Antibody Responses to Group A Streptococcal Infections in the Hamster

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The immune response after streptococcal infections of the skin and of the joints was studied in an experimental animal model. Hamsters were challenged intradermally or intra-articularly with different streptococcal serotypes, and antibodies for streptolysin O (ASO), deoxyribonuclease B (anti-deoxyribonuclease B), and group A carbohydrate (anti-group A CHO) were determined. After a single injection at either site, 7 of 48 animals (14%) developed group A-CHO antibodies; however, none of the animals developed detectable levels of ASO or anti-deoxyribonuclease B. After repeated injections of the skin or joint, anti-deoxyribonuclease B antibodies were detectable in 13% (4 of 30) and 30% (5 of 17) of the animals, respectively. Elevations of ASO occurred after repeated joint infections in 4 of 16 animals (25%), whereas none of 30 hamsters repeatedly infected intradermally developed antibodies against streptolysin O. For all three antibodies tested, elevated levels were more frequently noted after repeated joint infections than after repeated skin infections with the same streptococcal serotype. These data, similar to ones previously noted in human impetigo, indicate that ASO responses are feeble after streptococcal skin infections and that the site of infection per se, rather than the infecting strain, appears to be responsible for this poor response.

Variations in the immune response to different streptococcal antigens have been observed as related to the site of infection in humans (12). Rises in anti-streptolysin O (ASO) are slight in patients with streptococcal skin infections (6, 9, 11, 12), and antibodies to streptococcal nicotinamide adenine dinucleotidase occur irregularly in these patients (9). In contrast, anti-streptococcal deoxyribonuclease B (anti-deoxyribonuclease B) titers are regularly, and often markedly, elevated after streptococcal pyoderma (6, 9). Antibodies to all of these streptococcal products occur more consistently in patients with streptococcal pharyngitis (12).

The Syrian hamster has been used by us to study various aspects of experimental impetigo (2–5). The disease in the hamster simulates human impetigo in gross appearance, in the progression of the lesions in various stages, and in the histopathology of the process (3). During the course of these studies, no type-specific protection was detected in animals repeatedly infected with the same streptococcal serotype (3). The present studies were designed to assess the effect of the site of infection on antibody response to different streptococcal antigens in the hamster.

MATERIALS AND METHODS

Organisms. Four streptococcal strains, of different serotypes, were used in these studies. Strain 70-1090, an M-type 2 by the M-protein precipitation test and a T-type 2 by T-protein agglutination, was isolated from an ear culture. Strain 70-1155 is an M-type 4, T-type 4 and was recovered from a throat culture of a patient with pharyngitis. The M-type 12, T-type 12 strain (designated 70–711) and M-type 57, T-type 8/25/Imp. 19 strain (designated PF 1643) were isolated from blood and skin lesions, respectively, and have been utilized previously in the experimental impetigo model in the hamster (2–5).

Animal Inoculation. Experimental impetigo was produced in the hamsters as previously described (3). Each animal was injected at four sites by using 10⁶ colony-forming units of the various organisms contained in 0.1 ml of a broth culture. Attempts at producing respiratory infection with group A streptococci in this animal were unsuccessful after intranasal instillation and throat inoculation of all strains used. However, septic arthritis could be reproducibly effected in hamsters after intra-articular injection of 0.1 ml of culture containing 10⁶ colony-forming units of a logarithmic-phase streptococcal cell suspension. The forelimbs of each animal were simultaneously injected at each time.

Streptococcal antibodies. Antibody determinations
were performed by standard methods. ASO (8), anti-deoxyribonuclease B (10), and group A carbohydrate (CHO) antibodies (7) were assayed for in all sera.

RESULTS

Nine uninfected animals were bled, and antibody determinations were made to establish a norm for these control animals. ASO and anti-deoxyribonuclease B levels were uniformly < 50 in all animals. Group A CHO antibodies were 0.01 in five animals and 0.02 in four others. A pool of sera from 15 additional uninfected animals was made and used as a control for subsequent experiments. Levels of antibodies in this pool were the same as for the nine individual controls.

Groups of animals were infected once intradermally or intra-articularly with strains of different streptococcal M types, and after designated intervals they were bled to determine antibody titers (Table 1). No ASO or anti-deoxyribonuclease B titers were detectable in any of the animal sera. However, group A CHO antibodies were noted in 7 of the 48 animals. Values of 0.10 or greater were considered significant levels for this antibody.

Comparison of the capacity of the various streptococcal M types to elicit group A CHO antibody responses (Table 1) showed some differences. Whereas 25 to 30% of animals with skin infections due to M types 4, 12, and 57 developed group A CHO antibodies 3 to 4 weeks after infection, no such antibody was detected in any of eight hamsters infected intradermally with M-type 2. The number of animals infected with the various streptococcal types is too small, however, to lend statistical validity to these data.

All animals infected with M-type 12 (four animals) and M-type 57 (eight animals) streptococci intradermally and bled 3 weeks later had also been bled before infection (Table 1). The group A CHO antibody levels in all 12 animals were 0.02 or less prior to infection. The three animals with elevated titers 3 weeks after infection represent definite responses to the group A CHO. Because of the high mortality rate with repeated cardiac punctures, preinfection bleedings were not done in subsequent experiments, and the pooled sera from 15 uninfected animals was used as a control.

Two of eight animals infected intradermally with M-type 57 streptococci exhibited group A CHO antibody rises in 3 weeks postinfection; however, none of the 14 animals infected with the same streptococcal type and bled 8 weeks postinfection exhibited any such elevations. Whether some of these 14 animals had an antibody elevation earlier that subsequently declined is impossible to determine because serial bleedings were not done on these hamsters.

The possibility that these animals had had no previous exposure to group A streptococci might explain the relatively poor responses noted above. The effect of repeated infections with streptococci was therefore explored. A group of 30 hamsters was infected intradermally with M-type 57 streptococcus on three successive occasions with an interval of 3 weeks between infections. Spontaneous healing occurred each time in 7 to 10 days after infection. All animals were bled 4 weeks after the last infection, and antibody determinations were performed on these sera. Fig. 1 is a scattergram of the titers of the three antibodies in these animals. Four of the 30 animals demonstrated elevated anti-deoxyribonuclease B titers, whereas none had any detectable ASO levels. For the group A CHO antibody, 33% (10 of 30 hamsters) of the animals had levels of 0.10 or greater, four animals with levels of 0.10 and six with levels greater than 0.10. Among the animals showing elevated anti-deoxyribonuclease B titers, only one (with an anti-deoxyribonuclease B titer of 200) had a simultaneously high group A CHO antibody level (titer of 0.48).

TABLE 1. Streptococcal antibody elevations in hamsters after a single infection*

<table>
<thead>
<tr>
<th>Site of infection</th>
<th>Streptococcal type</th>
<th>Bleeding time after infection (weeks)</th>
<th>No. with elevated levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ASO</td>
</tr>
<tr>
<td>Skin</td>
<td>M2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Skin</td>
<td>M4</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Skin</td>
<td>M12</td>
<td>3*</td>
<td>4</td>
</tr>
<tr>
<td>Skin</td>
<td>M57</td>
<td>3*</td>
<td>8</td>
</tr>
<tr>
<td>Skin</td>
<td>M57</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Joint</td>
<td>M57</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>48</td>
<td>0</td>
</tr>
</tbody>
</table>

* Abbreviations: ASO, anti-streptolysin O; CHO, carbohydrate.

† Animals in these groups were bled before infection and at the indicated time after infection.
To investigate the effect, if any, of the site of infection on the subsequent development of antibodies against the streptococcal products or components, an additional group of 17 hamsters was infected intra-articularly with M-type 57 streptococci. The animals were infected on three successive occasions with a 3-week interval between challenges. Four weeks after the last infection all animals were bled. The results of the antibody titers on these animals are shown in Fig. 2. Five of the 17 hamsters exhibited elevated anti-deoxyribonuclease B titers. Of the 16 sera available for ASO determinations, 4 demonstrated an elevation. All but one of the 15 sera tested for group A CHO antibody showed levels of 0.10 or greater. The one animal that showed no group A CHO antibody elevation failed to respond to streptolysin O and deoxyribonuclease B also. Among the four animals with elevated ASO titers, two had simultaneous anti-deoxyribonuclease B elevations.

Comparison of the antibody elevations as related to the site of repeated infection is summarized in Table 2. For all three antibodies, titers were much more frequently elevated after repeated joint infections as compared with repeated skin infections. Of the 30 animals infected intra-dermally, 13 (43%) showed an elevation of one or more antibody. In marked contrast, 14 of the 15 animals (93%) infected intra-articularly demonstrated such elevations.

### Table 2. Streptococcal antibody elevations after repeated infections

<table>
<thead>
<tr>
<th>Determination</th>
<th>Antibody elevations</th>
<th>Skin&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Joint&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASO</td>
<td>0/30 (0%)</td>
<td>4/16 (25%)</td>
<td></td>
</tr>
<tr>
<td>Anti-deoxyribonuclease B</td>
<td>4/30 (13%)</td>
<td>5/17 (30%)</td>
<td></td>
</tr>
<tr>
<td>Anti-group A CHO</td>
<td>10/30 (33%)</td>
<td>14/15 (93%)</td>
<td></td>
</tr>
<tr>
<td>Any one or more of those above</td>
<td>13/30 (43%)</td>
<td>14/15 (93%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> All three antibody determinations were possible on only 15 sera. Abbreviations: ASO, anti-streptolysin O; CHO, carbohydrate.<br>
<sup>b</sup> Site of infection.

### DISCUSSION

Several studies have amply documented that the ASO titer is not a reliable serological test for the retrospective diagnosis of streptococcal pyodermia in man (6, 9, 11, 12). The results of the present studies indicate that ASO responses also vary in the hamster in relation to the site of infection. Respiratory infection with group A streptococci could not be produced in this animal, but septic arthritis occurred consistently after intra-articular injection. Although arthritis in the animal model may not be optimally comparable to pharyngitis in humans, the differences between
the skin and other sites in the body in relation to antibody responses exist both in humans and in the hamster. No elevated titers occurred in any of 30 animals repeatedly infected intradermally, whereas 25% (4 of 16) of the animals with intrarticular streptococcal infection showed elevated ASO levels. The same streptococcal strain (M-type 57) was used in this comparison, so that qualitative differences in streptolysin O production are obviated. Furthermore, approximately the same amounts of streptococcal cells were injected intradermally or intra-articularly, resulting in comparable streptococcal antigen masses.

Variations in anti-deoxyribonuclease B levels in relation to site of infection were less pronounced. More animals exhibited elevations after joint infections (30%) than after skin infections (13%). However, no quantitative differences among the titers were noted. The mean titer of anti-deoxyribonuclease B for animals infected intradermally and exhibiting an elevated level was 170 as compared with 164 for the intra-articularly infected hamsters with high titers.

Repeated challenges were necessary to elicit antibody responses for streptolysin O and deoxyribonuclease B in infected hamsters. This is probably a reflection of the fact that this animal is immunologically virgin as far as the group A streptococcus is concerned, and therefore repeated exposure to the antigenic mass may be necessary to elicit a response.

Antibodies against the group A CHO were the most frequently demonstrable ones in the present studies. After a single challenge, approximately 14% of animals infected intradermally or intra-articularly showed elevated group A CHO antibody levels. With repeated challenges, the frequency of responses increased. This was particularly pronounced after repeated intra-articular challenges where 93% of the animals exhibited significant levels of this antibody.

An adequate explanation for the feeble ASO responses after skin infection in humans and in this experimental model is still lacking. Generalized immunological unresponsiveness or a small antigenic mass associated with streptococcal skin infection are excluded as possible explanations for other streptococcal antibodies are regularly elevated after such infections (9, 12). Although streptococcal strains are known to vary in production of various extracellular products (13), such variance in production of streptolysin O is probably not responsible for the dichotomy of ASO responses between skin and throat infections. During an epidemic period (9) when a single streptococcal strain (M-type 49) was responsible for most of the infections of both the skin and the respiratory tract, ASO responses were significantly higher in patients who had respiratory and skin infections than in those with skin infections alone. Furthermore, in the present studies, the same streptococcal strain was injected intradermally or intra-articularly, yet differences in the ASO levels were noted between these two groups.

That the site of infection per se, rather than the infecting strain, may be the determining factor for this disparity of the ASO response is a reasonable assumption based on the experimental data presented here and previous clinical reports (9, 12). Conditions on or in the skin may not be optimal for streptolysin O production. Some lipids, notably cholesterol, inhibit streptolysin O activity both in vitro and in vivo (1). It is possible that skin lipids may interfere with the antigenicity of streptolysin O, resulting in a poor ASO response. Additional work is warranted to examine this possibility.

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LITERATURE CITED

