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Activity of Human IgG and IgA Subclasses in Immune Defense Against *Neisseria meningitidis* Serogroup B[1]

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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 nonoverlapping roles for IgG and IgA Abs in defense against meningococcal infections. *The Journal of Immunology,* 2001, 166: 6250–6256.

Infections caused by *Neisseria meningitidis* represent an important threat to children and young adults. Immunity to meningococcal infections is acquired during periods of nasopharyngeal colonization by commensal neisserial strains, which elicit protective Ab responses, primarily IgG, but also IgM and IgA (1, 2). Vaccination strategies aim at induction of such responses, directed against capsular polysaccharides or subcapsular Ags (3, 4). It has generally been acknowledged that a polysaccharide (or a protein conjugate derivative) vaccine cannot be developed against serogroup B, the most frequent cause of meningococcal disease in the Western world (5) because the antigenic determinants of its polysialic acid capsule are found in the developing brain (6). Experimental vaccines based on specific outer membrane proteins or outer membrane protein vesicles have recently been developed, yielding promising results (7).

IgG can initiate a plethora of effector functions, varying from activation of humoral immune functions by interacting with C1q and engagement of the classical complement pathway to initiation of cellular effector functions, including phagocytosis, Ab-dependent cell-mediated cytotoxicity, and respiratory burst. In contrast, 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)[3] and Ig-specific FcR (8, 9), which are present on most myeloid cells. In humans, one phagocyte IgA receptor, FcαRI (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. meningitides* 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 and different human constant regions (γ1–γ4, α1, α2) directed against a single epitope on PorA. Additionally, secretory IgA

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[3] Abbreviations used in this paper: CR, complement receptor; Por, porin; BHK, baby hamster kidney cells; MN12H2, mouse mAb directed to PorA from serogroup B (H44/76); HMN12H2, chimeric MN12H2 Abs, with mouse V genes and human constant regions; mIgA, monomeric IgA; dIgA, dimeric IgA; pIgA, polymeric IgA; SIgA, secretory IgA; SC, secretory component; IgA-J, IgA from supernatants of cells transfected with IgA (heavy and light chains) and human J chain; IgA-J-SC, IgA from supernatants of cells transfected with IgA (heavy and light chains), human J chain, and human SC; PMN, polymorphonuclear leukocytes.

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(SIgA) 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* (B:15:P1.7,16) was stored in Tryptoset 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% CO_2. Bacteria were then subcultured in Trypticase soy broth and grown to OD ~0.9 (620 nm). When indicated, bacteria were heat killed at 56°C (30 min) after they were washed in PBS supplemented with 1% BSA (fraction V; Boehringer Mannheim, Mannheim, Germany). For phagocytosis experiments, 10^{10} heat-killed bacteria in 0.5 ml PBS were labeled with the pH-stable green dye Alexa 488 (Molecular Probes, Leiden, The Netherlands). After a washing with PBS (1% BSA), heat-killed bacteria were aliquoted and stored at −70°C.

Generation of expression vectors

Cloning of genes for the Ab variable regions (V_L, V_H) of the mouse IgG2a Ab MN12H2 (17, 18), was performed as described (19, 20). Briefly, RNA was isolated, and V genes were amplified by RT-PCR, using oligo(T) primers and avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) for cDNA generation and *Taq* polymerase (Amplitaq; Perkin-Elmer, Norwalk, CT) and V gene-specific primers for PCR amplification (18). Variable regions were inserted into a pUC-HAV20 vector (20), to incorporate the HAVT20 leader sequence (21). After sequence verification using M13 primers and ABI-Big-Dye-terminator mix (Perkin-Elmer), HAVT20-V inserts were subcloned into relevant pNUT vectors encoding human constant regions for the κ-light chain and γ1, γ2, γ3, γ4, α1, and α2 heavy chains (20, 22). The panel of Abs derived from MN12H2 was renamed HMN12H2. Vectors used for the expression of human J chain and human secretory component (SC) were described in Ref. 23.

PCR and sequence reactions were performed in a GeneAmp PCR system 9700 Thermocycler (Perkin-Elmer), and sequence reactions were run and analyzed using an ABI 373 Stretch automated sequencing machine (Applied Biosystems, Foster City, CA).

Ig expression

Heavy and light chain pNUT expression vectors were cotransfected into BHK-21 cells by calcium phosphate precipitation. Positive cells were selected in 1 μM methotrexate (Emthexate PF; Pharmochemie, Haarlem, The Netherlands). Subsequently, IgA-producing baby hamster kidney (BHK) cells were transfected with a J-chain expression vector (IgA-J (23)) and selected using 100 μg/ml hygromycin B (Life Technologies, Gaithersburg, MD). To generate SIgA, the IgA-J-producing cells were additionally transfected with a SC expression vector (IgA-J-SC (23)) and selected using gradually increasing concentrations of histidinol (Sigma, St. Louis, MO; final concentration, 7 mM). For Ab production, cells were grown in roller bottles (Costar, Cambridge, MA) using IMDM (Life Technologies) supplemented with 7.5% heat-inactivated HyClone Fetal Clone1 serum (Greiner, Alphen aan den Rijn, The Netherlands) and 100 μM $ZnCl_2$ to increase production (24).

Ig purification

Supernatants from Ig-producing BHK cells were concentrated using a Prep/Scale TFF concentrator (Millipore, Bedford, MA). IgG supernatants were isolated by affinity chromatography using Sepharose-coupled protein G (Pharmacia, Piscataway, NJ). IgA and IgM were isolated from supernatants using Affi-T columns (BIOZyme, Landgraaf, The Netherlands) (25). Purified Abs were submitted to electrophoresis on 8–18% SDS gradient polyacrylamide gels (Pharmacia) and stained with Coomassie blue. Different molecular forms of IgA, isolated from IgA1-J-SC-transfected cells, were separated by HPLC on HR200 Superdex size exclusion columns (Pharmacia).

Serological assays

Concentrations of purified HMN12H2 Abs were determined by ELISA using human IgG myeloma κ subclass Abs (CLB, Amsterdam, The Netherlands), serum IgA (LC-V Behring Diagnostics, Frankfurt, Germany), and SIgA (Sigma) as references as standards. Briefly, Maxisorp ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with rabbit anti-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 HMN12H2 Abs and levels of opsonization, 10^8 heat-killed bacteria in 10-μl 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 10^4 bacteria was determined using FACS analyses on a FACSCalibur (Becton Dickinson).

Binding experiments

For determination of the relative affinities of chimeric human Abs, Maxisorp plates were coated with heat-killed bacteria (10^9 CFU/ml, 2 h at room temperature), and the reactivity of HMN12H2 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 V-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% CO_2, and 95% humidity.

Phagocytosis assays

Human neutrophils (PMN) were isolated by using Ficoll (Pharmacia)-Histopaque (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 (Micronics, Lelystad, The Netherlands). PMN (10^5) were added along with 5×10^6 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 FcγRIIa-H/H131, FcγRIIIb-NA1/NA2 individuals (PCR-allotyped) were used (26).

For quantification of bacteria associated with PMN (phagocytic index), the same FL1 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:

$$\text{Phagocytic index} = \text{CFU/100PMN} = \frac{\text{GM}^{\text{FL1}}\text{PMN(FL1/PMN)}}{\text{GM}^{\text{FL1}}\text{Bact(FL1/CFU)}} \times 100$$

in which GM^{FL1} 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 (10^7, heat-killed or alive) were preopsonized with the recombinant 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 10^6/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 ($\gamma1$–$\gamma4$, $\alpha1$, $\alpha2$). The identity of all meningococcal Abs was verified by sequencing (GenBank accession numbers G5542523 (V_L), G5542524 (V_H), AF237583 ($\gamma1$), AF237584 ($\gamma2$), AF237585 ($\gamma3$), and AF237586 ($\gamma4$)). 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. 1). As can be seen in Fig. 1*B* (and data not shown), IgA-J-SC-producing cells produced not only SIgA but also monomers, indicating, as has been described previously (23, 28), that single-cell assembly of SIgA 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. 2*A*). 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. 2*B*). 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. 2*C*). 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 antiserum (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).

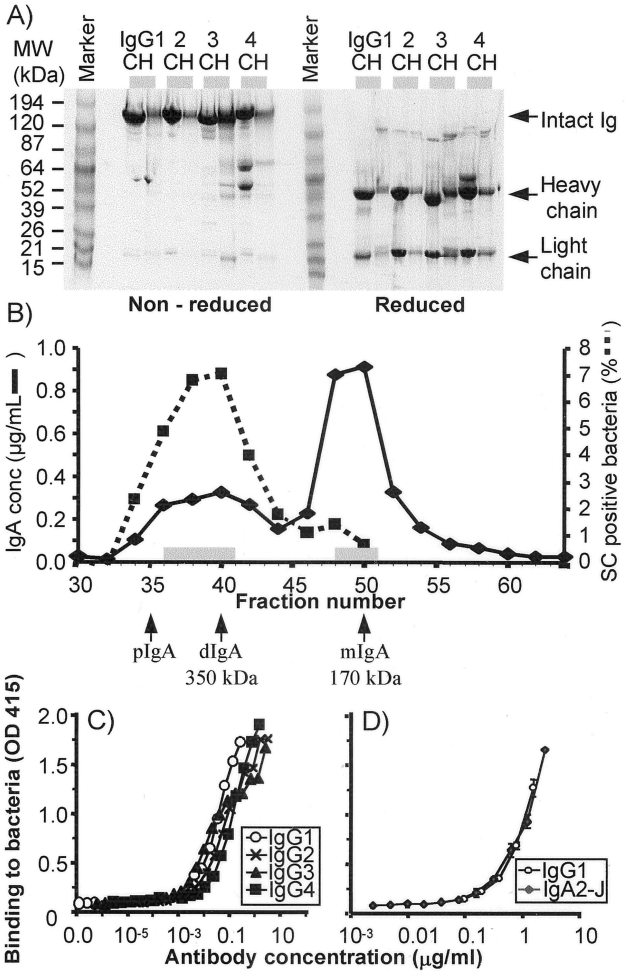

FIGURE 1. Molecular sizes and assembly of chimeric HMN12H2 Abs and Ag binding characteristics. *A*, Molecular size of chimeric IgG subclasses run under nonreduced and reduced conditions. Ten micrograms Abs were run on 8–18% SDS-PAGE and stained with Coomassie blue. C, Control human Abs, H, HMN12H2 recombinant Abs. *B*, Separation of Affi-T-purified IgA1, derived from IgA1-J-SC-transfected cells, on an HR-200 Superdex HPLC column. Total IgA concentrations (——) and percentage of bacteria staining positive for SC (····) with these fractions, measured by FACS. The elution profiles of reference standard IgA preparations (mIgA, dIgA, pIgA) is shown below the graph for orientation. Some of the IgA1-J-SC is monomeric, whereas higher molecular forms are associated with SC. ▨, Fractions that were pooled and concentrated for subsequent experiments (SIgA, fractions 36–41; mIgA, fractions 48–51). *C* and *D*, H44/76 meningococci-coated ELISA plates were incubated for 30 min with chimeric Abs over a wide concentration range. Abs were detected with alkaline phosphatase-conjugated anti-IgG (*C*) or FITC-labeled anti-κ light chain antiserum, followed by peroxidase-labeled anti-FITC Abs (*D*). Experiments were performed twice with similar results.

Respiratory burst

PMN respiratory burst activity was evaluated with live meningococci, chimeric IgG and IgA Abs and complement. In Fig. 3*A*, it

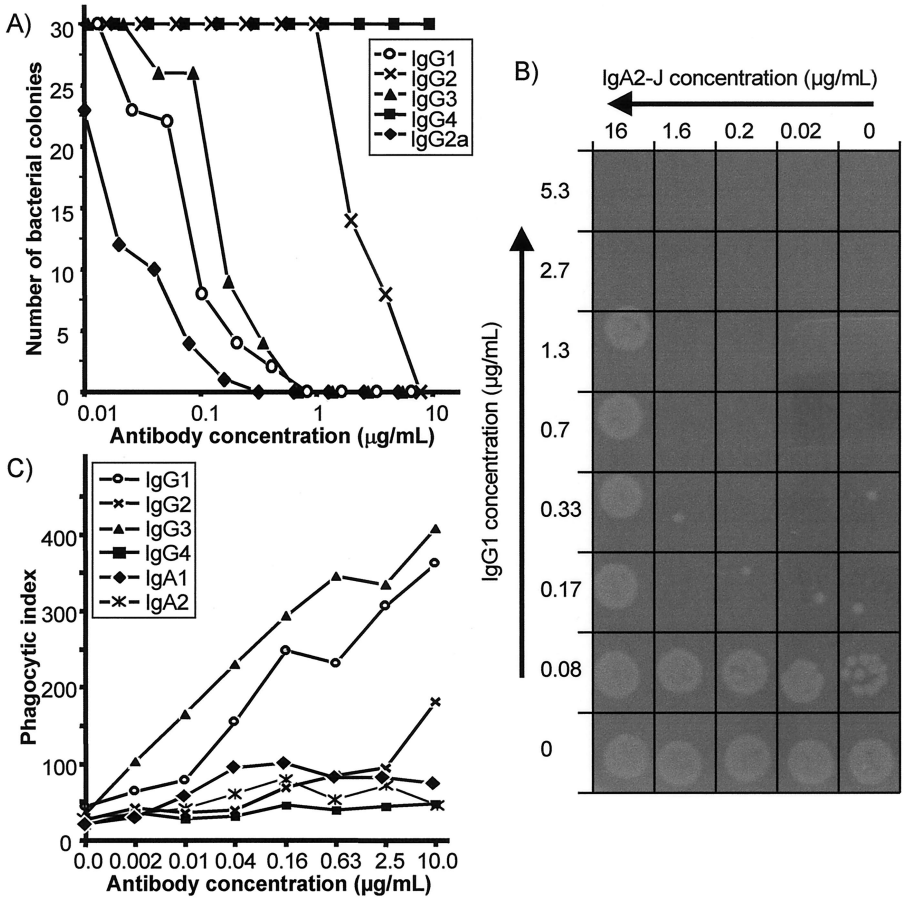

FIGURE 2. Bactericidal and phagocytic activity of chimeric IgG and IgA Abs. *A*, Human IgG-mediated killing of ~100 CFU in the presence of 12.5% serum, with no detectable Abs to H44/76 group B meningococci, as a complement source. The upper detection limit in our assay was ~30 CFU. Results are representative of four independent experiments. *B*, IgA2-J does not activate complement but blocks IgG1 complement-mediated killing of meningococci in a dose-dependent manner. Growth of bacterial colonies is visible only without IgG1, with IgA2-J, or with both IgG1 (low concentration) and IgA2-J (high concentration). Results are representative of two independent experiments. *C*, PMN phagocytic activity of Ig-opsonized meningococci using freshly isolated cells from an FcγRIIa-H/H131, FcγRIIIb-NA1/NA2 donor. Abs, bacteria, and PMN were incubated for 30 min at 37°C. Results are representative of three independent experiments using heat-killed, Alexa 488-labeled meningococci.

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 ($\leq$2 μg/ml) Ab concentrations, IgA stimulated respiratory burst as efficiently as IgG3, with somewhat delayed responses at higher concentrations ($\approx$20 μg/ml) (Fig. 3*B*). 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. 1*B*) (23). IgA2-J was also fractionated to separate mIgA2, dIgA2, and pIgA2 from each other. Phagocytosis of heat-killed meningococci with mIgA1 (Fig. 4*A*) resulted in significant phagocytosis ($p < 0.001$ compared with background), and to a similar extent as with unfractionated IgA1-J and IgA2-J (Figs. 4*A* and 2*C*). Similarly, mIgA2, dIgA2, and pIgA2 stimulated phagocytosis (Fig. 4*B*) and respiratory burst (data not shown). Remarkably, no phagocytic activity was found using SIgA1 (Fig. 4*A*). 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-nitrophenacetyl)-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. 1*B*). We determined the order of activity of

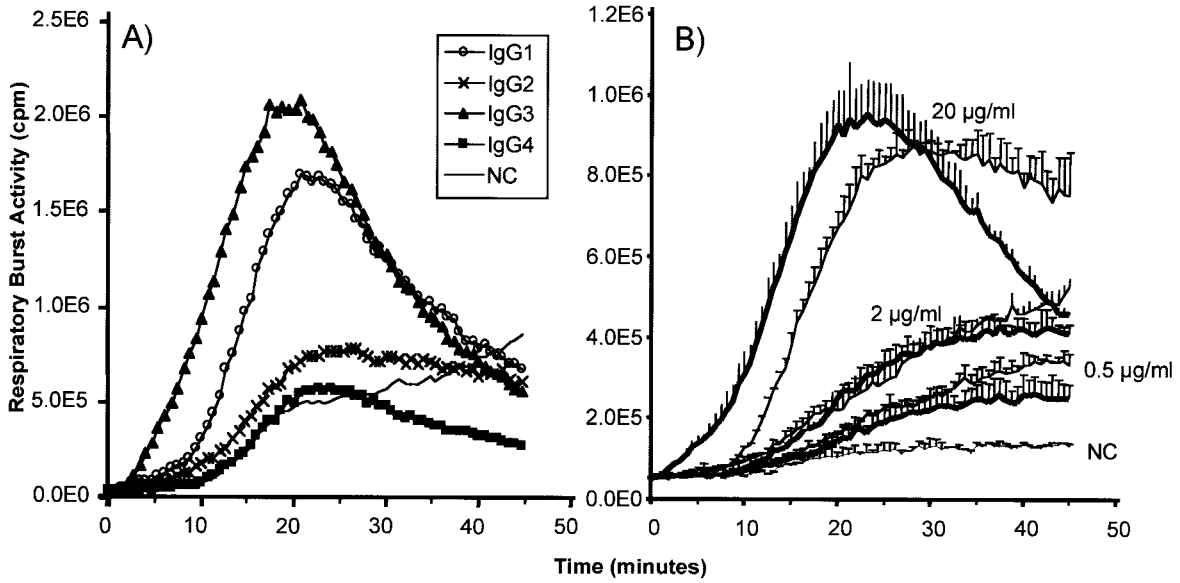

FIGURE 3. Respiratory burst stimulated by IgG and IgA opsonized H44/76 meningococci. *A*, Freshly washed 10^7 midlog-grown live bacteria were preopsonized for 30 min at 37°C with IgG (2 μg/ml) of different subclasses, in the presence of 12.5% human serum as source of complement. At the end of incubation, tubes were transferred into a luminometer, where luminol and 10^5 PMN were automatically injected at the initiation of measurements (time point zero). Experiments were performed twice, yielding similar results. *B*, Heat-killed bacteria were preopsonized for 30 min at 37°C with IgG3 (thick lines) or IgA2 (thin lines) at indicated concentrations. At low concentrations ($\leq$2 μg/ml), IgA2-J triggered a respiratory burst response similar to that of IgG3. At higher Ig concentrations, IgA2-J-induced respiratory burst had a lower peak than IgG3, with a lag period in both the early and late phase of the response. The experiment was performed in duplicate, and vertical lines represent SDs of the mean. NC, Negative control. Data are representative of four individual experiments.

IgG subclasses to activate human complement to be IgG1 = IgG3>IgG2≫IgG4 (Fig. 2*A*) (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 C1q 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 (FcγRIa; <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 FcγRIIIb (100–300,000 receptors/PMN), as well as FcγRIIa.

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. 3*A*). 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 ($\leq$2 μg/ml), IgA induced a respiratory burst similar to those of IgG3 Abs (Fig. 3*B*). At higher concentrations ($\approx$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 $\sim$80 K_d 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 (SIgA; Figs. 1*B* and 4). When incubated with heat-killed meningococci and PMN, all molecular forms of IgA facilitated phagocytosis and respiratory burst, except SIgA (Fig. 4), supporting the notion that PMN do not have a receptor for SIgA. 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 SIgA (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 SIgA does not interact with FcαRI is at variance with experiments performed with IgA-coated surfaces where both mIgA and SIgA were capable of initiating PMN respiratory burst (37). Together, these data support that FcαRI interacts solely with SIgA when coated on plastic or glass surfaces, but not when used as opsonin on the surface on invading pathogens. Thus, SIgA, 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.

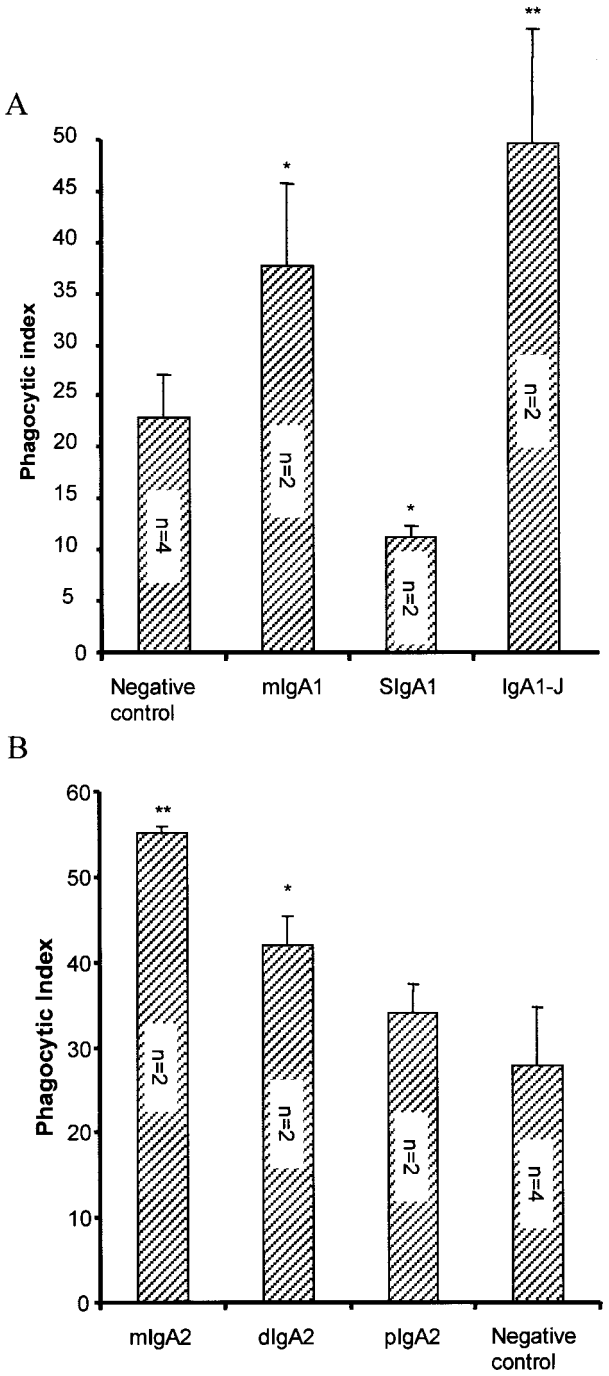

FIGURE 4. Monomeric, dimeric, and polymeric IgA, but not secretory IgA, stimulate PMN effector functions. *A*, mIgA1 is capable of inducing phagocytosis, whereas SIgA1 blocks meningococcal attachment to PMN. *B*, mIgA2, dIgA2, and pIgA2 stimulated PMN phagocytosis. Error bars represent SDs. Results are representative of two independent experiments. *, $0.05 \geq p > 0.01$; **, $0.01 \geq p > 0.001$ when compared with background.

In summary, we generated a unique panel of human IgG and IgA chimeric Abs that recognize PorA, a promising vaccine candidate for group B meningococci. We characterized their capacity to initiate humoral and cellular effector functions. IgG initiated efficient lysis of meningococci 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 mIgA, dIgA, and pIgA actively stimulated PMN functions, SIgA was unable to activate phagocytosis via FcαRI 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.

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