animal infected with staphylococci had absolute numbers greater than 500, with a mean of 1,534.

When M type 57 streptococci were grown in Todd Hewitt broth, washed twice in 0.85% saline, and then injected intradermally into hamsters, NBT responses 48 hr after infection were again seen (Fig. 5, first panel). The responses after the injection of washed cells were comparable to ones noted after injection of the same streptococcal strain grown in broth.

Since bacteremia was never detected in any of the infected animals, and since the skin infections in these animals are localized and not
extensive, it was of interest to assess whether extracellular products of the organisms might elicit an NBT response. Supernatant fluid from an 18-hr culture of M type 57 streptococci was sterilized by membrane filtration (Millipore Corp.). A group of six animals was injected with 0.1 ml of the filtered fluid at four sites each, and the NBT responses were determined 48 hr later. The results are shown in Fig. 5 (second panel). All animals exhibited responses with a mean NBT reduction of 21%. Such responses are quite comparable to ones seen after injection of live organisms. Animals injected with sterile Todd Hewitt broth or with normal saline showed no NBT responses.

**DISCUSSION**

The Syrian hamster was chosen for the present investigation in view of the findings of Stewart et al. (12) which indicate the similarity between human and hamster leukocytes in total number per cubic millimeter and in differential counts. Furthermore, previous work from this laboratory has shown that an experimental skin infection can be produced in the hamster consistently and that such infection mimics human impetigo in various parameters (2).

The results of the present report indicate that a definite NBT response occurs in the hamster after infection of the skin with group A streptococci or with *S. aureus*. An increase both in the percentage of NBT-positive leukocytes as well as in the absolute number of such cells has been demonstrated. It seems reasonable to assume that, in this animal, a negative NBT response is represented by 5% or fewer PMN-containing formazan deposits, since 35 of 37 uninfected animals had NBT responses of 4% or less. Of a total of 51 animals infected with either streptococci or *S. aureus*, 45 (90%) had NBT responses of 5% or greater.

In addition to the suitability of the hamster to study NBT reduction in vivo, the present report also strongly suggests that an increased reduction of NBT dye can occur after localized infection. The experimental disease in these animals is restricted to the epidermis and outer layers of the dermis (2), and no bacteremia was demonstrable in any of the infected hamsters. Blood stream invasion with bacteria is not necessary, therefore, for the stimulation of PMN to reduce NBT. This conclusion is further supported by the clinical studies in humans (4-6) where NBT-positive results were seen in bacterial infections with or without septicemia.

That certain bacterial products can stimulate NBT reduction in vitro has been well documented. Park and Good (9) demonstrated increased reduction of NBT dye using endotoxin. This was confirmed by Matula and Paterson (6) who also induced a positive NBT response after exposure of neutrophils in vitro to various bacterial culture filtrates. Our data indicate that extracellular products of M type 57 streptococci induce an NBT response in vivo, and that this response is comparable to what is observed after experimental infection with the same organism. It is reasonable to assume, therefore, that at least certain microorganisms that liberate extracellular products which can disseminate in vivo are capable of eliciting an NBT response even with a localized infection.

The spontaneous return of NBT response to normal levels by 96 hr after infection in this animal model is intriguing. Spontaneous healing of streptococcal skin infection in the hamster occurs by the 7th day after infection with a range of 5 to 10 days (1). Furthermore, lesions at 96 hr postinfection are grossly identical to ones 48 hr after infection (2). Products of the beta-hemolytic streptococcus that may stimulate NBT reduction in vivo could possibly be liberated only early in the infection. Alternately, such products could be neutralized in the host at a later stage either by local or by circulating factors. This temporal relationship of NBT responses to localized infection should be investigated further in humans. Elucidation of this observation may

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**Fig. 5.** Responses in NBT dye reduction to infection with washed, live streptococcal cells and to cell-free filtrates of a broth culture of the same organism.
offer an explanation for the more frequent association of NBT responses with systemic rather than localized infections.

The animal model described in this report should prove valuable to study various aspects of the kinetics of NBT reduction and to help delineate the bacterial and possibly nonbacterial products responsible for the stimulation of PMN to reduce the dye.

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LITERATURE CITED


