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Antibody-dependent Mononuclear Cell-mediated Antimeningococcal Activity: *COMPARISON OF THE EFFECTS OF CONVALESCENT AND POSTIMMUNIZATION IMMUNOGLOBULINS G, M, AND A*

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Antibody-dependent Mononuclear Cell-mediated Antimeningococcal Activity

COMPARISON OF THE EFFECTS OF CONVALESCENT AND POSTIMMUNIZATION IMMUNOGLOBULINS G, M, AND A

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ABSTRACT We have compared the abilities of immunoglobulin (Ig)G, IgM, and IgA to induce either mononuclear cell-mediated (complement-independent) or complement-mediated (cell-free) antibacterial activity against group C meningococci. In each of these assays, immunoglobulins purified from the sera of individuals immunized with meningococcal group C polysaccharide were compared with those purified from sera of patients convalescing from disseminated meningococcal disease. Our data support three conclusions. First, although nonbactericidal in cooperation with complement, IgA can induce cell-mediated antibacterial activity as well as IgG. Second, the amount of IgG required to induce cell-mediated antibacterial activity is similar to the amount required for complement-mediated killing. Third, although the amount of either postimmunization or convalescent IgM required to induce complement-mediated killing is 16- to 20-fold less than the amount of respective IgG required, IgM is inferior to IgG in its ability to induce cell-mediated antibacterial activity because in the cell-mediated system (*a*) postimmunization IgM is ineffective; (*b*) the amount of convalescent IgM required for minimal activity is eightfold more than the amount of convalescent IgG required; and (*c*) the maximal antibacterial index induced by convalescent IgM is 50% less than that which can be induced by IgG. These data suggest that IgG and IgA may play a greater role than IgM in mononuclear cell-mediated antibacterial host immune defense.

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INTRODUCTION

The antibacterial activities of immunoglobulin (Ig)G, IgM, and IgA have been previously investigated in various assays. IgG and IgM have each been shown to induce complement-mediated immune lysis of many bacteria, including group C meningococci (Mgc).[1] IgA, however, blocks this type of bactericidal activity (1). IgG can also promote complement-independent opsonophagocytosis of bacteria by polymorphonuclear leukocytes (PMN) (2–4). Bacterial opsonization by IgM, however, has with one exception (4) been demonstrated only in the presence of complement (3, 5). Although IgA-dependent phagocytosis has also been reported (2), the role of IgA in cooperation with PMN is still controversial, owing to phagocytic, studies in which IgA was either ineffective (3, 6, 7) or inhibitory (8). IgG and to a lesser extent IgA, have also been shown to induce complement-independent macrophage-mediated antibacterial activity (9).

We have previously demonstrated that among peripheral blood mononuclear cells, only K lymphocytes and monocytes are capable of complement-independent antibody-dependent cell-mediated (ADC) antibacterial activity against Mgc (10). The present study was undertaken to compare the abilities of IgG, IgM, and IgA purified from human meningococcal convalescent or postimmunization sera to induce ADC antibacterial activity.

METHODS

Bacteria. Pathogenic group C serotype 2 Mgc (*Neisseria meningitidis* strain 138-I), originally isolated from spinal

[1] *Abbreviations used in this paper*: ADC, antibody-dependent, cell-mediated; Csss, group C meningococcal polysaccharide; Mgc, group C meningococci; PMN, polymorphonuclear leukocytes.

fluid were stored and cultured for use as previously described (10). Just before the antibacterial assay, log-phase cultured Mgc were diluted in cold Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) to appropriate concentrations.

Fractionation and purification of immunoglobulins from human sera. Convalescent sera consisted of two pools, each containing equal parts of seven sera collected 7–13 d after infection from individuals hospitalized with disseminated disease due to group C, type 2 *Neisseria meningitidis*. The infecting organism isolated from each patient's blood or cerebrospinal fluid was identified, grouped, and typed as previously described (11, 12). Postimmunization sera obtained from two adult volunteers 3 wk after immunization with 50 μg of meningococcal polysaccharide vaccine, group C (Csss) (Meningovax-C, Merck Sharp & Dohme, Canada Ltd., Montreal, Quebec, Canada.) were not pooled before separation of immunoglobulin classes. IgM, IgA, and IgG were separated and purified from 3.0-ml aliquots of human sera using the previously described method of continuous, in-line chromatography over molecular sieve, ion-exchange, and immunoadsorbent affinity gels (11). Cross-contamination of IgM by IgA was ~2% by weight. IgA and IgG pools were free of contamination by the other two classes as measured by radial immunodiffusion. Tests of maintenance of biologic activity of the immunoglobulin fractions from the convalescent serum pool used here have been presented previously, and consisted of IgG- and IgM-dependent complement-mediated immune lysis of Mgc and IgA-dependent blocking of this immune lysis (1, 11).

Quantitation of specific antimeningococcal antibody. The radioactive antigen binding assay described by Brandt et al. (13) was used to determine the amount of specific anti-Csss antibody present in each immunoglobulin fraction.

The standard complement-dependent bactericidal test. The assay of Gold and Wyle (12) was used with only minor modification. To conserve materials and to run the test parallel to the cell-mediated assay, the reaction volumes (0.1 ml), concentrations of bacteria ($0.3–1.3 \times 10^4$/ml), and amounts of immunoglobulin used were identical to those used in the cell-mediated assay (described below).

Preparation of mononuclear cells. The nonadherent mononuclear cells used throughout this study were prepared as previously described (10). Summarized briefly, peripheral blood mononuclear cells, isolated from normal adults through dextran sedimentation followed by ficoll-hypaque density centrifugation, were incubated overnight in complete culture media consisting of RPMI 1640, Hepes buffer, L-glutamine, and 20% heat-inactivated fetal calf serum. The next day, the cells that did not adhere to the 75-cm^2 plastic incubation flasks were washed and then used in the cell-mediated antibacterial assay. As previously indicated (10), these nonadherent mononuclear cells contained 5±3% monocytes as determined by latex particle phagocytosis and myeloperoxidase or nonspecific esterase histochemistry. The remaining 95% of the cells were lymphocytes and ~14–22% of these lymphocytes were K cells, which lack readily detectable surface immunoglobulin and sheep erythrocyte receptors but do possess Fc receptors and are the major lymphocyte effector cells mediating ADC cytotoxicity (14, 15). We previously demonstrated that the antibacterial activity elicited with nonadherent mononuclear cells reflects effector activity mediated by both monocytes and K lymphocytes (10).

The ADC antibacterial assay. The assay used, a modification of the bactericidal assay reported by Steigbigel et al. (16), which measures leukocyte-mediated interference with bacterial growth, was previously described in detail (10). To conserve materials, the final volume of the reaction mixture in this study was usually limited to 0.1 ml. To maintain optimal proportions of reactants in the assay, 0.02 ml of the bacterial suspension (containing $0.3–1.3 \times 10^3$ Mgc), 0.02 ml of purified immunoglobulin (IgG, IgM, or IgA), 0.02 ml of fresh culture media, and 0.04 ml of nonadherent mononuclear cells (containing $2–4 \times 10^5$ cells) were added, in that order. The remainder of the assay and calculation of the antibacterial index were performed as previously described (10).

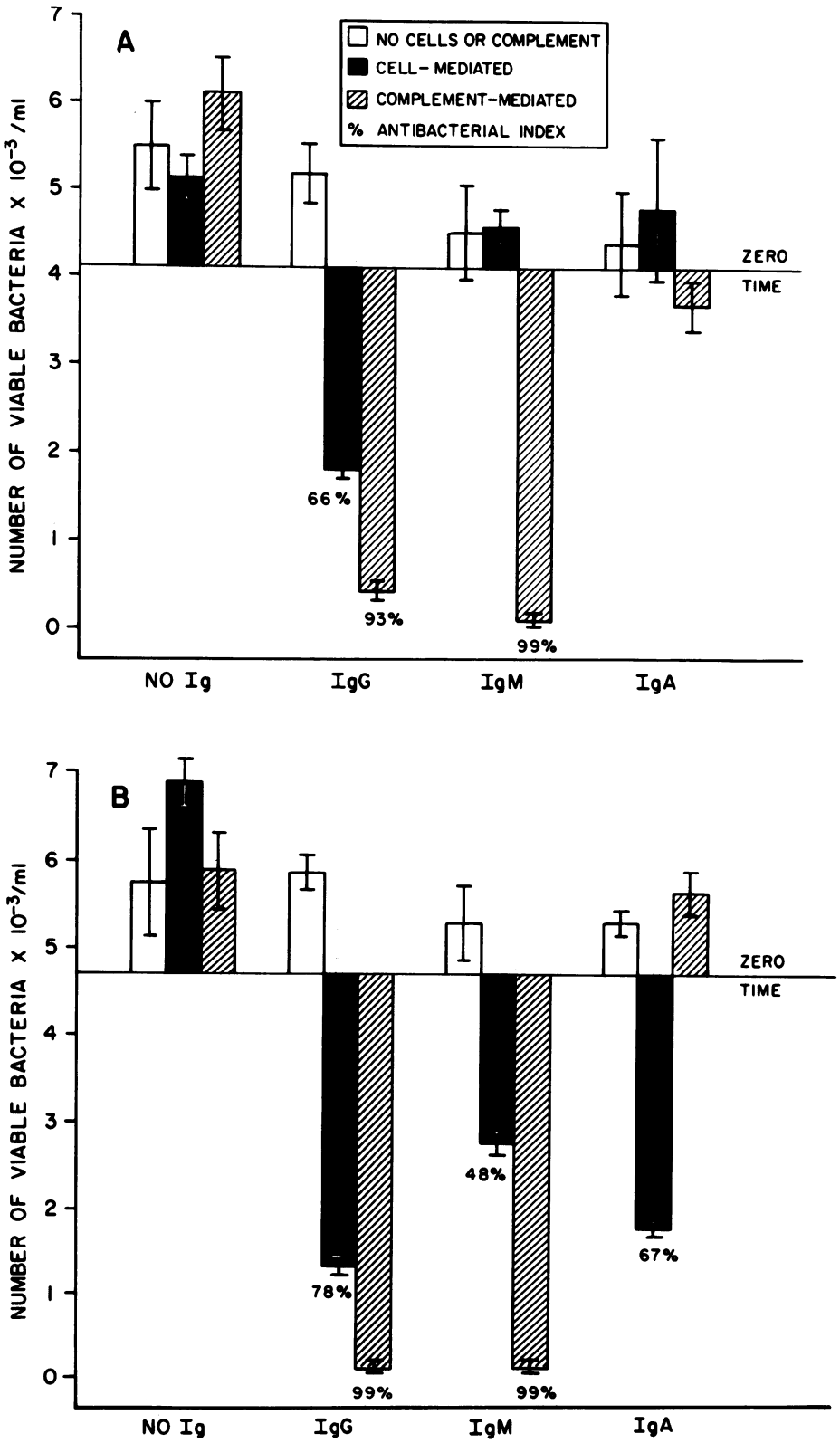
When testing for antigenic specificity, diluted heat-inactivated post-Csss immunization sera were preincubated with purified group C or group A meningococcal polysaccharide for 1 h at 37°C and then for 1 h at 4°C. The antigen-antibody mixtures were added in place of untreated antibody to assay tubes that would either contain (experimental) or lack (control) mononuclear cells.

RESULTS

Comparison of cell-mediated and complement-mediated antibacterial activity in cooperation with immunoglobulin purified from post-Csss immunization sera. When IgG, IgM, and IgA isolated from adult post-Csss immunization sera were tested in the cell-mediated assay, it was found that only IgG was capable of inducing ADC antibacterial activity (Fig. 1A). Either IgG or IgM, however, was able to induce complement-mediated killing of Mgc (Fig. 1A). Postimmunization IgA could not induce either cell-mediated or complement-mediated antibacterial activity (Fig. 1A). Bacterial viability was not diminished when Mgc were incubated with either IgG, IgM, or IgA in the absence of mononuclear cells and complement. Similarly, no antibacterial activity was present when Mgc were incubated with cells or complement in the absence of immunoglobulin.

Comparison of cell-mediated and complement-mediated antibacterial activity in cooperation with immunoglobulin purified from meningococcal convalescent sera. When IgG, IgM, and IgA isolated from adult meningococcal convalescent sera were tested, it was found that all three immunoglobulins were capable of inducing ADC antimeningococcal activity. Maximal ADC antibacterial indices induced by IgM, however, were consistently lower than those induced by either IgG or IgA (Fig. 1B). In contrast, either IgG or IgM could induce complement-mediated killing of Mgc, but IgA could not (Fig. 1B). As in the experiments performed with postimmunization immunoglobulin bacterial viability was not diminished when Mgc were incubated with any immunoglobulin in the absence of cells and complement, or when Mgc were incubated with cells or complement in the absence of immunoglobulin (Fig. 1B).

Evidence that antibody specificity directed against Csss induces ADC antibacterial activity. We wished to determine whether differences in the ADC antibacterial-inducing capabilities of immunoglobulins purified from post-Csss immunization sera (Fig. 1A) and convalescent sera (Fig. 1B) were related to the amount of specific anti-Csss antibody in the purified


A
NO CELLS OR COMPLEMENT
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% ANTIBACTERIAL INDEX
NUMBER OF VIABLE BACTERIA X 10^{-3}/ml
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66%
93%
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NO Ig
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NUMBER OF VIABLE BACTERIA X 10^{-3}/ml
ZERO TIME
78%
99%
48%
99%
67%
NO Ig
IgG
IgM
IgA

TABLE I

Inhibition of ADC Antibacterial Activity against Mgc by Group C, but Not Group A, Meningococcal Polysaccharide

Serum source	Inducing antibody	ADC antibacterial index: Without added antigen (control)*	ADC antibacterial index: Meningococcal antigen added* Group C	ADC antibacterial index: Meningococcal antigen added* Group A
Postimmunization	IgG	68±3	0	63±2
Convalescent	IgG	59±2	0	62±4
Convalescent	IgM	38±5	8±4	34±3
Convalescent	IgA	60±3	0	56±5

Data are mean±SEM.

* Immunoglobulins were preincubated without antigen (control) or with 10 μg of group C or group A meningococcal polysaccharide for 1 h at 37°C and then for 1 h at 4°C just before the experiment. In each instance the final volume of the test reaction mixture was 100 μl.

immunoglobulin samples used. Accordingly, we first wanted to determine whether the functional antibody in the ADC antibacterial assay was directed against the group C meningococcal capsular polysaccharide. The data from three experiments demonstrate that ADC antibacterial activity induced by either postimmunization or convalescent serum antibodies is totally abrogated by incubation with the homologous polysaccharide, Csss (Table I). As expected, this inhibition did not occur when antibodies were preincubated with heterologous polysaccharide derived from group A meningococci (Table I).

Quantitation of ADC antibacterial activity in cooperation with immunoglobulin purified from post-Csss immunization sera. As measured by the amount of Csss bound in a radioactive antigen binding assay, the purified IgG and IgM fractions of post-Csss immunization sera contained 25- to 100-fold more anti-Csss antibody/ml than were contained in IgA fractions. Because not >40 μl of undiluted immunoglobulin would be used in a 100-μl test reaction mixture, the maximum amounts of postimmunization anti-Csss IgG, IgM, and IgA that were present in the assays bound 18.0, 15.2, and 0.26 ng of Csss, respectively.

Data from five experiments in which postimmunization immunoglobulin was used are shown in Fig. 2A. ADC antibacterial activity induced by IgG was found to be directly proportional to the amount of anti-Csss antibody present. Maximal ADC antibacterial activity (78%) was induced in the presence of 18 Csss antigen binding units of IgG.

Postimmunization IgM, tested over a wide range of concentrations of anti-Csss antibody, was unable to induce ADC antibacterial activity. Although postimmunization IgA was also ineffective, the amounts of postimmunization IgA used contained only 0.065–0.26 Csss antigen binding units of anti-Csss antibody (Fig. 2A).

Quantitation of ADC antibacterial activity in cooperation with immunoglobulin purified from meningococcal convalescent sera. When IgG, IgM, and IgA were isolated from pooled meningococcal convalescent sera, large amounts of anti-Csss antibody were found not only in the IgG and IgM fractions, but also in the IgA fractions. Accordingly, the maximal amounts of convalescent anti-Csss IgG, IgM, and IgA that were used per test reaction bound 128, 24.8, and 16.0 ng of Csss antigen, respectively. Thus, more specific anti-Csss antibody could be present when any class of convalescent immunoglobulin was used instead of postimmunization immunoglobulin. This difference was most dramatic for IgA: in convalescent IgA the concentration of specific anti-Csss antibody was 60-fold greater than that in postimmunization IgA.

Data from 11 experiments (Fig. 2B) indicate that the magnitude of ADC antibacterial activity induced by each class of convalescent immunoglobulin was related

FIGURE 1 Comparison of cell-mediated and complement-mediated antibacterial activity induced by IgG, IgM, or IgA. Percentages denote antibacterial indices. Bars represent the mean±SEM of a representative experiment performed in triplicate. A. Immunoglobulins were derived from post-Csss immunization sera; the data are typical of five similarly performed experiments. B. Immunoglobulins derived from meningococcal convalescent sera; the data are typical of 11 similarly performed experiments.

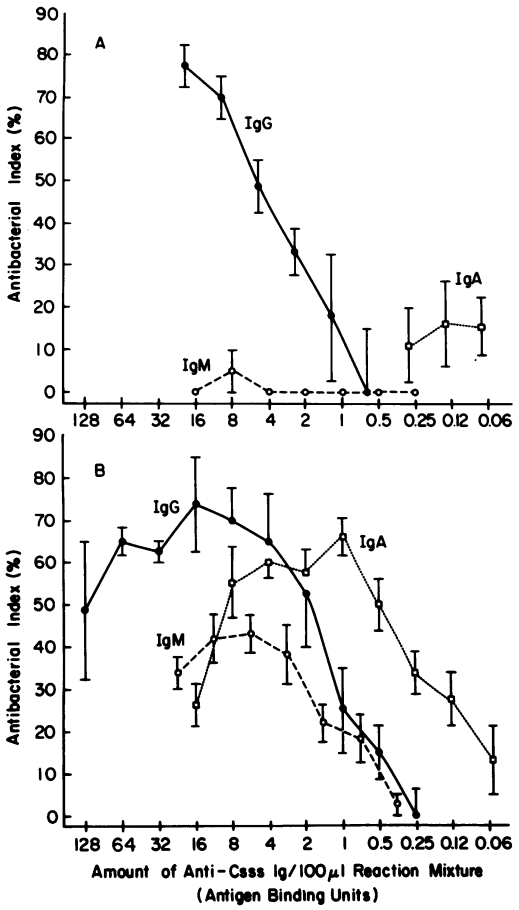

FIGURE 2 Quantitative analysis of antibody-dependent, cell-mediated antibacterial activity induced by IgG, IgM, or IgA. The magnitude of antibacterial activity is plotted against the quantity of specific anti-Csss antibody present in the reaction mixture. ●, IgG; ○, IgM; □, IgA. A. Immunoglobulins were derived from postimmunization sera; each point represents the mean±SEM of two to five separate experiments. B. Immunoglobulins were derived from meningococcal convalescent sera; each point represents the mean±SEM of two to five separate experiments.

to the concentration of anti-Csss antibody in the reaction mixture. Maximal IgG-induced ADC antibacterial activity averaged 73% and was found when 16 antigen binding units of anti-Csss antibody were present. Maximal IgM-induced ADC antibacterial activity averaged only 43% and was found when 6.2 antigen binding units of anti-Csss antibody were present. Maximal IgA-induced ADC antibacterial activity averaged 66% and required only one antigen binding unit of anti-Csss antibody. When 16 antigen binding units of convalescent anti-Csss IgA were used, a "prozone" effect of diminished ADC antibacterial activity was evident.

Quantitative comparison of cell-mediated and complement-mediated antibacterial activity. Presented in Table II are the minimal amounts of each immunoglobulin necessary to attain average minimal antibacterial indices > 40% in either complement-mediated or cell-mediated systems. Using this criterion, 2.6 and 3.0 Csss antigen binding units of postimmunization IgG were required for complement-mediated and cell-mediated antibacterial activity, respectively. Similarly, the amount of anti-Csss antibody in convalescent IgG that induced complement-mediated killing (1.4 antigen binding units) was comparable to the amount of anti-Csss antibody in convalescent IgG that induced cell-mediated antibacterial activity (1.6 antigen binding units).

IgM, however, was far more efficient in inducing complement-mediated killing than in inducing ADC antibacterial activity. The minimal amount of postimmunization IgM that induced antibody-dependent, complement-mediated antibacterial activity contained 0.16 antigen binding units of anti-Csss antibody. Nevertheless, these same samples of IgM were incapable of inducing cell-mediated antibacterial activity. Convalescent IgM induced complement-mediated killing of Mgc when as little as 0.07 antigen binding units of anti-Csss antibody were present. ADC antibacterial activity, however, was induced by these samples of IgM only when at least 3.1 Csss antigen binding units were present (Table II).

In contradistinction to both IgG and IgM, convalescent IgA (tested at anti-Csss antibody concentrations ranging from 0.25 to 8.0 antigen binding units per test mixture) was unable to induce any complement-mediated killing of Mgc (Table II), whereas this convalescent IgA induced cell-mediated antibacterial activity when as little as 0.37 antigen binding units of anti-Csss antibody were present (Table II). Postimmunization IgA was unable to induce either complement-mediated or cell-mediated antibacterial indices > 40% when tested at anti-Csss antibody concentrations ranging from 0.06 to 0.26 antigen binding units.

ADC antibacterial activity in the presence of both IgG and IgA. Because the IgA we used in this stock has been shown to block IgG- and IgM-dependent complement-mediated killing of Mgc (11), and because IgA has been reported to be capable of blocking PMN phagocytosis (8), we performed two experiments to determine whether our IgA and IgG were antagonistic when present together in the cell-mediated assay. As shown in Table III, the antibacterial indices elicited in the presence of both IgG and IgA were never less than those induced by either immunoglobulin alone.

DISCUSSION

In this paper we have demonstrated that IgA isolated from meningococcal convalescent sera, although unable to fix complement, was nevertheless highly

TABLE II

Comparison of Cell-mediated and Complement-mediated Antibody-dependent Antibacterial Activity in Cooperation with IgG, IgM, and IgA Purified from Either Post-Csss Immunization or Convalescent Sera

Immunoglobulin class	Source of sera	Lowest amount* of specific Ig (per 100-μl reaction mixture) required to induce an antibacterial index >40%	
		Cell-mediated (complement-free) antibacterial activity	Complement-mediated (cell-free) antibacterial activity
IgG	Post-Csss immunization	3.0	2.6
	Convalescent	1.6	1.4
IgM	Post-Csss immunization	>15.2(NAAD)‡	0.16
	Convalescent	3.1	0.07
IgA	Post-Csss immunization	>0.26(NAAD)‡	>0.26(NAAD)‡
	Convalescent	0.37	>8.0 (NAAD)‡

* Measured in Csss antigen binding units where 1 U equals that amount of immunoglobulin that binds 1 ng of Csss as detected in a radioactive binding assay.
‡ NAAD, no antibacterial activity (>40%) detectable.

capable of inducing mononuclear cell-mediated antimeningococcal activity in vitro. Moreover, the minimal necessary amount of IgA was at least fourfold and eightfold less than the amounts of convalescent IgG and IgM required, respectively. IgA isolated from post-Csss immunization sera, however, contained an insufficient amount of anti-Csss antibody and was consequently inactive in our assay. Although Reed (3), Quie et al. (6), Wilson (7), and Wilton (8) could not demonstrate IgA-dependent phagocytosis by PMN, they used "normal" colostral or serum IgA, whereas Bisno et al. (2) (who used postinfection IgA) concluded that IgA was highly opsonic. Similarly, Reynolds et al. (9) have shown that postinfection secretory IgA can opsonize bacteria for respiratory macrophages, albeit not as well as IgG. Thus, the source of IgA is of paramount importance in determining whether or not IgA is functional.

TABLE III

ADC Antibacterial Activity in the Presence of Both IgG and IgA

Experiment	Amount of specific Ig present per 100-μl reaction mixture*		Cell-mediated antibacterial index		
	IgG‡	IgA§	IgG present	IgA present	IgG + IgA present
				%	
1	9	1	71	51	79
	9	2	71	50	80
	9	4	71	48	76
	9	8	71	45	71
2	9	4	63	63	79
	9	8	63	63	68
	9	16	63	31	66

* Measurement in Csss antigen binding units where one unit equals that amount of immunoglobulin that binds 1 ng of Csss, as detected in a radioactive antigen binding assay.
‡ IgG was purified from post-Csss immunization serum.
§ IgA was purified from meningococcal convalescent sera.

The minimal amount of convalescent IgG or postimmunization IgG required to induce ADC antibacterial activity was similar to the amount of convalescent IgG or postimmunization IgG required for complement-mediated killing. IgM, in contrast, was far more efficient in cooperation with complement than it was in cooperation with cells. Convalescent IgM induced cell-mediated antibacterial activity only at concentrations 40-fold greater than that required for complement-mediated bactericidal activity, and postimmunization IgM was ineffective with cells even when used at concentrations 100-fold greater than that required for killing with complement. Moreover, even at optimal concentrations, the maximal magnitude of the ADC antibacterial indices induced by convalescent IgM were 40–50% less than that which could be induced by IgG or IgA. The hypoeffectivity of IgM that we found is consistent with previously reported results in complement-independent PMN-mediated phagocytic studies, wherein IgM was found to be either inactive (3, 5) or limited to inducing 25–50% of the activity that could be induced by IgG (4).

Although definitive explanation is lacking for this quantitative and qualitative functional deficiency of

IgM, three hypotheses can be seriously considered. First, owing to IgM's pentameric structure, there may be steric difficulties in maintaining the effector cell-IgM-target bacteria complex long enough for the antibacterial reaction to proceed successfully. Second, there may be a paucity of Fc-IgM receptors on mononuclear cells capable of effecting ADC antibacterial activity. Indeed, peripheral blood monocytes (17), like alveolar macrophages (9), lack Fc-IgM receptors. Certain T lymphocytes, however, do bear Fc-IgM receptors (18). Although it has been reported that these cells can mediate IgM-dependent ADC cytotoxicity, this reaction is apparently far less efficient than that induced by IgG (19).

The third hypothesis for the functional deficiency of IgM is that antibodies directed against subcapsular (protein and/or lipopolysaccharide) meningococcal antigens, although ineffective alone, may potentiate the effectivity of anti-Csss antibodies synergistically. This would explain the lack of effect of postimmunization IgM, inasmuch as anticapsular antibodies are not stimulated by immunization with Csss but are clearly present after infection, as shown by Zollinger et al. (20). Although we showed in this report (as did Roberts in his [5]) that activity in either postimmunization sera or convalescent sera is abrogated when anti-Csss antibodies are absorbed by free Csss, Frasch et al. (21) clearly demonstrated with a chick embryo model (in which antimeningococcal protection is due to complement-independent, reticuloendothelial cell-mediated activity) that anticapsular group-specific and antisubcapsular type-specific antibodies are strongly synergistic. This synergy would also explain why the minimal amounts of convalescent IgM or IgG needed to induce complement-mediated killing and the minimal amount of convalescent IgG needed to induce ADC antibacterial activity were roughly half the minimal amounts of respective postimmunization antibodies needed (see Table II).

Immunity against disseminated meningococcal infection has been correlated with the presence of antibody-dependent, complement-mediated bactericidal activity of human serum (22). Nevertheless, as emphasized by Goldschneider et al. (22), cell-mediated antibacterial activity is "undoubtedly of importance in confining and eliminating the meningococcus," particularly in tissues containing cells of the reticuloendothelial system. Since the ADC antibacterial system we have reported occurs without added complement, it is reasonable to suppose that this mechanism is physiologically more relevant to areas such as mucosal surfaces, where IgA, IgG, and mononuclear cells are present (9, 23, 24) but complement is functionally inadequate (23, 25). To assess appropriately the ability of mononuclear cells to limit bacterial multiplication among mucosal tissues (thereby decreasing the opportunity for systemic invasion), the ability of secretory immunoglobulins to induce local mononuclear cell-mediated antibacterial activity (particularly against enteric pathogens) should be examined. In this regard, Arnaud-Battandier et al. (24) have recently shown that intraepithelial gastrointestinal lymphocytes can mediate ADC cytotoxicity, and Fubara and Freter (26) have demonstrated that protection in enteric cholera infection is associated with a complement-independent antibacterial capability of secretory IgA that requires intact intestinal cells and is inhibited by the potent inhibitor of phagocytosis, iodoacetate. In addition, we have evidence that monocytes and K lymphocytes can mediate ADC antibacterial activity against the enteric pathogen *Shigella flexneri*.[2]

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