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Adenovirus Serotype 1 Does Not Act Synergistically with *Moraxella* (*Branhamella*) *catarrhalis* To Induce Otitis Media in the Chinchilla

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A chinchilla model of otitis media in which adenovirus compromise of the tubotympanum facilitates the subsequent induction of middle ear disease was used to investigate *Moraxella* (*Branhamella*) *catarrhalis* pathogenesis. Intranasally inoculated *M. catarrhalis* did readily colonize the nasopharynx of this host; however, despite evidence of viral infection and tubotympanal compromise, *M. catarrhalis* did not induce culture-positive otitis media in this model.

Moraxella (*Branhamella*) *catarrhalis*, a gram-negative diplococcus, has emerged as the third-ranking commensal inhabitant of the upper respiratory tract capable of inducing otitis media (OM), sinusitis, conjunctivitis, bronchitis, and lower respiratory tract infections (6, 10, 17, 20, 25). While the specific characteristics and pathogenic mechanisms of this microorganism are being investigated by several groups (1, 4, 7, 12–14, 19, 22–24, 27), developing an animal model of human disease due to *M. catarrhalis* has been difficult (8, 11, 31, 33). Previous attempts by our laboratories, and others, to model *M. catarrhalis*-induced OM via a variety of protocols have failed to demonstrate an active infection in either chinchillas (8), rats (33), or gerbils (11) after transbullar inoculation, despite the extremely large inocula used ($\sim 10^6$ to 10^9 CFU). Similarly, in a mouse model wherein a bolus ($\sim 10^5$ CFU) was inoculated directly into the lung, there was marked clearance of 8 of 10 *M. catarrhalis* strains within 6 h and complete clearance of all strains within 24 h (31).

Since increasing the inoculum further is not reasonable and there are no more-direct methods to challenge the respiratory tract, we attempted to model *M. catarrhalis*-induced OM using a dual-pathogen synergy model which relies exclusively on intranasal (IN) inoculation of both pathogens (30). Such synergy has been documented to occur in chinchillas, which were significantly more prone to OM due to *Streptococcus pneumoniae* when this organism was coinoculated with influenza A virus than they were when they received either agent alone (15, 16). Similarly, synergy was also noted with chinchillas dually challenged with adenovirus and nontypeable *Haemophilus influenzae* (NTHI) (30). The goal of the present study was to determine if we could expand the utility of this model for use in future studies of *M. catarrhalis* OM and its prevention. We hypothesized that establishment of an *M. catarrhalis* infection in the middle ear perhaps required predisposition of the host by viral compromise of either the structural and/or functional integrity of the tubotympanum or immune function (2, 3).

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Ten chinchillas (*Chinchilla laniger*) (mean weight, 570 g), free of signs of OM as determined by otoscopy and tympanometry, were used. A cohort of five animals received 6×10^6 50% tissue culture infective doses of adenovirus type 1 IN in 0.5 ml of minimum essential medium (MEM) (inoculum divided equally between nares) via passive inhalation on day −7 (3, 30). A control cohort of five chinchillas received an equal volume of sterile MEM (BioWhittaker, Walkersville, Md.) via an identical route (day −7). Adenovirus-inoculated animals were isolated to prevent cross-infection of controls. Seven days later (day 0), all 10 animals were inoculated IN with approximately 10^8 CFU of *M. catarrhalis* 1857 suspended in 0.5 ml of sterile saline. This strain is a minimally passaged pediatric middle ear effusion isolate described previously (8).

All animals were assessed daily (blindly, by the same observer) for signs of (i) OM as determined by otoscopy, wherein inflammatory changes were rated on a scale of 0 to 4+ (0 = normal, 1+ = minimal inflammation, 2+ = moderate inflammation with a minimal effusion volume, 3+ = significant inflammation with a moderate effusion volume, and 4+ = severe inflammation with perforation and discharge); (ii) general health or adenovirus infection as indicated by ruffling of fur, cornering behavior, conjunctivitis, labored breathing, or thickened nasal secretions; (iii) relative middle ear pressure as determined by tympanometry (normal range for chinchillas, −60 to +40 to daPa); and (iv) labyrinthine (inner ear) involvement (rated on a severity scale of 0 to 3+). These assessment criteria have been described previously (29, 30). On days 1, 4, 7, 10, and 14 postinoculation of *M. catarrhalis*, nasopharyngeal (NP) lavages were performed and bilateral epitympanic taps were attempted, even in the absence of an observable effusion. To semiquantitate the bacteria per milliliter of lavage fluid, as an indicator of the colonization status of both the nasopharynx and the middle ear, the collected lavage fluids were cultured on chocolate agar and *M. catarrhalis* colonies were identified by standard methods (8).

Mild inflammation of the tubotympanum, previously reported for this viral isolate (3), occurred in all adenovirus-inoculated animals starting approximately 4 days after virus inoculation (day −3) and was maintained until 7 days after bacterial inoculation (Table 1). Control animals demonstrated normal tympanic membranes until day −1, when an average inflammation value of 1+ was recorded and was likely due to repeated handling and examination. Clinical signs of illness

TABLE 1. Mean tympanic membrane inflammation scores

Cohort (n = 5)	Result[a] on indicated day relative to challenge with *M. catarrhalis*																			
	−7[b]	−6	−5	−4	−3	−2	−1	0[c]	1	2	3	4	5	6	7	8	9	10	13	14	15
M. catarrhalis only	0	0	0	0	0	0	1+	1+	1+	1+	1+	1+	1+	1+	1+	0	0	0	0	0	0
Adenovirus and *M. catarrhalis*	0	0	0	0	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0	0	0	0	0	0

[a] Scores indicating mild inflammation are shown in boldface type.
[b] Adenovirus inoculation.
[c] *M. catarrhalis* inoculation.

which would provide indirect evidence of an active adenovirus infection (or a lack thereof) were also recorded. On day −2 (5 days after virus inoculation), 9 of 10 ears were rated 1+, 9 of 10 ears were slightly retracted, two of five animals demonstrated cornering behavior, and two animals were febrile (temperatures of ≥102°F [38.9°C]) in the test cohort. Conversely, in the control cohort 3 of 10 ears were rated 1+, 3 of 10 ears were slightly retracted, one animal demonstrated cornering behavior, and no animals were febrile. The average middle ear pressure in adenovirus-inoculated chinchillas at the time of *M. catarrhalis* inoculation (day 0) was −7.2 daPa whereas that of sham-infected controls was +33.0 daPa, indicating the anticipated adenovirus-induced middle ear underpressure in test animals.

Mean CFU of *M. catarrhalis* per milliliter of NP lavage fluid per cohort are shown in Table 2. The values are typical for IN inoculation of chinchillas with *M. catarrhalis*: 24 h after inoculation (day 1), numbers of recoverable bacteria are generally 2 to 3 log units less than the number introduced; later, a stable colonization level of approximately 10^3 to 10^5 CFU/ml of lavage fluid is reached and maintained for a minimum of 2 weeks (unpublished data). The two cohorts had equivalent CFU of *M. catarrhalis* per ml recovered from the nasopharynx for 14 days after inoculation, indicating the organism's ability to colonize this site in chinchillas. Interestingly, we were able to obtain nearly pure cultures of *M. catarrhalis* (95 to 99% pure) from NP lavage fluids in 100% of animals from day 1 to day 4 of this study, after which time the percentage of *M. catarrhalis* colonies was gradually reduced to less than 10% on day 14. This phenomenon is quite atypical for NP lavage fluids obtained post-IN inoculation of chinchillas with either NTHI or *S. pneumoniae*: a significant amount and variety of normal flora are typically obtained along with the experimentally introduced bacterium when nonselective plating media are used. The mechanism behind this phenomenon is obscure at this time and cannot be attributed to adenovirus infection since it was noted with sham-inoculated animals as well.

While statistically significant differences ($P \leq 0.01$) between values for adenovirus-inoculated and sham-inoculated cohorts were noted on days 3 and 5 post-bacterial challenge, with results based on mean tympanic inflammation scores prerounding (1.4 versus 0.7 and 1.0 versus 0.5, respectively), no significant differences were noted on any other day of observation. In addition, no middle ear fluids were noted for or obtained from any animal (all attempted taps were "dry"), nor was any labyrinthitis noted for any animal throughout the duration of the study, which is consistent with the lack of otoscopic or tympanometric evidence of bacterial OM in either cohort.

Attempts to model disease induced by *M. catarrhalis* have not been successful to date. While these models have shown evidence of local inflammation at the site of bacterial introduction (8, 11, 31, 33), no evidence of significant bacterial multiplication postinoculation has been shown in any model and eradication of even large inocula of *M. catarrhalis* from either the tympanum or the lung has been unequivocally rapid. In addition, nearly equivalent inflammatory changes in the middle ear can be obtained via inoculation of formalin-inactivated bacteria (8) and isolated lipooligosaccharide (LOS) from NTHI (9), suggesting that these effects can be attributed to bacterial cell envelope components. The collective data obtained from mice, rats, gerbils, and chinchillas thus suggest that the existing animals models of *M. catarrhalis* infection are useful primarily for studies of colonization, bacterial clearance, or specific aspects of inflammation but clearly not for studies of pathogenesis, the kinetics of disease development, or therapeutics.

In the present study, we attempted to allow an upper respiratory tract infection induced by a pediatric adenovirus type 1 isolate to predispose the chinchilla host to *M. catarrhalis*-induced OM. This virus has been shown to act synergistically with NTHI residing in the nasopharynx to induce OM primarily by its effects of thickening of NP and bullar secretions, induction of underpressured middle ears, depression of ciliary beat frequency, disruption of transport function in the middle ear and the eustachian tube, and focal destruction of mucosal epithelium (3, 30). Despite evidence obtained in this study that chinchillas were colonized with *M. catarrhalis*, as well as indications that chinchillas were manifesting an adenovirus infection (as determined by previously established criteria [3, 30]), no synergistic effect on development of OM similar to that noted with NTHI was found under the conditions used.

Although it is possible that other adenovirus serotypes may be more effective than type 1, this possibility seems remote. It is more likely that another upper respiratory tract virus is perhaps involved in *M. catarrhalis*-induced OM (10, 26). Alternatively, *M. catarrhalis* may require the copresence of other bacterial pathogens, occurring either simultaneously or as a

TABLE 2. Mean CFU of *M. catarrhalis* per milliliter of NP lavage fluid

Cohort (n = 5)	Mean CFU on indicated day post-bacterial challenge (% *M. catarrhalis* colonies)				
	1	4	7	10	14
M. catarrhalis only	4.1×10^6 (95)	2.2×10^5 (90)	1.9×10^4 (46)	7.4×10^3 (<10)	2.4×10^4 (<10)
Adenovirus and *M. catarrhalis*	3.3×10^6 (96)	2.2×10^{5a} (90)	1.9×10^4 (36)	1.9×10^4 (15)	5.5×10^3 (<10)

[a] One animal deleted from study because of moribundity.

preexisting infection, to create optimal growth conditions for the succession of *M. catarrhalis*. This hypothesis is supported by culture data which indicate that *M. catarrhalis* is frequently cocultured from respiratory fluids (17, 25). Casselbrant et al. (5) found that in 13 of 30 (43%) middle ear fluid specimens which contained *M. catarrhalis*, this organism was present as part of a mixed bacterial population, similar to the findings of Van Hare et al. (32), who reported that *M. catarrhalis* was present in combination with either *H. influenzae* or *S. pneumoniae* or both in 21 of 61 (34%) respiratory fluid specimens. Sarubbi et al. (28) similarly reported mixed culture of *M. catarrhalis* from 47% of 457 adult respiratory secretion specimens positive for this organism, the remainder yielding pure cultures.

What is clear from our data is that while most clinical evidence suggests that a higher rate of colonization by *M. catarrhalis* occurs in conjunction with an increased incidence of OM (12, 18, 26), the induction of bacterial disease in the tympanum does not appear to be an inevitable event following successful NP colonization, even when there is a compromised primary middle ear defense mechanism (i.e., the ciliated eustachian tube in this model). Middle ear fluids were not collected in the above-mentioned colonization studies, and so the link between *M. catarrhalis* colonization and bacterial OM has not been directly demonstrated. In a recent study of high-risk Australian Aboriginal infants, in fact, *M. catarrhalis* colonization of the nasopharynx occurred as early as 8 days after birth; however, the authors did not find a strong association between OM and *M. catarrhalis* colonization alone, whereas a correlation was noted with either *S. pneumoniae*, NTHI, or mixed colonizations (21). Our data thereby support the widely recognized, yet unexplained, phenomena that despite there being a multitude of commensals inhabiting the nasopharynx at all times, (i) only a rather exclusive subset of the NP inhabitants successfully make up the triad of primary pathogens of OM and (ii) colonization does not per se increase the risk of OM (26).

In conclusion, there are no existing animal models of *M. catarrhalis* disease. Perhaps, because of our as yet unclear understanding of this organism's innate mechanism of pathogenesis, these models do not yet truly reflect the natural process by which *M. catarrhalis* gains access to and induces disease in the human respiratory tract. The chinchilla does, however, provide us with an excellent model of NP colonization by *M. catarrhalis*. Studies aimed at effectively inhibiting or disrupting this colonization phenomenon, a prerequisite for the development of OM, are ongoing.

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