Standardization of the Experimental Model of *Haemophilus ducreyi* Infection in Human Subjects

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Human volunteers were challenged with *Haemophilus ducreyi*. Twenty subjects were inoculated with 2 doses (~30 cfu) of live and 1 dose of heat-killed bacteria at 3 sites on the arm. Eight subjects were assigned to biopsy 1 or 4 days after inoculation, and 12 were biopsied after they developed a painful pustular lesion or were followed until disease resolved. Papules developed at 95% of 40 sites infected with live bacteria (95% confidence interval [CI], 83.1%–99.4%). In 24 sites followed to end point, 27% of the papules resolved, 69% (95% CI, 47.1%–86.6%) evolved into pustules, and 4% remained at the papular stage. Recovery rates for a standardized EDD of bacteria and examined whether lesion formation and outcomes could be considered statistically independent in individual subjects.

**Methods**

**Human volunteers.** Healthy adult men and women volunteers (≥18 years old) were recruited for the study. Enrollment procedures and exclusion criteria were described in detail elsewhere [6, 7].

**Bacteria.** Master stocks of *H. ducreyi* 35000HP (human-passaged) were prepared by expanding a single colony isolated from a subject (C-7) after 13 days of experimental infection with 35000 [7]. In broth, 35000 and 35000HP had identical growth rates. The isolates had identical outer membrane protein and lipooligosaccharide profiles (data not shown).

**Inoculation.** Bacteria were grown to mid-log phase and processed exactly as described previously [6, 7]. One to 4 volunteers were challenged in 12 different iterations with 2 identical doses of live and 1 dose of heat-killed bacteria at 3 sites on the same arm. Suspensions containing the target dose (1.8–4.4 × 10^4 cfu) were inoculated into the skin of the upper arm with an allergy testing device [6, 7]. The device delivers ~1:1000 of solution loaded on its tines into the skin [6, 7]. Thus, the target EDD (18–44 cfu) of bacteria is likely to be 1000-fold less than that loaded on the tines.

**Study design and statistics.** We did not know whether lesion formation at 2 sites in an individual subject would be statistically
Results

Human subjects. Twenty-nine adults were enrolled in the study (13 men, 16 women; 8 black, 20 white, 1 Hispanic; mean age 34.3 ± 8 years). One volunteer withdrew from the study prior to inoculation. Another was excluded because she was taking ibuprofen regularly for arthritis.

Clinical response to experimental infection. Each volunteer was inoculated with 2 doses of live and 1 dose of heat-killed H. ducreyi 35000HP. Four volunteers received a lower EDD (4 or 11 cfu) than the target dose. Erythematous papules developed at 7 of 8 sites inoculated with live H. ducreyi and resolved in 2 to 7 days. These subjects were treated with ciprofloxacin, released from the study, and excluded from further analysis.

Twenty volunteers were challenged with the target EDD (range, 18–44 cfu; mean ± SD, 27.5 ± 9.8) of 35000HP (table 1). In an individual subject, lesion formation was sometimes discordant. Concordance did not differ from that expected by chance, and sites were assumed independent. Thus, the infection rate was determined based on all sites inoculated. Papules developed in 1 day at 38 (95%) of 40 sites (95% CI, 83.1%–99.4%). No lesions occurred at sites inoculated with heat-killed bacteria.

Eight subjects were assigned to biopsy 1 day and 4 days after inoculation. Since infection was terminated prior to achievement of the clinical end point, data from these 8 subjects were not used to calculate lesion outcome.

Twelve subjects were followed for 2 weeks or until they achieved the clinical end point. Nine of the 12 had concordant outcomes; in other words, both papules resolved or both papules evolved into pustules. Two of the 12 subjects had discordant outcomes; a painful pustule developed at 1 site while the second lesion resolved or remained a papule. One of the 12 subjects developed a papule at only 1 site, which resolved in 3 days. Since lesion outcome in the 2 sites in individual subjects appeared independent, the pustule formation rate was based on 23 papules followed to end point. Six (27.3%) of the papules resolved in 3–5 days (4.3 ± 1), and 1 papule remained at the papular stage through the observation period. The other 16 papules evolved into pustules in 2–5 days (3.5 ± 1). The pustule formation rate was 69.6% (95% CI, 47.1%–86.8%). None of the pustules resolved within 3–11 days (7.2 ± 2.4) of observation.

Recovery of H. ducreyi from experimental lesions. The recovery rate of H. ducreyi from surface cultures ranged from 14% to 41.2% between day 1 and day 7 (figure 1). H. ducreyi was recovered intermittently (67 of 174 cultures) from 11 (65%) of 17 lesions that did not resolve. However, none of the 6 papules that resolved were culture-positive (0 of 27 cultures

### Table 1. Outcome of human inoculation experiments with H. ducreyi.

<table>
<thead>
<tr>
<th>Duration of infection</th>
<th>No. of sites inoculated</th>
<th>Initial papule</th>
<th>Final outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Papule</td>
</tr>
<tr>
<td>End point</td>
<td>24</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>1 day</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>4 days</td>
<td>8</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 1. % of positive surface cultures after inoculation with live H. ducreyi. Nos. above bars represent lesions cultured on that day.
obtained when disease was present). The difference in the recovery rate of *H. ducreyi* from lesions that did not resolve and from lesions that resolved was statistically significant (Fisher’s exact test, *P* = .014). Cultures (n = 104) of sites inoculated with heat-killed bacteria were sterile.

*H. ducreyi* was recovered from 0 of 4 papules and 3 of 5 pustules that were semiquantitatively cultured. The range of *H. ducreyi* recovered varied from 6.50 × 10^2 to 3.75 × 10^3 cfu per gram of tissue. *H. ducreyi* was recovered from 1 of 3 papules and 9 of 10 pustules that were cultured qualitatively. Hence, the overall recovery rate of *H. ducreyi* from all biopsies was 80% (12/15) for pustules and 14% (1/7) for papules (Fisher’s exact test, *P* = .007).

Complications. Three volunteers were inoculated with a mixed culture of *H. ducreyi* and *Pseudomonas aeruginosa*. The *Pseudomonas* isolate was sensitive to ciprofloxacin, and the EDD of the contaminants was 10 cfu. These volunteers developed disease typical of experimental chancreoid, and the EDD of the contaminants was 10 cfu. These volunteers were treated with 2 doses of oral ciprofloxacin. Their lesions resolved, and the subjects were released from the study.

All subjects were contacted 6 months after participation in the study. Two volunteers developed hypertrophic scars at the biopsy sites and were treated with intralesional injections of triamcinolone. The hypertrophic scars became flat after treatment.

**Discussion**

In dose-response trials with *H. ducreyi* 35000, papule and pustule formation rates could not be determined due to the small sample size. In this study, inoculation of a mean EDD of 27.5 ± 9.8 cfu of 35000HP caused papule formation at 95% of 40 sites (95% CI, 83.1%–99.4%) infected with live bacteria. For 23 papules followed to the clinical end point, the pustule formation rate was 69% (95% CI, 47.1%–86.6%). *H. ducreyi* 35000 and 35000HP had similar outer membrane and lipooligosaccharide profiles and growth rates and appeared to be equally virulent based on historical controls in the human challenge model.

Several subjects followed to clinical end point had discordant outcomes, where 1 papule developed into a pustule while a second papule resolved or remained at the papular stage. None of the pustules resolved within the observation period of 3–11 days. These findings suggest that local factors play a role in the resolution of the disease and that the immune system may clear disease at the papular but not the pustular stage.

We recovered *H. ducreyi* intermittently from the surface of 11 of 17 pustules and nonresolving papules and from 0 of 6 papules that resolved. Thus, *H. ducreyi* was shed intermittently from sites with established infection and not from sites where infection was transient. *H. ducreyi* was recovered from 3 of 5 pustules and 0 of 4 papules that were quantitatively cultured.

An EDD of 18–44 cfu was injected into the skin of the subjects, and entire pustules were estimated to contain 7.2 × 10^2 to 6.0 × 10^3 recoverable cfu. The overall recovery rate of *H. ducreyi* from biopsies was 80% for pustules and 14% for papules. Taken together, the data indicate that *H. ducreyi* replicate between the papular and pustular stages.

Inadvertent infection with a mixed bacterial culture occurred once during this study. The contaminant was probably introduced into the inoculum during processing of the mid-log phase culture. We revised our inoculum preparation protocol and have safely infected an additional 45 volunteers in 19 different iterations. However, our informed consent statements note the potential risk of inadvertent infection with contaminants.

None of the volunteers developed fever or lymphadenopathy. No secondary transmission occurred in 125 patient-days of observations. However, 2 volunteers developed hypertrophic scars at sites that were biopsied. To date, we have completed several trials of human experimental infection with *H. ducreyi* [6–10]. Of 65 subjects who were challenged, 53 underwent biopsy, and 7 (13.2% of those biopsied) formed hypertrophic scars. Hypertrophic scars formed only where clinical disease was present and a biopsy was done. Of 15 blacks who were biopsied, 5 (33%) formed hypertrophic scars; of 36 whites who were biopsied, 2 (5.5%) formed hypertrophic scars (*P* = .018). Seven of 28 women formed hypertrophic scars, whereas 0 of 25 men formed hypertrophic scars (*P* = .034). Hypertrophic scars are known to form more commonly in blacks and Asians than in whites [11, 12], and there is no known association between gender and hypertrophic scarring [11]. Thus, the increased prevalence in minorities is probably significant, while the gender differences may be spurious. We now include the above data in our revised informed consent statements. At the intake visit, volunteers are shown pictures of scars that result from participation in the study, including those with good cosmetic results and hypertrophic scars before and after treatment with intralesional triamcinolone. Volunteers are asked not to participate in the studies if the possible cosmetic results are unacceptable to them.

In summary, these data have laid the foundation for other experimental infection trials with *H. ducreyi*. An EDD of ≤15 cfu of *H. ducreyi* generally causes only transient infection [7]. Inoculation of an EDD of 100–1000 cfu causes a pustule formation rate of 100%, but disease progresses too rapidly to mimic natural infection [6]. Inoculation of ~30 cfu causes a papule formation rate of 95% and a pustule formation rate of 69%. Thus, for chemoprophylaxis or vaccine trials, subjects are inoculated at 2 sites with 30–60 cfu to ensure that a susceptible subject will become infected [9]. Lesion outcome at multiple sites inoculated with the same isolate in an individual subject may be independent. For comparison of an isogenic mutant and its parent, subjects are infected at multiple sites with the parent and the mutant, as opposed to inoculating subjects with 1 dose of each isolate [10]. If the mutant is avirulent at an EDD
of 30–60 cfu, the EDD of the mutant can be increased into the $10^2$ to $10^3$ cfu range to assess if the isogenic mutant is severely impaired in virulence. However, the EDD of the parent must be held in the 30 cfu range or disease will progress at these sites too rapidly to allow sufficient time to observe a subject.

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References