Binding properties and anti-bacterial activities of V-region identical, human IgG and IgM antibodies, against group B Neisseria meningitidis

T.E. Michaelsen*†1, Ø. Ihle*, K.J. Beckstrøm*2, T.K. Herstad*, R.H. Sandin*, J. Kolberg* and A. Aase*

*Division of Infectious Disease Control, Norwegian Institute of Public Health, PO Box 4404 Nydalen, N-0403 Oslo, Norway, and †Department of Pharmacognosy, Institute of Pharmacy, University of Oslo, Norway

Abstract

We have constructed chimaeric (ch) mouse/human antibodies with identical binding regions isolated from the V-genes of two mouse parent hybridoma cell lines, with specificity against the P1.7 and P1.16 epitopes on the outer-membrane protein PorA on meningococci. The chimaeric antibodies can be used to analyse relationships between specificity, binding activity (avidity and kinetics), isotype (antibody class and antibody subclass) and in vitro anti-bacterial activity of meningococcal antibodies. The antibody sets represented the human isotypes IgG1, IgG3 and IgM, which dominate during immune response against protein antigens. The binding activities were quite similar for all these isotypes, surprisingly also for the pentameric IgM. Interestingly, monomeric IgM, prepared from pentameric IgM by partially reduction and alkylation, had similar binding activities as the original pentameric IgM. Regarding in vitro anti-bacterial activity, chIgG1 was superior in SBA (serum bactericidal activity) compared with chIgG3, while chIgG3 was more efficient in OP (opsonophagocytosis; measured by flow cytometry) than chIgG1. ChIgM showed slightly higher SBA than chIgG1 on molar basis, and much higher OP than chIgG3 and chIgG1. A lower concentration of antibodies was needed against the P1.16 than against the P1.7 epitope to induce SBA, but this was not the case for OP.

Immune defence against meningococci

Immune defence against systemic meningococcal disease depends on recognition of bacterial surface antigens by antibodies, followed by the activation of complement leading to bacteriolysis, also called SBA (serum bactericidal activity), and/or OP (opsonophagocytosis) leading to intracellular killing of the bacteria by phagocytes. The class 1 outer-membrane porin protein, PorA, is expressed by almost all meningococcal strains [1,2], and it can induce bactericidal antibodies in humans and mice [3–7] and is therefore considered an important vaccine antigen [8,9].

Construction of vectors and cell clones producing chimaeric (ch) IgG1, IgG3 and IgM antibodies

The V-region genes of the anti-P1.7 and anti-P1.16 mAbs (monoclonal antibodies) were subcloned into expression vectors [10] containing human Ca and γ 1, γ 3 and μ genes respectively. The vectors were subsequently used to transfect NSO cells [11]. Clones were screened for the production of chimaeric antibodies reacting with strain 44/76.

Key words: antibacterials, bactericidal activity, human IgG, human IgM, meningococcal disease, opsonophagocytosis.

Abbreviations used: SBA, serum bactericidal activity; OP, opsonophagocytosis; ch, chimaeric; mAb, monoclonal antibody.

To whom correspondence should be addressed (e-mail terje.e.michaelsen@fhi.no).

Present address: Institute of Immunology, National Hospital, Oslo, Norway.

IgM showed the highest OP activity of the isotypes

OP activity was measured by flow cytometry using live, logarithmic-phase-grown meningococci as target cells, and human peripheral blood polymorphonuclear cells as effector cells [12–14]. The chIgG3 molecules showed a higher OP activity compared with the corresponding chIgG1 molecules (Table 1). Interestingly, the chIgM molecules were the most active in this respect. The hierarchy of OP was thus chIgM ≫ chIgG3 > chIgG1 (Table 1).

The highest serum bactericidal activities were found among the antibodies against the P1.16 epitope

SBA assay was performed against the vaccine strain 44/76 [15], examining a two-fold dilution series of the antibody preparations, and adding external human plasma, without any bactericidal activity, as the complement source [16]. The chIgG1 antibodies were more potent in SBA than the corresponding chIgG3 antibodies, against both the P1.7 and P1.16 epitopes. The hierarchy of SBA consequently was chIgM > chIgG1 > chIgG3 (Table 1). The SBA induced by the chimaeric antibodies against the P1.16 epitope were at least 10 times more efficient than the corresponding antibodies against the P1.7 epitope (Table 1).
Table 1 | SBA, OP and avidity index (AI) of chIgG1, chIgG3 and chIgM against the P1.7 and P1.16 epitopes on the PorA protein
SBA and OP columns indicate the lowest concentration (nM) of the antibodies able to induce SBA or OP. The AI columns indicate the lowest concentrations (M) of NH4 SCN giving a 50% reduction in absorbency in ELISA. The numbers are means from 2–4 independent experiments.

<table>
<thead>
<tr>
<th></th>
<th>SBA (anti-P1.7)</th>
<th>OP (anti-P1.7)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>5.6</td>
<td>8.1</td>
<td>2.5</td>
</tr>
<tr>
<td>IgG3</td>
<td>20.9</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>IgM pentamer</td>
<td>1.2</td>
<td>0.039</td>
<td>2.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SBA (anti-P1.16)</th>
<th>OP (anti-P1.16)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>0.3</td>
<td>8.6</td>
<td>2.6</td>
</tr>
<tr>
<td>IgG3</td>
<td>1.1</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>IgM pentamer</td>
<td>0.029</td>
<td>0.015</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Table 2 | Inhibition capacity in flow cytometry of pentameric IgM, monomeric IgM and IgG3 tested against biotin-labelled monomeric IgM antibodies (184,F-12) against the P1.16 epitope
Data are means from 2–3 independent experiments.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM pentamer</td>
<td>8.9</td>
</tr>
<tr>
<td>IgM monomer</td>
<td>9.1</td>
</tr>
<tr>
<td>IgG3</td>
<td>11.9</td>
</tr>
</tbody>
</table>

IgG, IgM pentamers and IgM monomers have similar functional affinity (avidity)
The functional affinity of chIgG and chIgM was primarily tested by the thiocyanate elution method [17]. The results showed similar avidity indexes for chIgM and chIgG1/chIgG3 (Table 1). In order to verify these somewhat unexpected results, some antibodies were further tested by binding and inhibition in flow cytometry using biotin labelled and unlabelled antibodies including monomeric IgM. Monomeric IgM was prepared by partial reduction and alkylation using 10 mM dithiothreitol followed by 24 mM iodoacetamide and further isolated by gel filtration on a Superdex 200 column. Inhibition was measured by incubating meningococci with various concentrations of unlabelled antibodies (inhibitor) for 30 min at 37°C before adding an optimal dilution of biotin-labelled monomeric IgM. After further incubation for 60 min at 37°C, the meningococci were centrifuged and washed before adding an optimal dilution of fluorescein-labelled streptavidin and incubated for 30 min at 37°C. The concentration (9–12 µg/ml) needed to inhibit by 50% the binding of biotin-labelled monomeric IgM, was essentially the same for IgM pentamers, IgM monomer and IgG3, indicating similar functional affinity against the intact bacteria (Table 2). Direct binding curves against intact inactivated bacteria measured in flow cytometry also indicated binding activity of monomer IgM compared with the two other antibody molecules (Figure 1).

Discussion
Antibodies probably protect against systemic meningococcal disease by inducing complement-mediated bacteriolysis (SBA), and/or by inducing intracellular destruction (OP) of the bacteria by phagocytes [18–22]. The isotype of the antibody governs the effector functions, which are assigned to the Fc region of the molecules [23]. In the present study, we have isolated the V-region genes of two mouse mAbs against an epidemic group B meningococcal strain, 44/76, used to produce a Norwegian group B meningococcal vaccine [24]. One of the mAbs is directed against the P1.16 epitope and the second against the P1.7 epitope, located to the V2 and V1 extracellular loop of the PorA molecule, respectively [7].

The anti-bacterial activities (SBA and OP) of antibodies directed against meningococci are very complicated on the molecular level. The activities kill bacteria, making them unable to form colonies on agar plates (SBA) or there is a respiratory burst of human polymorphonuclear leucocytes following internalization and killing of bacteria (OP). SBA involves the antibody-binding region (Fab) and its effector region (Fc), which upon engagement [23] recruits the complement system from the initial phase (complement factor 1, C1, activation) to the final phase (membrane-attack-complex formation) resulting in holes in the bacterial membrane [25]. Tools to study the two- and three-dimensional interactions at all the steps are largely lacking. However, the antibody–antigen interaction is getting clearer from X-ray diffraction analysis, where both the antigen and the antibody adapt to each other during the interaction. This
is also shown for a peptide mimicking the P1.16 epitope described here [26]. Regarding the OP activity, it also involves the Fab and Fc parts of the IgG [23]. The Fc region in this case interacts with Fc receptors on polymorphonuclear cells [27], which initiate internalization of the meningococci and start an intracellular chain of events leading to respiratory burst [28].

In this report we have focused on the end results of antibacterial activities of human monoclonal antibodies of IgG1, IgG3 and IgM isotypes against two different epitopes, P1.7 and P1.16, present on the outer-membrane protein PorA on meningococci. By this strategy we could analyse the influence of isotype upon antibody binding activity and upon bacterial killing activity. The three-dimensional position of the two epitopes studied here is not known in detail except the loop position (loop 1 for P1.7 and loop 4 for P1.16) [7]. The results described could thus be an initial guide for a detailed two- and three-dimensional study of antibodies interacting and killing bacteria.

ChIgG1 performed slightly better than chIgG3 in SBA, when directed against both the P1.16 and P1.7 epitopes. Human IgG1 seems therefore to achieve complement more efficiently than human IgG3 when directed at PorA on meningococci. On the other hand, chIgG3 performed better than chlgG1 in OP. A similar study also showed a better SBA of chIgG1 compared with chlgG3, and chlgG3 was better than chlgG1 in OP [29]. In this report we extend this IgG isotype pattern of biological activity also include P1.7 specificity and also IgM.

The P1.16 epitope was more sensitive than the P1.7 epitope as a target for the SBA assay, since a 10-times-lower antibody concentration was needed to achieve SBA against the P1.16 epitope compared with the corresponding antibodies against the P1.7 epitope. Possibly the lower SBA activity of antibodies to the P1.7 epitope compared with those against P1.16 could be due to more distant localization relative to the bacterial membrane of the P1.7 epitope [7]. Antibodies to the P1.7 epitope might therefore induce deposition of the membrane attack complexes far away from the bacterial membrane, leading to less bacterial lysis. Only future X-ray analysis of PorA will show if this interpretation is correct. Similarly, the OP activity induced by the interaction between opsonized bacteria and FcRs and/or complement receptors on phagocytes will likely not depend on the relative distance from the bacteria membrane and the target epitope. Thus, antibodies to the P1.7 and P1.16 epitopes (Table 1) induce OP equally well. It would even be beneficial for OP that the target epitope is distal to the bacterial membrane as this would probably facilitate the interaction with complement receptors/FcRs.

IgM is part of the primary immune response and usually has low intrinsic affinity. In our case both the IgM and IgG have the same intrinsic high affinity as they share the V-regions made possible by DNA technology. We could thus study the binding properties and anti-bacterial activity of human IgM relative to IgG. Interestingly, we did not observe the expected enhanced avidity of pentamer chIgM compared with monomer chlgG [30]. Equal functional affinity was observed by the isothiocyanate method [17] and confirmed by binding and inhibition in flow cytometry. A more direct analysis of the minor importance of the IgM pentamer structure in our test system was performed using monomeric IgM achieved by partially reduction and alkylation. The monomeric IgM showed essentially the same binding properties as the original pentameric IgM, in several systems. These data would indicate that IgM does not always have a favourable binding activity against bacteria when the target is a protein epitope, at least not for antibodies with high intrinsic affinity, as in our case. The increase in avidity claimed for IgM, might only be valid for antibodies against abundantly expressed antigens such as carbohydrate [30], and possibly only for low-avidity antibodies. Lack of avidity as a bonus effect upon polymerization for high-affinity antibodies has been reported in parallel with such a bonus effect for low-affinity antibodies [31].

Regarding the in vitro anti-bacterial activity of chlgM, we observed that our chlgM antibodies had only slightly higher bactericidal activity on a molar basis than chlgG1, in contrast to switch variants of rat IgM and IgG against lipopolysaccharide of Escherichia coli, where rat IgM was 100–1000 times more efficient in complement-mediated bacteriolysis than IgG [32]. Perhaps IgM requires a high density of antigens to activate complement more efficiently than IgG. On the other hand, the chlgM antibodies had at least a 50-times-higher OP activity on a molar basis than chlgG3 in our test system. A similar high OP activity of human chlgM over human IgG1 has been observed for anti-carbohydrate antibodies against the Gram-positive group B streptococci [33,34].

The molecular interaction of pentamer IgM relative to monomer IgG with micro-organisms and its consequences for anti-microbial activity is very intriguing and deserves special attention to better understand antibody immune protection against infectious diseases. Apart from being a guide to the development of meningococcal vaccines and antibody preparations for therapy and prophylaxis against meningococcal disease, such information will be of interest to combat emerging diseases like AIDS (due to HIV) and SARS (due to SCV; SARS-associated corona virus) [35].

We gratefully acknowledge the technical assistance of Ms Gunnhild Rødal in creating the mouse mAbs, and Dr Lars Norderhaug for supply of the pLNOX and LNOH2 vectors.

References