Microbial Pathogenesis 46 (2009) 253–260

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

IgA1 antibodies specific for outer membrane protein PorA modulate the interaction between *Neisseria meningitidis* and the epithelium

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ARTICLE INFO

Article history:
Received 25 October 2008
Received in revised form
18 December 2008
Accepted 29 January 2009
Available online 5 February 2009

Keywords:
Immunoglobulin A
Neisseria meningitidis
Mucosal immunity
Antibodies
PorA

ABSTRACT

Despite high carriage rates of *Neisseria meningitidis*, incidence of meningococcal disease remains low, partially due to development of natural immunity. We have previously demonstrated an inverse relationship between salivary anti-meningococcal IgA and disease incidence, but little is known about the contribution of IgA to immunity at mucosal surfaces. Here we show strong immunoreactivity by human salivary IgA against the meningococcal outer membrane porin, PorA. Monomeric anti-PorA IgA1 (humanized chimeric antibodies) but not IgG increased the association of unencapsulated serogroup B *N. meningitidis* (H44/76) with Chang (conjunctival) but not with either Detroit (pharyngeal) cells or with A549 (alveolar) epithelial cells. Association of encapsulated *N. meningitidis* was not increased. Epithelial binding of IgA was Fc fragment dependent and not inhibited by IgM. Together these data suggest the presence of a specific epithelial IgA receptor that could influence the effect of both naturally acquired and vaccine induced IgA antibodies at the epithelial surface.

1. Introduction

Neisseria meningitidis is an important cause of meningitis in children and young adults worldwide [1,2]. However, although asymptomatic carriage is frequent [3], the development of meningococcal disease is relatively rare. In the UK it has been estimated that most individuals experience up to 10 meningococcal carriage episodes during their lifetime [4], while disease develops in only 2–5 individuals per 100,000 per year. The disparity between frequency of carriage and disease may be partly accounted for by the low virulence of many carriage strains but also suggests a role for naturally acquired protective immunity [2,5,6].

Naturally developed immunity is believed to occur through multiple colonisation events at the mucosal surface by *N. meningitidis*, related organisms such as *Neisseria lactamica* and other antigenically cross-reacting flora [7]. There is considerable evidence that either naturally acquired or vaccine-induced circulating complement fixing IgG antibody, high in bactericidal activity, protects against invasive disease [1,6,8,9]. We have previously demonstrated T-cell mediated immunological memory to *N. meningitidis* at the mucosal level which has a predominately proinflammatory phenotype. We have shown that the magnitude of these responses is highly regulated and increases with age [10,11]. IgA is the major antibody at mucosal surfaces in the upper respiratory tract and mucosal clearance through the elaboration of secretory IgA is considered to be important in limiting meningococcal colonisation and preventing early invasion. We have previously reported a link between salivary anti-meningococcal IgA levels and the incidence of meningococcal infection in the population [2]. However, the target meningococcal antigens for this IgA and the mechanisms of this immunity are uncertain.

IgA consists of two subclasses, IgA1 and IgA2, differing almost exclusively by a 13 amino acid sequence present in the hinge region of IgA1 [12], the most common isotype at most mucosal surfaces [13]. The hinge region is the site of cleavage for IgA1 proteases expressed by a number of pathogens including *N. meningitidis* and *Streptococcus pneumoniae* [14,15]. Serum IgA is mainly monomeric whereas mucosal IgA is mostly dimeric, synthesized by B cells located in the mucosal associated lymphoid tissue (MALT) [16]. During transcytosis of IgA through epithelial cells, the extracellular domain of the receptor for dimeric IgA, the polymeric Ig receptor (PIgR), is cleaved from the cells (secretory component) and remains complexed with IgA (secretory IgA).

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doi:10.1016/j.micpath.2009.01.009

We created a panel of humanized chimeric antibodies with identical mouse V regions, and different human constant (C) regions against a single PorA epitope (P1.16 in surface loop four) [17]. Meningococcal PorA is a membrane spanning protein with eight well conserved surface exposed loops [18] with the exception of two hyper variable regions (VR1 and VR2) [19]. While chimeric IgG1 and IgG3 were both good activators of complement mediated lysis, neither IgA1 nor IgA2 activated complement but blocked IgG mediated killing in a concentration dependent manner. In the present study we have shown strong immunoreactivity by human salivary IgA against the meningococcal outer membrane porin, PorA. We demonstrate that anti-PorA IgA1 antibodies increase meningococcal association with Chang epithelial cells and suggest that this interaction occurs through a specific IgA receptor.

2. Results

2.1. Anti-PorA IgA is highly represented in human saliva

We have previously reported the presence of IgA to a range of meningococcal outer membrane proteins saliva samples obtained from adults and children [2]. Subsequent experiments were carried out to determine the targets of IgA antibodies. Densitometry analysis of OMV Western blots (Fig. 1a), with proteins identified by band size, demonstrated strong immunoreactivity of salivary antibodies against PorA and to a lesser extent PorB (Fig. 1b). This was despite very similar levels of PorA and PorB in OMVs [20]. Antibodies to RmpM (putative peptidoglycan binding protein [21]), Opa, Opc and putative OMP P1 were also detected in some subjects (Fig. 1a and data not shown).

2.2. Monomeric IgA1 (mIgA1) but not IgG1 increases meningococcal association with Chang epithelial cells

To determine the potential functional effects of anti-PorA antibody on meningococcal interactions with the mucosal barrier, we initially co-cultured with H44/76 (P1.7,16-2 serosubtype) with humanized mIgA1 antibody and Chang epithelial cells. The chimeric antibody is highly specific for the PorA surface loop four expressed by this strain and does not bind another commonly used serogroup B clinical strain *N. meningitidis* MC58 (B.15.P1.7,16 immunotype L3) with a single D to N amino acid substitution in the DTNNN consensus sequence (results not shown) [22]. The Chang cell line was selected because it expresses low levels of CEACAMs which are potent mediators of Opa-dependent epithelial adherence and invasion [23–25]. Time course experiments using an unencapsulated H44/76 derivative revealed an increase in meningococcal–epithelial association over 3 h which was further enhanced by specific mIgA1 (Fig. 2a) (9.881×10^4 cfu/well ± SEM 1.9×10^4 vs 16.97×10^4 cfu/well ± SEM 0.7813×10^4 with specific mIgA1, $P = 0.0435$). This antibody-mediated increase in association was not seen with humanized IgG specific for the same epitope of the PorA loop region (Fig. 2b) (SEM = 0.5025 ± 4.334). Association assays using $F(ab)_2$ fragments showed that the effects of mIgA1 on meningococcal association with Chang cells are Fc fragment dependent (Fig. 2b).

It has previously been shown that capsulation reduces adherence of meningococci to Chang epithelial cells [24–26]. In this study the effect of monomeric IgA1 (mIgA1) on the capsulated parent *N. meningitidis* H44/76 strain was examined but no increase in epithelial association was observed.

2.3. Modulation of meningococcal association with other epithelial cells by mIgA1

To investigate the influence of anti-PorA mIgA on meningococcal interactions with epithelial cells expressing intermediate and high levels of CEACAM, association experiments were conducted with A549 (alveolar) and Detroit (pharyngeal carcinoma) cells characterised for their receptor expression previously. Mean fluorescence intensity for CEACAM expression was approximately 5 for A549 cells, compared with 1 for Chang cells and 22 for Detroit cells [25]. Although pre-incubation of unencapsulated H44/76 with specific mIgA1 resulted in a two-fold increase in association with Chang cells (29.29×10^4 cfu/well vs 66.35×10^4 cfu/well with specific mIgA1, $P = 0.05$), significant increases with A549 cells (82.16×10^4 vs 87.4×10^4 respectively, $P = 0.7535$) or Detroit cells (91.42×10^4 vs 89.3×10^4, respectively, $P = 0.9314$) were not seen (Fig. 3).

2.4. Characteristics of IgA binding to Chang cells

Further analysis of IgA binding to Chang was carried out using commercially available serum-derived IgA (mainly monomeric, IgA1 and IgA2), colostrum-derived IgA (mainly polymeric, IgA1 and IgA2). Both serum and colostrum-derived IgA bound to a sub-population (approximately 22%) of Chang cells (Fig. 4a and b). Chimeric anti-PorA mIgA1 was also shown to bind Chang cells similarly whereas human serum-derived IgG did not (data not shown). To investigate the possibility that meningococci upregulate IgA receptor expression on epithelial cells, Chang cells

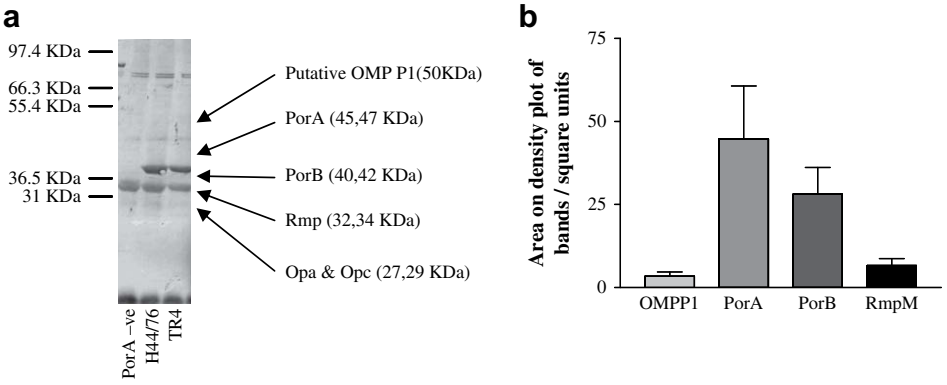

Fig. 1. Meningococcal antigen specificity of salivary IgA (a) Western blot with saliva from a representative individual. From left to right, the lanes contain the PorA negative OMV, WT H44/76, and WT TR4. OMP indicated are based on molecular weights: putative OMP P1 (50 kDa), PorA (46 kDa ± 1), PorB (41/38 kDa ± 1), Rmp (33 kDa ± 1) and Opa/Opc (28 kDa ± 1) [55]. (b) Density plot of Western blot bands. Columns represent the mean of 5 individual blots measuring anti-meningococcal salivary antibody specificities, 2 children and 3 adults. SEM is shown by vertical bars.