Susceptibility of brain and skin to bacterial challenge

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The brain is a uniquely protected organ. Once the protective barriers are overcome, the brain is susceptible to bacterial infection. Using a reproducible rat model, the susceptibility of brain tissue to challenge by S. aureus or E. coli was quantitatively compared to that of skin. Brain was significantly more susceptible to the presence of bacteria than was the skin of the scalp. The development of infection in skin required at least $10^5$ organisms, while brain infection could be produced with as few as $10^2$ organisms.

KEY WORDS □9 brain susceptibility □9 brain abscess □9 infection □9 S. aureus □9 E. coli

The brain is often referred to as an immunologically privileged site. Compared to other body tissues, the immune response of the brain is significantly less, a fact that is attributed to the unique features of a blood-brain barrier and the lack of a lymphatic system. The consequences of these phenomena on the susceptibility of brain tissue to bacterial challenge as compared to other soft tissues have not been documented by quantitative bacteriology.

Clinically, parenchymal brain infections or abscesses are relatively infrequent when compared to the occurrence of infections and abscesses in other soft tissues. The protective isolation of the brain by skin, skull, and meninges may account for this low rate of brain infection. In addition, the blood-brain barrier protects the brain from blood-borne bacteria. However, once these barriers are overcome, the immunological inferiority of the brain may result in increased susceptibility to infection. In this study, the susceptibility of brain and skin to bacterial challenge was quantitated.

Materials and Methods

Bacterial Preparation

The bacteria used in the present study were

Staphylococcus aureus* and Escherichia coli (sero-

* S. aureus bacteria (No. 2801) obtained from the Center for Disease Control, Atlanta, Georgia.

type 06: non-motile). They were maintained on blood agar plates, and selected colonies were transferred by wire loop into broth culture before each experiment. The bacteria were grown in trypticase soy broth for 18 hours with agitation at a temperature of $37^\circ$C, and then collected by centrifugation. The bacterial button was washed twice with saline, and the bacteria were resuspended in 2 ml of saline and then serially diluted 1:10 to $10^5$. The number of bacteria present in the stock suspension and the dilutions were determined by plating aliquots from dilutions $10^{-4}$, $10^{-5}$, and $10^{-6}$ on blood agar plates. After 18 hours of incubation at $37^\circ$C, the colony-forming units on the appropriate dilution were counted, and the number in the other dilutions were derived by appropriate calculations.

Animal Preparation

Adult female Sprague-Dawley rats (300 gm in weight) were anesthetized by an intraperitoneal injection of sodium pentobarbital (38 mg/kg). The hair was clipped from the top of the head with electric shears and the scalp depilated with Surgex. The skin was prepared with iodophor and neutralized with sterile sodium thiosulfate solution (1%). The animals were then secured in a stereotaxic head holder. Separate groups of animals were used for skin injections and brain injections in order to eliminate the possibility of metastatic infection. Each group consisted of a minimum of eight animals, and each animal within the group received the same inoculum of bacteria. The inocula ranged from $10^1$ to $10^6$ bacteria.
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per μl of isotonic saline. A dose-response curve for both skin and brain was generated.

**Bacterial Challenge**

The technique for injecting bacteria into brain has been described in detail previously. In summary, 1 μl of saline containing a known number of bacteria was injected stereotaxically into the frontal lobe over a 60-minute period. Quantitation of bacteria before and after the injection showed no significant decrease in the number of viable organisms during the injection interval.

Injections into skin were performed with a 10-μl syringe and a No. 26 needle which was mounted on a stereotaxic head holder. The needle was lowered to the surface of the skin of the scalp and then moved 0.8 mm into the dermal tissue. After 2 minutes had elapsed, 1 μl of isotonic saline containing a known number of bacteria was slowly injected into the tissue. The appearance of a well formed bleb indicated an appropriate intradermal scalp injection. After another 2 minutes, the needle was slowly withdrawn from the skin.

The results were reported as the mean logarithm of bacteria recovered from the injection site. When infection occurred, it presented as a well defined, localized abscess. In order to standardize the quantitative procedure, standard skin and brain biopsies were taken which were designed to include the entire abscess. This procedure yields the total number of bacteria present in the abscess, and eliminates the variability of bacterial density when the number of bacteria per gram of biopsy specimen is reported.

Differences between the infection rates of skin and brain at a specific bacterial inoculum were compared for statistical significance using a chi-square test. Similarly, the differences in the number of viable bacteria recovered from skin and brain were compared using a nonpaired Student’s t-test. The “p” values reflect the Bonferroni correction for multiple testing.

**Results**

Brain tissue was much more susceptible to bacterial challenge than was skin. Injection of 10⁴ organisms of *S. aureus* into skin did not result in infection (Fig. 1

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**Analysis of Tissue Response**

Four days after the injection procedure, the animals were sacrificed by an overdose of intraperitoneal sodium pentobarbital (200 mg/kg). The scalp was sterilized by washing with iodophor, followed by neutralization with sterile sodium thiosulfate solution (1%). Using aseptic technique, the injection area and a 2 mm margin of uninvolved skin were excised and placed in a sterile petri dish. The skin biopsy was dissected through the center of the injection site and observed for the presence of purulent exudate. When pus was observed, the injection site was considered infected. The skin specimen was then placed in a sterile tube with 5 ml of isotonic saline and homogenized with a Polytron grinder.† The number of viable bacteria in the injection site was quantitated by standard serial dilution and plating techniques.

A similar procedure was used to evaluate the inflammatory response of brain tissue following bacterial challenge. Using aseptic technique, the scalp was incised and reflected and the calvaria removed. The brain was excised, placed in a sterile petri dish, and sectioned sagittally in the midline. A coronal cut was then made in the right hemisphere through the injection site, and the brain tissue was examined for the presence of purulent exudate. When pus was observed, the brain was considered to be infected. The number of viable bacteria in the injected brain tissue was then determined in a manner similar to that described previously for the analysis of skin.

†Polytron grinder manufactured by Brinkmann Instruments, Westbury, New York.

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Fig. 1. Graphs showing the tissue response of brain and skin 4 days after challenge with different inocula of *S. aureus*.
and Table 1. In contrast, similar injections into brain tissue always resulted in infection. Only when skin was injected with $10^8$ organisms of *S. aureus* did a 100% infection rate occur. Even when brain tissue was injected with as few as $10^5$ organisms of *S. aureus*, two of 11 animals developed a brain abscess.

The high incidence of brain infection was correlated with large numbers of bacteria still present in the tissue after 4 days. When skin was injected with $10^6$ organisms of *S. aureus*, the tissue defenses prevented their proliferation, and by the 4th day had reduced their numbers to an average of about $10^2$. In contrast, brain tissue defenses were less effective and the bacteria proliferated, with an average of $10^6$ organisms still present after 4 days. The brain tissue was not able to maintain and eliminate bacteria in $58\%$ of the animals challenged with as few as $10^2$ bacteria. Injection of $10^2$ organisms of *S. aureus* into the brain still resulted in an average recovery 4 days later of $10^{2.7}$.

The differences in response between skin and brain were much more dramatic when the tissues were injected with *E. coli* (Fig. 2 and Table 2). Skin was very resistant to contamination with *E. coli*. Even when skin was injected with $10^8$ organisms of *E. coli* there was no visual sign of purulent exudate. With an injection of $5 \times 10^4$ *E. coli*, 100% of the injected brains became infected. With as few as 500 organisms of *E. coli*, three of 24 brains became infected.

Quantitative bacteriology correlated with the clinical observations. The number of *E. coli* remaining in skin 4 days after injection was significantly reduced from the original inoculum. In contrast, the mean number of *E. coli* recovered from brain tissue after 4 days was always higher than the original inoculum.

**Discussion**

This study clearly demonstrates that brain is much more susceptible to bacterial challenge than is skin. Using quantitative bacteriology, injections of $10^4$ *S. aureus* or $10^6$ *E. coli* did not cause infection in skin. In contrast, brain tissue was susceptible to as few as $10^2$ *S. aureus* or *E. coli*.

Several factors are unique to the brain which may account for this susceptibility. Of primary importance is the lack of an adequate inflammatory and im-
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### Table 2

<table>
<thead>
<tr>
<th>Inoculum (log of bacteria)</th>
<th>No. of Rats</th>
<th>Tissue Injected</th>
<th>Gross Infection</th>
<th>Viable Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive (%)</td>
<td>log ± SE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td>$1 \times 10^6$</td>
<td>17</td>
<td>skin</td>
<td>0</td>
<td>4.4 ± 0.2</td>
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<tr>
<td></td>
<td>18</td>
<td>brain</td>
<td>100</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>$5 \times 10^5$</td>
<td>8</td>
<td>skin</td>
<td>0</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>brain</td>
<td>100</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>$5 \times 10^4$</td>
<td>8</td>
<td>skin</td>
<td>0</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>brain</td>
<td>100</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>$5 \times 10^3$</td>
<td>19</td>
<td>brain</td>
<td>42</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>$5 \times 10^2$</td>
<td>24</td>
<td>brain</td>
<td>12</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td>$5 \times 10^1$</td>
<td>11</td>
<td>brain</td>
<td>0</td>
<td>6.4 ± 0.2</td>
</tr>
</tbody>
</table>

munological response to the bacterial insult. Because of the absence of a lymphatic system, the ability to recognize these foreign cells and stimulate the humoral and cellular defense mechanisms is markedly inhibited. Even after recognition has occurred, the penetration of specific antibodies and activated leukocytes into the brain may be significantly inhibited by the presence of the blood-brain barrier.

The minimal reaction of brain tissue to microorganisms as compared to other soft tissues was well documented by the histological study of Levine, et al. Injection of Cryptococcus neoformans into subcutaneous tissue resulted in a prompt outpouring of polymorphonuclear leukocytes, followed by lymphocytes, plasma cells, and macrophages, with fibrosis and encapsulation. In contrast, injection of the organisms into brain tissue resulted in a delayed, weak, and irregular reaction.

Factors other than the immunological isolation of brain may also play a role in the lower threshold for brain to infection. For example, Sherman stated that the inability of a space to distend with advancing infection or suppuration leads to the interruption of local blood flow and, subsequently, to tissue necrosis. Unlike most other soft tissue, the brain is confined within a rigid space and prevented from expanding. Moreover, the physical structure and composition of the brain tissue are intrinsic properties that may dispose the brain to infection. Without the extensive connective tissue matrix present in other soft tissue, the nervous system cannot mechanically restrict the spread of proliferating bacteria. Thus, Smith, et al., referred to the brain as an open area in which bacteria can easily multiply and spread, while skin and muscle are considered closed areas in which bacterial expansion is restricted.

The relative contribution of various factors to lowering the threshold of infection for brain is unclear. However, the development of a simple reproducible model for brain abscess and the use of quantitative bacteriological and immunological techniques will allow the evaluation of the role that each of these parameters is playing in brain infection.

### References


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