Passive Transfer of Resistance to Frambesial Infection in Hamsters

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The immune mechanism by which hamsters acquire resistance to infection with Treponema pertenue, the causative agent of frambesia, or yaws, has not been elucidated. Serum or cells (spleen or lymph node) obtained from hamsters resistant to frambesial infection were transferred to normal syngenic recipients, who are subsequently infected with T. pertenue. The following parameters were used to measure the ability of immune serum or cells to confer resistance on recipient hamsters to frambesial infection: inhibition of the development of cutaneous lesions, decreased weight, and number of treponemes in the inguinal lymph nodes. This investigation demonstrated that immune serum conferred protection on recipient hamsters infected with T. pertenue. Discontinuation of the administration of immune serum (18 days after frambesial infection) did not result in the development of cutaneous lesions. Since the inguinal lymph nodes contained a sizeable number of treponemes (2.6 x 10⁶), immune serum failed to prevent frambesial infection. Recipients of immune spleen or lymph node cells initially developed frambesial lesions 9 days after infection. The frambesial lesions began to resolve 12 to 14 days after infection and by day 21 had completely regressed. These results illustrated that humoral factors and cells are involved in resistance of the hamster to frambesial infection.

The mechanism by which humans or experimental animals acquire resistance to treponemal infection has not been elucidated. A major obstacle in delineating the immune mechanism has been the unavailability of suitable inbred animal models. Inbred guinea pig and mouse strains are available; however, clinical manifestations of treponemal disease have not been regularly induced in these animal species (3, 7, 23, 26, 32, 35, 36). Although the rabbit is considered the animal model of choice for investigation of treponemal infections (1, 18, 32), inbred strains are not readily available. The most direct method to determine the mechanism of immunity would be to confer resistance on normal recipients by transferring serum or cells from animals immune to treponemal infection. The unavailability of inbred strains compromises immunological studies involving the transfer of acquired resistance to treponemal infection. The efficacy of treponemal immune cells or serum cannot be gauged in recipient outbred animals due to allogeneic differences. Inbred hamsters, however, are readily available and develop extensive chronic skin lesions after infection with Treponema pertenue, the causative agent of frambesia, or yaws (5, 9, 10, 12, 14, 32; B. J. Rosenau, Ph.D. thesis, Harvard University, Cambridge, Mass., 1953).

The purpose of this investigation was to determine the mechanism by which frambesial hamsters develop resistance to reinfection with T. pertenue. Serum or cells obtained from hamsters resistant to frambesial infection were transferred to normal syngenic recipients, who were subsequently infected with T. pertenue. The ability of immune serum or cells to inhibit the development of frambesial lesions would provide evidence as to the mechanism by which the host acquires resistance to infection with T. pertenue.

MATERIALS AND METHODS

Animals. Inbred hamsters CB/Ss Lak(CB) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Hamsters weighing 80 to 100 g were housed six per cage at an ambient temperature of 18°C, a condition which facilitates the development of cutaneous lesions (14).

Organism. T. pertenue Haiti B was obtained from Paul H. Hardy, Jr. (Johns Hopkins University, Baltimore, Md.) and was maintained by passage in hamsters. The inguinal lymph nodes were removed aseptically 3 to 4 weeks after intradermal (i.d.) infection, teased apart in sterile saline, and filtered through 60-mesh stainless-steel wire. After centrifuging at 270 x
g for 3 min to remove cellular debris, the number of treponemes in the supernatant was determined by dark-field microscopy.

**Infection of the hamster with T. pertenue.**

Hamsters used in this investigation were infected i.d. in the inguinal region with $10^6$ T. pertenue. Cutaneous lesions regularly developed in all hamsters in 16 to 17 days after infection. The development of cutaneous lesions was not the only clinical manifestation of frambesial infection, since the inguinal lymph nodes increased in weight and teemed with treponemes (13, 32). These three parameters were used in this investigation to evaluate the response of hamsters adoptively immunized with frambesial immune cells or serum.

**Preparation of spleen, lymph node, or bone marrow cells.** Pooled single-cell suspensions of spleen and lymph node cells were prepared by teasing apart these tissues with forceps and gently pressing through a stainless-steel 60-mesh wire into RPMI 1640 medium. Bone marrow cells were harvested from the femora and tibia by flushing the marrow cavities with RPMI 1640. Single cell suspensions were obtained by aspiration and expulsion through a 18-gauge needle. The number of viable nucleated cells was determined by the trypan blue exclusion test.

**Immune serum.** Immune serum was obtained from the following group of frambesial hamsters. Hamsters were treated with penicillin (5,000 U) 6 months after frambesial infection. Two weeks later the cured hamsters were divided into two groups. One group was reinfect ed i.d. with T. pertenue and was shown to be resistant to the development of frambesial lesions. The other group was bled by intracardiac puncture to obtain pooled immune serum. The sera were pooled, sterilized by filtration (0.22 μm; Millipore Corp.), and stored at $-20^\circ$C until use. The pooled immune serum had a micro-hemagglutination assay-T. pallidum (MHA-TP) antibody titer of 1:10240.

**Irradiation of hamsters.** Hamsters were exposed in Lucite containers to 950 rads of gamma irradiation by using a Gammator Cs-137 irradiator (Isomedix, Inc.). The mid-phantom dose rate was 35 krads/h. Hamsters exposed to 950 rads of irradiation survived for only 9 to 14 days. Hamsters survived the lethal effects of irradiation after reconstitution with $10^6$ normal hamster bone marrow cells. Intradermal infection of reconstituted and non-reconstituted irradiated hamsters with $10^6$ T. pertenue resulted in the development of cutaneous lesions 9 days after infection. In contrast, non-irradiated hamsters infected with $10^6$ T. pertenue developed cutaneous lesions 16 to 17 days after i.d. infection (see section on infection of hamsters with T. pertenue). Although inbred hamsters were used in this investigation, hamsters (recipient) were irradiated as an additional precaution to guarantee the survival of transferred immune or normal cells.

**Serological test.** The Sera-Test treponemal antibody test (MHA-TP) was obtained from Ames Co. (Elkhart, Ind.). The MHA-TP test is manufactured by Frijizoki Pharmaceutical Co., Ltd., Shinjuku-ku, Tokyo 161, Japan. The test was performed as described by the manufacturer with one exception. Serum obtained from frambesial hamsters was serially diluted with absorbing diluent to obtain quantitative titers.

**Statistical analysis.** The analysis of variance was used. The Fischer least significant difference test (29) was used to examine pairs of means when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 before the initiation of the experiments.

**RESULTS**

A large group of hamsters was infected i.d. in the inguinal region with $10^6$ T. pertenue. Six months after infection, hamsters were treated with penicillin (5,000 U) to terminate infection. Pooled immune serum or cells (spleen or lymph node) were obtained simultaneously from one group of these hamsters 2 weeks after treatment with penicillin. The remaining group of hamsters was reinfected with T. pertenue and failed to develop cutaneous lesions. Normal serum or cells (spleen, lymph node, or bone marrow) were obtained from hamsters matched for age and treated 2 weeks previously with penicillin. The following experiments utilized this group of normal or frambesial immune hamsters as donors of serum or cells.

**Passive transfer of resistance to frambesial infection with serum.** The purpose of this experiment was to determine whether treatment of hamsters with immune serum would protect animals from a subsequent infection with T. pertenue. Two groups of five hamsters were injected intravenously with immune or normal serum (0.5 ml) at 3-day intervals for 3 weeks. Three days after the first injection of immune or normal serum hamsters were infected i.d. with $10^6$ T. pertenue. Concomitantly, a third group of five hamsters that received no serum was infected with T. pertenue.

The time and the number of frambesial lesions that developed in hamsters receiving immune, normal, or no serum are shown in Table 1. Lesions failed to develop in hamsters receiving immune serum. The average time for the development of lesions in hamsters receiving no serum or normal serum was 16 ± 2 and 17 ± 2 days, respectively. Discontinuation of the administration of immune serum (18 days after

**Table 1. Development of frambesial lesions in hamsters that received immune or normal serum and were challenged i.d. with $10^6$ T. pertenue**

<table>
<thead>
<tr>
<th>Expt groups</th>
<th>No. of lesions/total sites inoculated</th>
<th>Avg time of lesion development (days)</th>
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</thead>
<tbody>
<tr>
<td>Immune serum</td>
<td>0/10</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Normal serum</td>
<td>10/10</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Control (no serum)</td>
<td>10/10</td>
<td>16 ± 2</td>
</tr>
</tbody>
</table>

*There were five hamsters per group. T. pertenue was inoculated at two sites per hamster.*
frambesial infection) did not result in hamsters developing frambesial lesions after prolonged observation (4 weeks).

These results demonstrated that the development of frambesial lesions in the hamster was influenced by the prior administration of immune serum. Immune serum, however, did not prevent infection of the hamster with *T. pertenue* (Table 2). The lymph nodes of hamsters that received immune serum increased in weight and contained a sizeable number of treponemes (2.6 × 10⁸) 21 days after infection. The increase in weight and the number of treponemes detected in the lymph nodes of passively immunized hamsters were significantly reduced (*P < 0.01*), when compared to the weight and number of treponemes detected in the lymph nodes of hamsters that received normal or no serum (Table 2). This suggested that hamsters receiving immune serum were partially protected from infection with *T. pertenue*.

Passive transfer of resistance to frambesial infection with cells. The previous experiment demonstrated that hamsters receiving immune serum were partially protected from a subsequent infection with *T. pertenue*. The purpose of this experiment was to determine whether cells obtained from hamsters resistant to frambesial infection would also confer resistance on normal recipients to infection with *T. pertenue*. Pooled splenic (10⁸) or lymph node (10⁸) cells from normal hamsters or hamsters resistant to frambesial were transferred to irradiated bone marrow (10⁸) reconstituted hamsters and subsequently infected i.d. with 10⁶ *T. pertenue*. As additional controls, the remaining group of hamsters that served as donors of immune or normal cells was also infected with *T. pertenue*.

Frambesial lesions which began as small scaly patches were detected 9 days after infection in all hamsters infused with immune or normal cells. These cutaneous lesions enlarged and became necrotic in recipients of normal cells (spleen or lymph node) 14 to 16 days after infection. In hamsters that received immune cells (spleen or lymph node), the frambesial lesions reddened and enlarged until 12 to 14 days after infection and then regressed. The normal cell donors developed lesions 16 to 17 days after infection. In contrast, no cutaneous lesions were detected in the group of hamsters that served as donors of immune cells.

The ability of immune or normal cells to influence the progression of frambesial lesions, the weight, and the number of treponemes in the lymph nodes of recipient hamsters 21 days after infection are shown in Table 3. The results clearly show that the recipients or donors of immune cells have a statistically significant reduction (*P < 0.001*) of cutaneous lesions and weight of lymph nodes when compared to the donors or recipients of normal cells. In addition, the number of treponemes detected in the lymph nodes of hamsters receiving immune cells was significantly reduced (*P < 0.001*) when compared to hamsters infused with normal cells. No treponemes were detected by dark-field microscopy in the lymph nodes of hamsters that received immune spleen cells or donated immune cells. A large number of treponemes (1.1 × 10⁸) was detected in the lymph nodes of the normal cell donors. These results suggest that resistance to frambesial infection can be conferred on recipient hamsters with immune cells. When this experiment was replicated, a similar effect of immune or normal cells on the progression of

<p>| Table 2. Effect of immune serum on the average weight and number of treponemes per lymph node* |</p>
<table>
<thead>
<tr>
<th>Expt groups</th>
<th>Avg wt of lymph node (g)</th>
<th>Approx no. of treponemes × 10⁶/lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune Serum</td>
<td>0.73</td>
<td>26</td>
</tr>
<tr>
<td>Normal Serum</td>
<td>0.093</td>
<td>1240</td>
</tr>
<tr>
<td>Control (no serum)</td>
<td>0.104</td>
<td>800</td>
</tr>
<tr>
<td>*At 21 days after infection.</td>
<td></td>
<td></td>
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<tr>
<td>*There were three hamsters per group.</td>
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<td></td>
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<tr>
<td>*The standard error associated with each mean was 0.009.</td>
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<tr>
<td>*Treponemes were estimated by darkfield microscopy.</td>
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</tbody>
</table>

<p>| Table 3. Ability of immune cells to influence the progression of frambesial lesions, the weight, and number of treponemes in the lymph nodes of recipient hamsters 21 days after infection |</p>
<table>
<thead>
<tr>
<th>Expt groups</th>
<th>No. of lesions/total sites inoculated*</th>
<th>Avg wt of lymph node (g)</th>
<th>Approx no. of treponemes × 10⁶/lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipients of: Immune spleen</td>
<td>0/6</td>
<td>0.022</td>
<td>0</td>
</tr>
<tr>
<td>Immune lymph node</td>
<td>1/6</td>
<td>0.026</td>
<td>1.2</td>
</tr>
<tr>
<td>Recipients of: Normal spleen</td>
<td>6/6</td>
<td>0.101</td>
<td>11.0</td>
</tr>
<tr>
<td>Normal lymph node</td>
<td>6/6</td>
<td>0.119</td>
<td>51.0</td>
</tr>
<tr>
<td>Immune cell donors</td>
<td>0/6</td>
<td>0.018</td>
<td>0</td>
</tr>
<tr>
<td>Normal cell donors (pure controls)</td>
<td>6/6</td>
<td>0.116</td>
<td>1147.0</td>
</tr>
<tr>
<td>*There were three hamsters per group. <em>T. pertenue</em> was inoculated at two sites per hamster. Lesions developed in all hamsters 9 to 16 days after infection.</td>
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<td></td>
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<tr>
<td>*Standard error associated with each mean was 0.013.</td>
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<td>*Treponemes were estimated by darkfield microscopy.</td>
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frambesial lesions in recipient hamsters was observed (Fig. 1).

DISCUSSION

Treponemal infection of the hamster has been virtually ignored during the last 2 decades. One of the reasons for this decreased research enthusiasm for this infection has been the paucity of human frambesial disease. Frambesial infection is a relatively insignificant public health problem in the United States (16) and is endemic in only a few areas of the Americas (16) and the world (15, 24). Second, the hamster has not been shown regularly to develop clinical manifestations of syphilitic disease (3, 18, 32, 35, 37). Since syphilis is considered a major public health problem, utilization of the hamster as an experimental model of treponemal disease has not received great encouragement.

The immune mechanism by which the hamster acquires resistance to frambesial infection is unclear (17, 19-21; Rosenau, Ph.D. thesis). The purpose of this investigation was to determine the mechanism by which frambesial hamsters acquire resistance to reinfection with T. pertenue. The most direct method to determine the mechanism of immunity would be to confer resistance on normal recipients by transferring serum or cells from animals immune to frambesial infection. In this investigation serum or cells obtained from hamsters resistant to frambesial infection were transferred to normal syngenic recipients, who were subsequently infected with T. pertenue. The ability of immune serum or cells to inhibit the development of cutaneous lesions and decrease the weight and number of treponemes in the lymph nodes provided evidence as to the mechanism of acquired resistance to infection with T. pertenue.

This investigation clearly has shown that serum obtained from hamsters immune to frambesia can confer partial protection on recipient hamsters infected with T. pertenue. Administration of immune serum to hamsters infected with T. pertenue inhibited the development of frambesial lesions (Table 1). Immune serum, however, did not prevent infection of the hamster with T. pertenue since the inguinal lymph nodes contained a sizeable number of treponemes (Table 2). These results suggested that antibody-mediated immunity was involved in resistance of the hamster to frambesial infection.

Similar observations on the effect of passive transfer of immune serum had been made involving T. pallidum (4, 6, 25, 28, 34). When administration of syphilitic immune serum to rabbits was discontinued, lesions (chancrets) developed. In this investigation withdrawal of treatment with immune serum (18 days after infection) did not result in hamsters developing frambesial lesions even after prolonged observation (4 weeks). This difference in the development of lesions between species after withdrawal of immune serum may be related to the type and quantity of antibody induced in donor animals. It is important to note that passive immunization of hamsters or rabbits did not

FIG. 1. Influence of immune spleen and lymph node cells on the progression of frambesial lesions in representative hamsters. Experimental groups included hamsters that received normal spleen (1), normal lymph node (2), immune spleen (3), and immune lymph node cells (4). Groups (5) and (6) represented hamsters that donated immune or normal (pure control) cells, respectively. Photograph of hamsters was taken 21 days after i.d. infection with T. pertenue.
prevent infection, suggesting that antibody is not completely treponemical.

Passive transfer of immune spleen or lymph node cells also conferred protection on recipient hamsters infected with *T. pertenue*. Similar observations have been reported by Guerraz et al. (8). Recipients of immune spleen or lymph node cells initially developed frambesial lesions 9 days after infection. These frambesial lesions, however, began to resolve 12 to 14 days after infection and by day 21 had completely regressed (Table 3). A possible explanation for the early onset and regression of frambesial lesions in recipients of immune cells is that sufficient antibody was not initially produced by immune cells to prevent the development of lesions. Once sufficient treponemal antibody became available, the lesions regressed. This explanation may also clarify the observation that hamsters infused with immune spleen cells appeared to be slightly more resistant to frambesial infection than recipients of immune lymph node cells (Table 3). Enhanced protection conferred by immune spleen cells might be explained on the basis of rapid antibody production; however, no significant difference was detected in the MHA-TP antibody titer (1:1,280) when serum from recipients of immune spleen or lymph node cells were compared 21 days after infection (R. F. Schell and J. P. Babu, unpublished observations).

Recently, Metzger and Smogor (22) demonstrated that normal rabbits infused with syphilitic immune lymph node cells were protected from a subsequent i.d. challenge with *T. pallidum*. Although these adoptive transfer studies were performed using outbred rabbits, the authors concluded that cell-mediated immunity was involved in protection of rabbits from syphilitic infection. In contrast, Baughn et al. (2), using inbred rabbits, transferred syphilitic immune spleen cells and failed to protect recipient rabbits from infection with *T. pallidum*. Baughn et al. (2) concluded that cell-mediated mechanisms do not play a central role in immunity to syphilis. The results of this investigation clearly showed that immune spleen or lymph node cells conferred protection on inbred hamsters challenged with *T. pertenue*. The successful transfer of resistance to frambesial infection using immune cells, however, does not mean that resistance is T-cell mediated (cell-mediated immunity). Additional experiments are needed that will selectively remove thymus-dependent cells from the immune spleen or lymph node cell populations before any conclusions can be drawn as to the effect of T-cell-mediated immunity on frambesial infection.

Although immune serum and cells conferred resistance on recipient hamsters to infection with *T. pertenue*, one might question whether the immune mechanism involved in frambesial infection is applicable to the other treponematoses, in particular, syphilis. This question can not be completely answered, although there is evidence to suggest that frambesial infection induces cross-immunity to syphilitic infection (17, 19, 20, 27, 31–33). It is well-documented that patients who have frambesia are protected against developing syphilis (11, 27, 30). Likewise, rabbits infected with yaws or syphilis show a substantial degree of immunity to challenge with heterologous treponemes (20, 33). Demonstration of cross-immunity between the treponematoses suggests that similar immune responses are induced by the primary infection with the various treponemes. Therefore, experimental frambesial infection of the hamsters might be an alternative model to elucidate the immune response mechanism by which humans recover from treponemal and, more specifically, syphilitic infection.

Finally, the results of this investigation demonstrated that hamsters have a number of parameters that can be utilized for evaluating the efficacy of a potential treponemal vaccine and monitoring the immune response to infection with *T. pertenue*. These parameters include: (i) the inhibition of cutaneous lesions, (ii) the weight, and (iii) the number of treponemes per inguinal lymph node. The inhibition of lesions has been the primary parameter by which the immune response of the rabbit (treponemal animal model of choice) has been used to monitor treponemal vaccination or infection. Since inbred rabbits are not readily available, hamsters possess some distinct advantages for determining the immune mechanism to treponemal infection.

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LITERATURE CITED