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Serum antibody response to *Moraxella catarrhalis* proteins OMP CD, OppA, Msp22, Hag, and PilA2 after nasopharyngeal colonization and acute otitis media in children

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ABSTRACT

Background: There is no licensed vaccine for *Moraxella catarrhalis* (*Mcat*), which is a prominent bacterium causing acute otitis media (AOM) in children and lower respiratory tract infections in adults. Nasopharyngeal (NP) colonization caused by respiratory bacteria results in natural immunization of the host. To identify *Mcat* antigens as vaccine candidates, we evaluated the development of naturally induced antibodies to 5 *Mcat* surface proteins in children 6–30 months of age during *Mcat* NP colonization and AOM.

Methods: Human serum IgG against the recombinant *Mcat* proteins, outer membrane protein (OMP) CD, oligopeptide permease (Opp)A, hemagglutinin (Hag), Moraxella surface protein (Msp)22, and PilA clade 2 (PilA2) was quantitated by using an ELISA assay.

Results: There were 223 *Mcat* NP colonization episodes documented in 111 (60%) of 184 children in the study. Thirty five *Mcat* AOM episodes occurred in 30 (16%) of 184 children. All 5 *Mcat* candidate vaccine antigens evaluated stimulated a significant rise in serum IgG levles over time from 6 to 36 months of age ($P < 0.001$), with a rank order as follows: Msp22 = OppA > OMP CD = Hag = PilA2. Children with no detectable *Mcat* NP colonization showed a higher serum IgG level against OppA, Hag, and Msp22 compared to those with *Mcat* NP colonization ($P < 0.05$). Individual data showed that some children responded to AOM with an antibody increase to one or more of the studied *Mcat* proteins but some children failed to respond.

Conclusions: Serum antibody to *Mcat* candidate vaccine proteins OMP CD, OppA, Msp22, Hag, and PilA2 increased with age in naturally immunized children age 6–30 months following *Mcat* NP colonization and AOM. High antibody levels against OppA, Msp22, and Hag correlated with reduced carriage. The results support further investigation of these vaccine candidates in protecting against *Mcat* colonization and infection.

Abbreviations: *Mcat*, *Moraxella catarrhalis*; AOM, acute otitis media; NP, nasopharyngeal; OP, oropharyngeal; OMP, outer membrane protein; *Spn*, *Streptococcus pneumoniae*; NTHi, non-typeable *Haemophilus influenzae*; OppA, oligopeptide permease A; Msp, Moraxella surface protein; MID, Moraxella IgD-binding protein; Hag, hemagglutinin; PilA2, PilA clade 2; mo, months; MEF, middle ear fluid; *E. coli*, *Escherichia coli*; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; ICH, International Conference on Harmonisation; ANOVA, analysis of variance; GAM, generalized additive model.

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1. Introduction

Moraxella catarrhalis (*Mcat*) is a Gram-negative diplococcus that commonly colonizes mucous membranes of the human nasopharynx [1]. *Mcat* is also a transmittable pathogen responsible for various respiratory infections in children and adults resulting in a significant medical and economic burden worldwide [1–3]. Our recent studies revealed that *Mcat* now has overtaken *Streptococcus pneumoniae* (*Spn*) and non-typeable *Haemophilus influenzae* (NTHi) as the most frequent cause of episodic and recurrent acute otitis media (AOM) in children [4]. AOM and all respiratory bacterial infections begin pathogenesis with nasopharyngeal (NP) colonization. However, colonization is mostly asymptomatic; only when the condition of the host is altered will *Mcat* invade the middle ear, causing AOM or the bronchi and lungs, causing acute exacerbations of chronic bronchitis in adults.

Mcat vaccine development is currently moving from antigen identification to clinical trial. A number of potential vaccine antigens of *Mcat* have shown significant immunogenicity and protective effectiveness in various animal models [5–7]. Several prior studies have detected antibody responses to *Mcat* proteins in humans [5–11]. Some *Mcat* proteins have been eliminated as vaccine candidates due to surface epitope heterogeneity or variable expression. Desirable *Mcat* candidate antigens should be conserved among strains and immunogenic in children and adults. In the work reported here, we studied 5 *Mcat* protein vaccine candidates: outer membrane protein (OMP) CD, oligopeptide permease A (OppA), a non-lipidated form of Msp22 which we named Msp22NL, a truncated form of MID/Hag (Hag5–9), and PilA clade 2 (PilA2). OMP CD is a porin and adhesin and is highly conserved with exposed epitopes on the bacterial surface [12]. OppA is an oligopeptide binding protein which is located on the surface of *Mcat* and is involved in a number of functions of bacterial physiology including nutrient acquisition and persistence in the respiratory tract [13,14]. Msp22 is a putative outer membrane lipoprotein which may be involved in the transport of divalent cations across the outer membrane [15]. MID/Hag is an autotransporter outer membrane adhesin protein and hemagglutinin. It contains regions of highly conserved and moderately conserved domains [16]. PilA2 is the major pilin subunit that is conserved and essential for genetic transformation, adherence to eukaryotic cells and biofilm formation [17].

For *Mcat* vaccine development it is important to know whether a target antigen is immunogenic in the human host in the age time frame when vaccination is anticipated. The results from that knowledge would be to expect natural priming and boosting of vaccine responses caused by natural colonization. Therefore, we examined the antibody responses in young children after natural *Mcat* exposure by asymptomatic NP colonization and after a local infection, AOM. To our knowledge, this is the first study to prospectively compare the development of naturally induced antibodies to these 5 *Mcat* OMPs simultaneously in a single cohort of children 6–30 months of age during NP colonization and AOM. Specifically, we compared: (1) Changes of serum IgG antibodies to proteins OMP CD, OppA, Msp22, Hag, and PilA2 in children when their age increased from 6 to 30 months old; (2) Differences in antibody levels between children with NP colonization of *Mcat* and those with no NP colonization of *Mcat* at age 6–30 months old; (3) Differences in antibody levels during acute onset of AOM versus convalescence; (4) Variations in individual antibody responses following AOM.

2. Materials and methods

2.1. Subjects and sampling

2.1.1. Patient population

The samples collected and analyzed were obtained during a prospective study supported by the National Institute of Deafness and Communication Disorders, as previously described [18,19]. Healthy children without previous episodes of AOM were enrolled at 6 months of age from a middle class, suburban socio-demographic pediatric practice in Rochester, NY (Legacy Pediatrics) during June, 2008 to March, 2014. For this study we assessed a total of 184 children followed prospectively until 30 months of age. Serum samples, and NP and oropharyngeal (OP) cultures were obtained 7 times during the study period at 6, 9, 12, 15, 18, 24, and 30 months of age. During the study period whenever children in this group experienced an AOM episode a confirmatory tympanocentesis was performed and MEF samples microbiologically assessed; plus serum, NP, and OP cultures were obtained. The study was approved by the Rochester General Hospital Research Subjects Review Boards and written informed consent was obtained for participation and all procedures.

2.1.2. Sample collection

Serum, NP, OP and middle ear fluid (MEF) sampling was conducted as previously described [18].

2.1.3. Microbiology

Bacteria were isolated as previously described [18].

2.2. Mcat *protein expression and purification*

Recombinant *Mcat* proteins OMP CD [12], OppA [14], Hag5–9 (truncated Hag protein) [16], and PilA2 [17] were expressed and purified as previously described. We designed and constructed an expression plasmid for the gene of non-lipidated Msp22 which we named Msp22NL in a vector, pET303 with His-tag at its C-terminal. The sequence of the cloned *msp22nl* was verified by DNA sequencing. The plasmid was transformed into *E. coli* expression strain one shot BL21 (DE3) (Life Technologies, Grand Island, NY) and the Msp22NL was then expressed and purified by standard methods [14]. All the protein antigens and their purity were characterized by using SDS-PAGE along with Western blot (Fig. 1S).

2.3. Enzyme-linked immunosorbent assay

Protein-specific antibody concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using purified recombinant proteins. Ninety six-well Nunc MaxiSorp plates were coated with 1 μg/mL of individual proteins (100 μL/well) in phosphate-buffered saline (PBS, pH 7.4) and incubated at 37 °C for 1 h. After five washes, the plates were blocked with 10% fetal bovine serum (FBS) in PBS (pH 7.4) at 37 °C for 1 h (200 μl per well). After washing, 100 μl of serum 2-fold serially diluted at a starting dilution of 1:50 (in PBS–10% FBS) was added to each well. Human serum IgGs, Carimune (CSL Behring AG, Bern, Switzerland) and Gammagard (Baxter, Deerfield, IL) were used as references and in-house control sera with high and low titers were run on each plate. The plates were incubated at room temperature for 1 h followed by the addition of affinity purified goat anti-human IgG antibody conjugated to horseradish peroxidase (Bethyl Laboratories, Montgomery, TX) as a secondary antibody. The reaction products were developed with TMB Microwell Peroxidase Substrate System (KPL, Gaithersburg, MD), stopped by addition of 1.0 M phosphoric acid and read by a Spectramax 340PC plate reader (Molecular Devices, Sunnyvale, CA) using a 450-nm filter.

To provide quantitative results on antibody concentrations, the level of the specific antibody present in the unknown sample was determined by comparison to an internal reference serum (Carimune for OMP CD and Gammagard for OppA, Msp22, Hag, and PilA2). The levels of IgG in the reference serum were quantitatively measured by using a human IgG ELISA quantitation kit (Bethyl laboratories). A four-parameter logistic-log function was

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