Activity of Human IgG and IgA Subclasses in Immune Defense Against *Neisseria meningitidis* Serogroup B


*J Immunol* 2001; 166:6250-6256; doi: 10.4049/jimmunol.166.10.6250
http://www.jimmunol.org/content/166/10/6250

**References**
This article *cites 40 articles*, 21 of which you can access for free at:
http://www.jimmunol.org/content/166/10/6250.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IgA does not bind C1q and thus cannot activate the classical complement pathway, whereas IgM is extremely potent in activation of complement but does not interact with phagocytic cells. Opsonized microorganisms interact with complement receptors (CR) and Ig-specific FcR (8, 9), which are present on most myeloid cells. In humans, one phagocyte IgA receptor, FcγRI (CD89), has been cloned and characterized (10), whereas the IgG receptor family consists of various members. We currently recognize three classes of leukocyte FcγR, namely, FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) (8). Polymorphonuclear leukocytes (PMN) are considered to be crucial effector cells for antibacterial responses and bear both FcR and CR that mediate activatory signals when cross-linked. Receptor cross-linking leads to degranulation, formation of oxygen radicals, and phagocytosis, which may result in destruction of opsonized microorganisms (9).

The relative contribution of each Ig isotype to host immunity against N. meningitidis has received remarkably little attention. Serogroup B-specific IgG has been shown to initiate complement-mediated lysis of bacteria, as well as leukocyte effector functions such as phagocytosis and respiratory burst (11–13). Specific IgA has been implicated as a risk factor for disease, because it was shown to impede IgG-mediated bactericidal activity (14), whereas other studies documented its capacity to initiate leukocyte-mediated antimeningococcal activity (15).

Porin (Por) A is a subcapsular protein Ag of group B N. meningitidis that induces Ab formation upon natural infection (16), and is considered a meningococcal vaccine candidate (7). To study Ab effector functions to encapsulated bacteria in detail, we generated a panel of chimeric Abs with identical mouse variable regions against a single epitope on PorA. Additionally, secretory IgA

Both IgG and IgA Abs have been implicated in host defense against bacterial infections, although their relative contributions remain unclear. We generated a unique panel of human chimeric Abs of all human IgG and IgA subclasses with identical V genes against porin A, a major subcapsular protein Ag of Neisseria meningitidis and a vaccine candidate. Chimeric Abs were produced in baby hamster kidney cells, and IgA-producing clones were cotransfected with human J chain and/or human secretory component. Although IgG (isotypes IgG1–3) mediated efficient complement-dependent lysis, IgA was unable to. However, IgA proved equally active to IgG in stimulating polymorphonuclear leukocyte respiratory burst. Remarkably, although porin-specific monomeric, dimeric, and polymeric IgA triggered efficient phagocytosis, secretory IgA did not. These studies reveal unique and non-overlapping roles for IgG and IgA Abs in defense against meningococcal infections. The Journal of Immunology, 2001, 166: 6250–6256.
(SlgA) molecules were generated by cotransfecting IgA-producing cells with the human J chain and the extracellular domain of the human polymeric Ig receptor (secretory component, SC). Our findings provide new insights into the roles of IgG and IgA Abs in antibacterial immunity.

Materials and Methods

Bacteria

The H44/76 strain of N. meningitidis (B15:Pl1.7,16) was stored in Tryptosecht broth (Becton Dickinson, Franklin Lakes, NJ) at −70°C. The day before use, bacteria were plated onto GC agar plates and incubated overnight at 37°C in 5% CO₂. Bacteria were then subcultured in Trypticase soy broth and grown to OD₆₀₀ (Nunc, Roskilde, Denmark) were coated overnight at 4°C with rabbit anti-

Serological assays

Concentrations of purified HMIN12H2 Abs were determined by ELISA using human κ-chain antiserum (Dako, Glostrup, Denmark). Subsequently, controls (1 μg/ml) and samples were serially diluted in ELISA plates. For detection, alkaline phosphatase-conjugated rabbit anti-human IgG, IgA, and IgM serum (Dako) was used. Reactions were developed with p-nitrophenyl phosphate in diethanolamine buffer (Sigma), and OD values were obtained at 405 nm. Ab concentrations were calculated from standard curves.

To confirm the isotypes of HMIN12H2 Abs and levels of opsonization, 10⁷ heat-killed bacteria in 10-ml volumes were incubated with 5 μl of 10 μg/ml Abs for 30 min at 4°C, washed twice, and incubated with FITC-labeled Abs to IgG1, IgG2, IgG3, IgG4, IgA1, SC (unlabeled) (Sigma) or anti-IgA (Cappel, West Chester, PA). After a washing, the resulting fluorescence of 10⁷ bacteria was determined using FACS analyses on a FACSCalibur (Becton Dickinson).

Binding experiments

For determination of the relative affinities of chimeric human Abs, Maxi-
sorp plates were coated with heat-killed bacteria (10⁶ CFU/ml, 2 h at room temperature), and the reactivity of HMIN12H2 isotypes was examined by incubating Abs over a broad concentration range. Abs were detected using FITC-labeled anti-κ Abs (Southern Biotechnology Associates, Birmingham, AL), followed by peroxidase-labeled anti-FITC Abs (Boehringer Mannheim) or alkaline phosphatase-conjugated rabbit anti-human IgG, IgA, and IgM (Dako).

Bactericidal activity

Serum-mediated lysis of meningococci was determined by serially diluting chimeric Abs in 96-well bottom-plates (Greiner) using ~100 CFU H44/76 group B meningococci per well, with 12.5% human serum as a source of complement. The serum was obtained from an individual without detectable H44/76 meningococcal group B Abs. Plates were incubated for 30 min, and 7-μl volumes (~30 CFU) were transferred to GC agar plates (Oxoid, Basingstoke, U.K.) and incubated overnight. All incubations were performed at 37°C, 5% CO₂, and 95% humidity.

Phagocytosis assays

Human neutrophils (PMN) were isolated by using Ficoll (Pharmacia-His-
topaca) (Sigma) gradients, followed by hypotonic lysis of residual RBC in water (for 30 s at 4°C). Abs were serially diluted in 1-ml polypropylene tubes (Micronex, Lelystad, The Netherlands). PMN (10⁦⁶) were added along with 5 x 10⁶ Alexa 488-labeled bacteria (see above) in final volumes of 100 μl and incubated at 37°C for 30 min. After a washing, samples were resuspended in 300 μl FACs buffer (PBS, supplemented with 1% BSA and 0.1% azide), and fluorescence intensities of PMN were measured by flow cytometry. Ig concentrations were adjusted to 1 μg/ml in assays using fractionated IgA. For all experiments, cells from FcyRIIA-H/H131, FcyRIIb-N1A1/N2A1 individuals (PCR-allotyped) were used (26).

For quantification of bacteria associated with PMN (phagocytic index), the same FLS FACs settings used for measurements of PMN samples, were also used to measure fluorescence intensities of bacteria, and numbers of bacteria/PMN were calculated as follows:

\[ \text{Phagocytic index} = \frac{\text{CFU} \times 100}{\text{PMN}} = \text{GM}^{\text{PL}} \times 10 \]

in which \( \text{GM}^{\text{PL}} \) is the geometric mean of fluorescent intensity (FL1). Thus, the mean fluorescent intensity of a single PMN is then divided by the geometric mean fluorescent intensity of a single CFU to calculate the mean number of bacteria associated with PMN.

Respiratory burst

Meningococci (10⁷, heat-killed or alive) were preincubated with the re-
combiant Abs in volumes of 50 μl for 30 min at 37°C in IMDM supplemented with 1% FCS. When human serum (12.5%, without detectable H44/76 Abs) was used as a source of complement, it was included for the last 5 min of incubation. Tubes were then transferred to a luminometer (Autolumat LB 953, Berthold, Germany), in which chemiluminescence responses were measured every min for 45 min after injection of 100 μl 10⁴/ml PMN and 600 μl 180 μM luminol solution (Sigma) (27).

Statistical analyses

For comparison between total fluorescence of PMN after phagocytosis of fluorescent bacteria, we used Student’s t tests after testing for equal variance. Significance was accepted at \( p < 0.05 \).
Results

Generation of chimeric Abs

The variable regions of a well-characterized Ab (MN12H2) (17, 18) directed against the class 1 outer membrane protein PorA of group B meningococci were cloned. The light chain variable region (V_L) was joined with a human κ constant region, and the heavy chain variable region (V_H) with different human heavy chain constant regions (γ1–γ8, α1, α2). The identity of all meningococcal Abs was verified by sequencing (GenBank accession numbers G5542523 (V_L), G5542524 (V_H), AF237583 (γ1), AF237584 (γ2), AF237585 (γ3), and AF237586 (γ4)). V_L-κ and V_H heavy chain vectors were cotransfected into BHK cells, with or without plasmids coding for the human J chain, and/or human SC for IgA (IgA-J or IgA-J-SC). After affinity purification, Abs were assessed for correct molecular assembly and size. The concentration of whole Abs were determined in anti-κ-anti-Fc-AP sandwich ELISA using well-characterized human myeloma Abs as references.

Ag recognition by chimeric Abs

The characteristics of both recombinant IgG and IgA molecules were similar to those of reference Abs (Fig. 1A). As can be seen in Fig. 1B (and data not shown), IgA-J-SC-producing cells produced not only SlgA but also monomers, indicating, as has been described previously (23, 28), that single-cell assembly of SlgA is not 100% efficient. Because this resembles the situation in human serum, we used total IgA (mIgA, with some dIgA) preparations in the remainder of the study, unless otherwise stated. Isotypes were verified by isotype-specific mAbs, in both ELISA and flow cytometric analyses (data not shown). Binding of chimeric Abs to meningococci could be blocked by the parental mouse IgG2a monoclonal and by each of the IgG and IgA chimeric Abs (data not shown). All chimeric Abs recognized the P1.16 epitope of PorA (17) as determined in ELISA by binding to peptides representing this epitope (data not shown). Binding curves of IgG and IgA chimeric Abs to whole bacteria, using anti-κ Abs for detection, were indistinguishable (Fig. 1, C and D).

Complement lysis

We studied humoral effector functions of the Ab panel by assessing their abilities to kill bacteria via activation of human complement. Human serum with undetectable Ig levels to H44/76 meningococci (determined by ELISA) was the source of complement. The parental mouse IgG2a was highly active in this assay (Fig. 2A). Human IgG3 and IgG1 exhibited similar activity, whereas IgG2 had approximately one-tenth activity. At a concentration of 0.5 µg/ml, HMN12H2 IgG3 and IgG1 triggered killing of 90% of the bacteria. Human IgG4 did not show detectable bactericidal activity. Heat inactivation of serum abolished Ab induced bactericidal activity (data not shown). In accord with previously reported data (14), neither IgA1 nor IgA2 mediated bacterial killing via complement, but both blocked IgG-mediated killing in a concentration-dependent manner (Fig. 2B). We found that 16 µg/ml IgA2 was sufficient to completely block IgG1-mediated lysis up to a concentration of ~1.5 µg/ml. Higher concentrations of IgG1 overcame this blocking effect.

Phagocytosis

We studied phagocytosis of heat-killed meningococci by freshly isolated human PMN with each of the IgG and IgA Abs. Human IgG1- and IgG3-mediated efficient phagocytosis, whereas IgG2 and IgA triggered lower levels of bacterial uptake (Fig. 2C). Human IgG4 exerted minimal activity. To confirm that the activity measured in our assays represented true phagocytosis, internalization was determined by comparing cells incubated with opsonized Alexa488-labeled bacteria after incubation at 4°C (attachment) or 37°C (phagocytosis), using PE-labeled anti-human-κ antisera (29) (data not shown). Because PMN express an IgG receptor (FcγRIIa, CD32) that interacts with human IgG2 and IgG3 in a polymorphic way (26), we studied PMN from both allotypes. Interaction of human IgG2-opsonized N. meningitidis was lower for FcγRIIa-R/R131 than IIa-H/H131 and similar, albeit smaller, differences were observed for IgG3 (data not shown).

Respiratory burst

PMN respiratory burst activity was evaluated with live meningococci, chimeric IgG and IgA Abs and complement. In Fig. 3A, it...
can be seen that, in the presence of complement, IgG1 and IgG3 potently stimulated respiratory burst, whereas IgG2 was less active. The activity mediated by IgG1 and IgG3 was highly up-regulated by complement (2-fold), whereas the IgG2 activity was only moderately enhanced (data not shown). Human IgG4 exhibited minimal activity in this assay.

Both IgA1 and IgA2 efficiently triggered PMN respiratory burst using heat-killed bacteria. At low, albeit physiological (≤2 μg/ml) Ab concentrations, IgA stimulated respiratory burst as efficiently as IgG3, with somewhat delayed responses at higher concentrations (≥20 μg/ml) (Fig. 3B). Unlike IgG-triggered respiratory burst, the activity of IgA-opsonized meningococci was not markedly enhanced by complement (data not shown).

Interaction of mIgA, dIgA, pIgA, and SIgA with FcαRI on PMN
IgA exists as several distinct molecular forms in vivo, mIgA, dIgA, pIgA, and SIgA (30). Dimeric J-chain-containing IgA is normally associated with SC on mucosal surfaces. To compare phagocytic activity of the different molecular forms, we fractionated IgA1-J-SC on HPLC columns to separate monomeric (fractions 48–51) and dimeric SC-containing forms (fractions 36–41) (Fig. 1B) (23). IgA2-J was also fractionated to separate mIgA2, dIgA2, and pIgA2 from each other. Phagocytosis of heat-killed meningococci with mIgA1 (Fig. 4A) resulted in significant phagocytosis (p < 0.001 compared with background), and to a similar extent as with un-fractionated IgA1-J and IgA2-J (Figs. 4A and 2C). Similarly, mIgA2, dIgA2, and pIgA2 stimulated phagocytosis (Fig. 4B) and respiratory burst (data not shown). Remarkably, no phagocytic activity was found using SIgA1 (Fig. 4A). These data indicate SC of SIgA to block interaction of IgA with FcαRI on PMN.

Discussion
In the present work, we studied the biological functions of IgG and IgA subclasses against group B meningococci. Effector functions of IgG subclasses (IgG1–4) have previously been compared using hapten (4-hydroxy-3-nitrophenylacetyl)-specific recombinant chimeric Abs. Such studies were performed with targets like human erythrocytes (31) and encapsulated bacteria (12). The limitation of these earlier studies is that the epitopes recognized were randomly dispersed on both capsular and subcapsular molecules. In addition, a comparison with human IgA subclasses has not been performed. The importance of studying Ab effector functions recognizing only subcapsular Ags is exemplified for group B meningococci, where vaccine developments will not focus on capsular polysaccharides in the foreseeable future. By generating a series of chimeric human IgG and IgA Abs carrying the same V genes, recovered from a well-characterized mouse mAb (17, 18), we studied anti-bacterial functions of Abs recognizing a surface protein on N. meningitidis used in experimental group B vaccines, PorA (7).

This work confirmed some of the IgG effector functions documented before (12, 31). It also yielded data that shed new light on the impact of IgA-mediated effector functions against encapsulated bacteria. First, we demonstrated our panel of chimeric Abs to exhibit correct molecular structure and assembly (Fig. 1) and to bind with similar affinity (Fig. 1, C and D) to the P1.16 epitope located in surface loop 4 of PorA serosubtype P1.7,16 (17, 18). Our IgA preparations (a portion of which has dimeric/polymeric properties) exhibited similar binding curves to whole bacteria as IgG (Fig. 1, C and D), which is probably explained by the fact that most IgA was monomeric (Fig. 1B). We determined the order of activity of
IgG subclasses to activate human complement to be IgG1 = IgG3 > IgG2 > IgG4 (Fig. 2A) (12, 31). IgA Abs have been reported capable of activating the alternative complement pathway (32). As previously described (14), however, IgA Abs did not mediate bactericidal activity and furthermore blocked IgG-mediated complement activation, likely by interfering with IgG access to PorA. An alternative explanation may be that IgA binding resulted in reduced accessibility of Clq to IgG Fc tails by steric hindrance (33, 34).

We showed that the relative ability of IgG Abs to trigger phagocytic activation (IgG3 > IgG1 > IgG2 > IgG4) correlated with predictions based on knowledge on FcγR expression levels (9, 35) and their relative affinities for IgG subclasses (36). The relatively low number of receptors for IgG2 (FcγRIIa; 30–60,000 receptors/PMN), IgG4 (FcγRIIb; <300 receptors/PMN), and IgA (FcαRI; 6–7,000 receptors/PMN) readily explains the relatively low phagocytic activities of IgG2, IgA, and IgG4. In addition, the potent capacity of IgG1 and IgG3 Abs correlates with expression levels and binding specificity of FcγRIIa (100–300,000 receptors/PMN), as well as FcγRIIIa.

FcR cross-linking reflects to some extent their ability to activate intracellular signaling cascades, leading to phagocytosis, degranulation, and eventual killing of microorganisms. We quantitated PMN activation by measuring the ability of opsonized live meningococci to stimulate PMN respiratory burst. The chimeric IgG subclasses stimulated PMN in a similar order as observed in phagocytosis (Fig. 3A). Additionally, we found a major increase in the IgG3 and IgG1 responses (and a minor increase for IgG2) in the presence of complement. This effect was absent for IgG4 and IgA Abs (data not shown). IgA that showed only moderate phagocytic activity and stimulated respiratory burst effectively. At lower concentrations (<2 µg/ml), IgA induced a respiratory burst similar to those of IgG3 Abs (Fig. 3B). At higher concentrations (~20 µg/ml), IgA still induced an efficient respiratory burst, albeit with a slower response time than IgG3. This is likely a reflection of lower FcαRI expression levels, compared with those of IgG receptors on PMN (8, 9). These results are in agreement with those of others reporting IgA to be more potent than IgG in initiating PMN respiratory burst (10, 15, 37).

At mucosal surfaces, J-chain-containing dimeric IgA is associated with the extracellular part of the polymeric Ig receptor (SC), where an ~80 KDa SC covalently associates with dimeric IgA (28, 30). The “docking site” on IgA for FcαRI has been mapped to the boundary of CH2 and CH3 (38, 39), a location occupied by SC (40). To evaluate the effect of SC on IgA-FcαRI binding, we fractionated IgA1 isolated from supernatants of IgA1-J-SC and that of IgA2-J-transfected BHK cells. In this way, we were able to isolate mIgA, dIgA, pIgA, and dimeric IgA containing SC (SlgA; Figs. 1B and 4). When incubated with heat-killed meningococci and PMN, all molecular forms of IgA facilitated phagocytosis and respiratory burst, except SlgA (Fig. 4), supporting the notion that PMN do not have a receptor for SlgA. These results agree well with other studies performed in our laboratory, where various microorganisms were opsonized with commercially available polyclonal human serum IgA or milk derived SlgA (41). We extend these studies now by showing that not only mIgA2 but also dIgA2 and pIgA2 that can stimulate PMN functions, demonstrating that it is not the dimeric or polymeric nature of the IgA that is responsible for the lack of binding to CD89, but SC. However, the observation that SlgA does not interact with FcαRI is at variance with experiments performed with IgA-coated surfaces where both mIgA and SlgA were capable of initiating PMN respiratory burst (37). Together, these data support that FcαRI interacts solely with SlgA when coated on plastic or glass surfaces, but not when used as opsonin on the surface on invading pathogens. Thus, SlgA, which serves as a “first-line” defense molecule, seems to have antiinflammatory properties, whereas serum and subepithelial IgA can interact with human PMN (via FcαRI) to stimulate potent responses.
In summary, we generated a unique panel of human IgG and IgA chimeric Abs that recognize PorA, a promising vaccine candidate for group B meningococcus. We characterized their capacity to initiate humoral and cellular effector functions. IgG initiated efficient lysis of meningococcal via complement that furthermore enhanced PMN effector functions. IgA did not mediate complement-dependent bactericidal activity and blocked IgG-mediated complement lysis effectively. Despite a moderate phagocytic capacity, IgA proved most potent in stimulating PMN respiratory burst. Comparison of IgA1 and IgA2 effector functions revealed them to be equally effective using heat-killed Neisseria. However, further studies are needed to evaluate the influence of IgA1 protease expressed by live bacteria (42). Remarkably, whereas mlgA, dlgA, and plgA actively stimulated PMN functions, SlgA was unable to activate phagocytosis via FcαR1 on PMN.

This study established the existence of several differences between IgG and IgA Abs and uncovered an unanticipated level of complexity in the biology of these Abs that are relevant for our understanding of antibacterial immune defense.

Acknowledgments

We thank Drs. Maria Eugenia Rodriguez, Anника Petterson, and Thorstein Prinz for help with bactericidal assays; Drs. Jos van Strijp, Kok van Kessel, and Erik Heezius for expert advice on respiratory burst measurements; and Betsy Kuipers, Germaine van den Dobbelsteen, Jan Poolman, and Loek van Alphen for supplying the MN12H2 hybridoma.

References


38. Carayannopoulos, L., J. M. Hexham, and J. D. Capra. 1996. Localization of the binding site for the monocyte immunoglobulin (Ig) A-Fc receptor (CD89) to the domain boundary between c-α-2 and c-α-3 in human IgAL1. J. Exp. Med. 183:1379.

