A New Human Colonization Model for Nontypeable *Haemophilus influenzae*

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(See the major article by Winokur et al on pages 728–38.)

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Nontypeable *Haemophilus influenzae* are small gram-negative bacteria that colonize the upper respiratory tract of humans, beginning at a very early age [1]. Although these organisms are normally commensals, when host defenses are compromised by underlying medical conditions such as malnutrition, immunodeficiency, chronic lung disease, or acute viral infection, *H. influenzae*–associated disease may ensue [2–4]. The contribution of nontypeable *H. influenzae* to the disease burden of children with otitis media is substantial. Among children in the developed world, this organism is currently responsible for an estimated 40%–50% of the cases of acute otitis media and an even higher percentage of cases of chronic and recurrent disease [5, 6]. In the adult population, particularly among patients with chronic obstructive pulmonary disease, nontypeable *H. influenzae* are major contributors to the ongoing disease process, particularly during the acute exacerbations that characterize the disease in many patients [4, 7]. Much has been learned about the molecular pathogenesis of disease caused by nontypeable *H. influenzae* in the past 2 decades, using a variety of in vitro models and in vivo models [8]. The long-term goal of much of this work has been to gain sufficient knowledge about the disease process, such that vaccines or other novel therapies can be developed to prevent nontypeable *H. influenzae*–associated disease in the future [9]. However, one criticism of this work has been that the findings may not be truly relevant to understanding human disease because many of these earlier studies have been conducted in nonhuman systems. Thus, the study reported by Winokur et al in this issue of the *Journal*, in which they describe the development of a new human model of nasopharyngeal colonization with nontypeable *H. influenzae*, is particularly timely and has the potential to be very important for the field [10].

In their newly described human colonization model, Winokur et al used a single well-characterized strain of nontypeable *H. influenzae* to intranasally inoculate a small group of human volunteers. The challenge strain was genetically modified to make it streptomycin resistant. This allowed the investigators to efficiently recover the organism from nasopharyngeal specimens collected from subjects over the course of the study. The investigators also used a carefully planned dosing algorithm that consisted of stepwise up-and-down dosing inocula, such that doses of bacteria for later volunteers were dependent on colonization success or failure in earlier study subjects. This approach allowed the investigators to quickly determine the dose that would be expected to lead to successful colonization of most study subjects and minimized the overall number of volunteers required for the project.

Fifteen volunteers took part in the study and, depending on their place in the study sequence, were scheduled to receive 1000–100 000 colony-forming units (CFU) of the nontypeable *H. influenzae* test strain that was administered by nose drops to each nares. The actual inoculum received by each volunteer was monitored and in most instances reasonably approximated the intended dose. Nine of the fifteen study subjects were successfully colonized with the bacteria, and the chances of success generally correlated with increasing doses of the bacteria. The investigators estimated a human colonizing dose 50 (HCD_{50}) of 1991 CFU and a HCD_{90} of 150 314 CFU, although each value was associated with relatively wide confidence intervals, reflecting the variability observed in the study group as a whole. Nasal wash specimens were collected from each volunteer on days 3–6

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following inoculation. In individuals who were successfully colonized, positive results were generally observed for nasal wash specimens obtained by days 3 or 4 after inoculation, and results for specimens obtained on successive monitoring days were also positive. Bacterial concentrations in the wash specimens appeared to increase with time, although precise quantitation of bacterial densities in the specimens was not performed. All subjects received antibiotic treatment with levofloxacin to eliminate colonization after the day 6 specimens were obtained and recovered uneventfully.

Although nasopharyngeal acquisition of common respiratory tract bacteria has generally been thought to be an asymptomatic process, it was notable that a majority of study subjects experienced mild-to-moderate upper respiratory tract symptoms following inoculation. None of the study subjects developed a documented fever, but 5 subjects developed headache, 6 subjects developed rhinorrhea or nasal congestion, and 1 subject developed mild-to-moderate sore throat. There appeared to be a close temporal relationship between the development of sore throat and the recovery of nontypeable *H. influenzae* from the nasal wash specimens. Furthermore, resolution of sore throat symptoms occurred in the majority of colonized subjects coincident with receipt of levofloxacin, again supporting a causative role for bacterial colonization in the development of symptoms.

Demonstration of interaction of the nasopharyngeal bacteria with the host immune system is important in validating the model and in justifying use of the model in future investigations. Not surprisingly, given the ubiquity of nontypeable *H. influenzae* in the normal nasopharyngeal flora, a screening enzyme-linked immunosorbent assay detected antibody to whole bacterial cells in preinoculation serum samples from all subjects. However, only colonized subjects demonstrated ≥4-fold increases in titers of the major antibody classes, with 7 of 9 subjects demonstrating such an increase and with IgG being the class most likely to show a 4-fold rise.

The work presented by Winokur et al represents an important first step in the development and application of this unique model. Many questions that could not be directly addressed previously can now be examined in this model system. The investigators appropriately chose to begin their studies by using a previously well-characterized bacterial strain [11, 12]. In future studies, evaluation of mutants derived from the parent strain for their ability to colonize human volunteers should allow the investigators to quickly assess the relative importance of the many surface adhesins and other *H. influenzae* virulence factors that have been previously defined in other model systems [13–15]. Other investigators studying the related organism *Haemophilus ducreyi* have had great success defining the relative importance of its potential virulence factors for humans by evaluating *H. ducreyi* mutants in a different human challenge model [16].

The nontypeable *H. influenzae* colonization model would also seem to hold great promise for the evaluation and comparison of the many vaccine candidates being studied for prevention of nontypeable *Haemophilus*–associated disease [9]. Despite many years of research, there is still no well-recognized correlate of protection against nontypeable *H. influenzae*–associated disease in humans. This has made it very difficult to objectively assess and compare the many vaccine candidates that have been studied over the years. The colonization model developed by Winokur et al may finally give investigators the ability to critically evaluate the protective potential of the leading vaccine candidates prior to progressing to human clinical trials [17, 18]

Despite the promise of the model, there are still many questions and challenges that will need to be addressed and further explored as the work moves forward. Some of these relate to the nontypeable *H. influenzae* organisms themselves and others relate to the human subjects that would form the basis of future investigations. Nontypeable *H. influenzae* are a very diverse group of organisms with the ability to rapidly change their genetic makeup via natural transformation [19, 20]. One must always be cautious about drawing broad conclusions about experimental results on the basis of studies of a single strain, which may be more or less representative of the bacterial population as a whole. While the data derived from the colonization model is valuable in its own right even with the one prototype strain described in the article by Winokur et al, the validity and generalizability of future studies would be strengthened by the inclusion of additional strains. This concern would apply not only for studies of virulence traits, but also for studies of potential vaccine candidates whose antigenic properties and expression levels may vary considerably from strain to strain [9].

Another potential challenge in the development and interpretation of the colonization model concerns the effect of preexisting immunity on the success or failure of colonization. The investigators allude to this point in their discussion, noting that one volunteer who received an inoculum well above the predicted HCD_{90} challenge dose was not successfully colonized. Without good understanding at this point as to what specific antibodies or other immune factors are most important in preventing colonization, it may be difficult to reliably prevent this same sort of problem from occurring in future studies. Hopefully, as their work goes forward, these investigators will be able to better define correlates of protection against colonization on the basis of either analysis of preexisting antibody profiles or assessment of protection offered by individual candidate vaccines.

Even with the potential problems that must still be overcome, the development of the nontypeable *H. influenzae* human colonization model must still be seen as a significant advance for the field. The model will, for the first time, provide investigators with a system in which

experimental questions related to nontypeable *H. influenzae*–associated disease pathogenesis and vaccine efficacy can be objectively addressed with confidence that the results may have direct applicability to understanding and preventing disease in humans. Winokur et al are to be commended for this important piece of work.

Notes

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