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SUBCLASS RESTRICTION OF MURINE ANTI-CARBOHYDRATE ANTIBODIES[1]

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Examination of the subclass distribution of murine antibodies directed against groups A and C streptococcal carbohydrate, α-(1→3) dextran and phosphocholine yields the surprising observation that these carbohydrate antigens stimulate IgG responses largely restricted to the rare IgG3 subclass. This subclass restriction is particularly impressive in light of the low circulating levels of IgG3 in nonimmune mouse serum and the failure of a variety of other antigens including proteins and aromatic haptens to stimulate IgG3 antibody production. Attempts to alter the subclass restriction of antibodies with carbohydrate specificity by immunization with carbohydrate-coupled protein have been unsuccessful and indicate that immunoregulation of subclass expression probably occurs at the level of the antibody forming (B) cell. It is therefore conceivable that V_H regions of murine immunoglobulins may be restricted to particular IgG subclasses. A similar type of subclass restriction has been reported in human and rat anti-carbohydrate antibodies. This recruitment of a minor immunoglobulin isotype by carbohydrate antigens in several species further supports the concept of immunoregulation at the level of subclass, and suggests that these and other mammals may share a structurally similar isotype with perhaps a common evolutionary origin.

7S immunoglobulins of most species can be separated into distinct subclasses on the basis of antigenic and physicochemical properties (1, 2). Although in many cases these subclasses display differentiable biologic characteristics (3), the significance of multiple Cγ regions remains obscure.

Interestingly, a variety of studies have shown that certain antigens elicit antibody responses largely restricted to a single subclass. Thus subclass predominance has been reported in human antibodies to diphtheria and tetanus toxins (IgG1, 4), Rh factor (IgG1 and IgG3, 5), measles virus (IgG1 in patients with multiple sclerosis or subacute sclerosing panencephalitis, 6), coagulation factor VIII (IgG4, 7), and carbohydrate antigens (IgG2, 4). Since identical immunoglobulin variable region determinants have been shown to be associated with multiple, different antibody classes (8–10), the regulation of expression of particular antibody subclasses has been presumed to be independent of the antibody-combining site. Comparison of subclass distributions in normal and T cell-deficient mice coupled with analysis of the influence of antigen form and route of administration on subclass expression (11–13) suggested that a T cell regulatory mechanism or some aspect of antigen processing might account for the antigen-induced preeminence of specific IgG subclasses.

In the mouse, four isotypes of IgG designated IgG1, IgG2a, IgG2b, and IgG3 have been identified (14, 15). Of these, IgG3 is poorly represented in normal sera, comprising at most a few percent of nonimmune mouse IgG (15).

In this report, we examine the subclass distributions of murine antibodies raised against a variety of antigens and observe that bacterial carbohydrates stimulate IgG responses largely restricted to the rare IgG3 subclass. Human antibodies to dextrans, levans, and teichoic acids (4) as well as to group-A streptococcal carbohydrate (16) have been reported to be restricted mainly to the human IgG2 subclass. Similarly, rat anti-group A streptococcal antibodies (17) and anti-phosphocholine antibodies (G. der Balian, personal communication) are almost entirely confined to the rat IgG2c subclass. Restriction of equine anti-pneumococcal antibodies to an unusual type of aggregating immunoglobulin, which is poorly represented in normal horse serum, has also been reported (18). In each of these species, carbohydrate antigens appear to stimulate an IgG response largely restricted to a single isotype that represents a minor, though variable, proportion of normal serum IgG. These observations support the notion of an immunoregulatory mechanism acting at the level of subclass. In this report we present preliminary evidence that suggests that subclass selection by specific antigens most likely occurs at the level of antibody-forming (B) cell.

MATERIALS AND METHODS

Animals and plasmacytomas. Adult A/J, BALB/cByJ, SWR/J and 129/J mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. AKR/Nat animals were obtained from the National Laboratory Animals Co., O'Fallon, Mo. The BALB/c plasmacytomas MOPC-11 (γ2b, K) and J606 (γ3, K)

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were obtained from Litton Bionetics, Inc., Kensington, Md., under National Cancer Institute Contract NO1-CB-92142 and were maintained by serial passage in BALB/cByJ mice. Purified myeloma proteins from MOPC-21 (γ1, K), MOPC-195 (γ2b, K) and FLOPC-21 (γ3, K) were purchased from Litton Bionetics, Inc.

Antigen preparation and immunizations. Antibodies to bovine γ-globulin (BGG)[6] and ovalbumin (OVA) were raised by intraperitoneal immunization with 100 μg antigen emulsified in complete Freund's adjuvant (CFA), followed by secondary immunization 6 weeks later of 100 μg antigen in incomplete Freund's adjuvant (IFA). Antibodies to groups A and C streptococci were produced by repeated immunization with appropriate vaccines as has been described (19, 20). AKR/Nat and A/J anti-PC antibodies were obtained by intramuscular immunization with pneumococcal vaccine R36A (21) emulsified in CFA, followed by 2° and 3° i.p. immunizations in saline. Anti-α-(1→3) dextran (DEX) antibodies were raised in BALB/cByJ and 129/J mice by combined immunization with dextran B1355 (gift of Dr. Allene Jeanes) and *Escherichia coli* strain B (Calbiochem, La Jolla, Calif.) as has been described (22). DNP-derivatized keyhole limpet hemocyanin (KLH) and OVA were prepared by conventional techniques (23) and were injected i.p., 100 μg per mouse, initially in CFA, secondarily in IFA, and thereafter in saline at 2-week intervals. DNP-lysine was coupled to B1355 dextran with cyanogen bromide. Tyraminated group-A streptococcal carbohydrate (24) was azo-coupled to BGG in a ratio of 8 μg GAC (see Abbreviation) to 5 mg BGG (kind gift of Dr. G. der Balian). The resultant conjugate was administered in 100-μg doses at weekly intervals initially in CFA, secondarily in IFA, and thereafter in saline.

Antibody purification. BGG and OVA-specific antibodies were eluted from BGG or OVA-Sepharose (25) by using 0.1 M glycine buffer, pH 2.3. Antibodies to group-A streptococci were purified on N-acetyl glucosamine Sepharose (NAG-Seph) and eluted with free *N*-acetyl glucosamine (19). Antibodies with specificity for group-C streptococcal carbohydrate were purified by glycine elution from group-C streptococcal vaccine. PC-specific antibodies were purified on PC-Sepharose and eluted with 10^{-2} M PC hapten (26). Antibodies directed against α-(1→3) dextran were purified from dextran-derivatized Sepharose (27) by using glycine buffer. Antibodies with specificity for DNP were purified on a DNP-lysine-Sepharose column (25) and eluted with glycine buffer. Purity of glycine-eluted antibodies was verified in each case by repassage on immunoadsorbents. Purified anti-GAC and anti-PC antibodies were isoelectric focused and visualized by ^{125}I-antigen overlay (19, 28) to ensure that IgG subpopulations were not selected by the affinity-chromatography purification. Purified antibodies were separated into IgM and IgG-enriched fractions by sucrose density gradient sedimentation (19). IgG subclass analysis was then performed on the pooled, antigen-specific IgG fractions.

Preparation of subclass-specific antisera. The preparation of rabbit anti-mouse IgG subclass antisera by using BALB/c myeloma proteins as immunogens has been previously described (29). Briefly, anti-IgG1 serum was produced by immunization of a rabbit with 500 μg of purified M21 in CFA. After 3 months this animal was boosted with 1 mg of the γ-globulin fraction of serum from an animal bearing the tumor MOPC-300 (γ1, K). The resultant antiserum was rendered subclass-specific by precipitation with serum from animals bearing the plasmacytomas LPC-1, MOPC-11, and J606. Anti-IgG2a serum was raised in a rabbit by immunization with the γ-globulin fraction of serum from animals bearing the plasmacytomas MOPC-173 and LPC-1. This antiserum was absorbed with S200, M11, and J606. Rabbit anti-mouse IgG3 was obtained by immunization with purified F21 followed by absorption with normal mouse serum. Antisera to mouse IgG2b were obtained from Gateway Immunosera, Cahokia, Ill.

Radioimmunoassay of subclass levels. Solid-phase radioimmunoassay for mouse IgG subclasses was performed by using our specific rabbit antisera adsorbed on the surface of polystyrene tubes (Falcon Plastics, Oxnard, Calif.) or in polyvinyl chloride microtiter trays (Cooke Engineering, Alexandria, Va.) (30). A 1:10,000 dilution of the 50% $(NH_4)_2SO_4$ precipitate of each antiserum is used to coat the plastic surface for 6 hr at 20°C. This solution is then decanted and the plastic surface washed briefly in 1% bovine serum albumin in 0.005 M phosphate-buffered saline, pH 7.4 (1% BSA in PBS). Inhibitors are then titrated in 3- or 4-fold dilutions in 1% BSA and allowed to interact overnight with the antiserum-coated plastic in the presence of 100,000 counts per minute of ^{125}I-labeled proband at a specific activity of about 20 μCi per μg (31). Probands for the IgG1, IgG2a, IgG2b, and IgG3 assays were ^{125}I-S200, ^{125}I-H1, ^{125}I-M195, and ^{125}I-J606, respectively. Uninhibited binding of the proband was typically between 10% and 20% of added counts per minute; nonspecific binding of proband to plastic was less than 0.5% of added counts per minute. All assays were performed in triplicate with both positive and negative controls included in each assay iteration. Inhibitor concentrations were standardized by total protein concentration according to $O.D._{280}$ and subclass representation was determined at the 50% inhibition point of each isotype assay.

Determination of idiotypes on anti-group A streptococcal antibodies. Hyperimmune anti-group A streptococcal sera from 10 A/J mice were pooled and the NAG-specific antibodies purified by affinity chromatography (19). 400 μg of this preparation emulsified in CFA was used to immunize a guinea pig (Eldridge Rabbitry, Belleville, Ill.). Two months later this animal was boosted with the same antigen dose in IFA and 10 days later the animal was bled. The resultant antiserum was absorbed with normal serum and with J606. Idiotypic assays were performed in polyvinyl chloride microtiter plates by using a 1:3000 dilution of the 50% $(NH_4)_2SO_4$ precipitate of the adsorbed guinea pig anti-idiotypic antiserum with the ^{125}I-labeled, purified, pooled, A/J anti-GAC antibody as proband. Maximum binding was 15% and minimum binding 0.5% of added counts per minute.

RESULTS

Specificity of the radioimmunoassay for subclass. Figure 1 documents the specificity of our antisera to mouse IgG1, IgG2a, IgG2b, and IgG3 isotypes. In each case, the 50% inhibition point for myeloma proteins of the correct subclass occurs at a protein concentration less than 3% of that required for similar inhibition by myeloma proteins of different subclasses. Also shown in Figure 1 are the inhibition profiles of pooled AKR/Nat anti-PC antibodies tested in our radioimmunoassay system. Clearly the majority of these specific antibodies are of the IgG3 subclass (a complete report of murine 7S antibodies to PC is in preparation).

Subclass selection by carbohydrate antigens. The subclass

[6] Abbreviations used in this paper: BGG, bovine γ-globulin; CFA, complete Freund's adjuvant; DEX, B1355 α-(1→3) dextran; GAC, group-A streptococcal carbohydrate; IFA, incomplete Freund's adjuvant; NAG, *N*-acetyl glucosamine; OVA, ovalbumin; PBS, 0.005 M phosphate, 0.15 M NaCl, pH 7.4; PC, phosphocholine.

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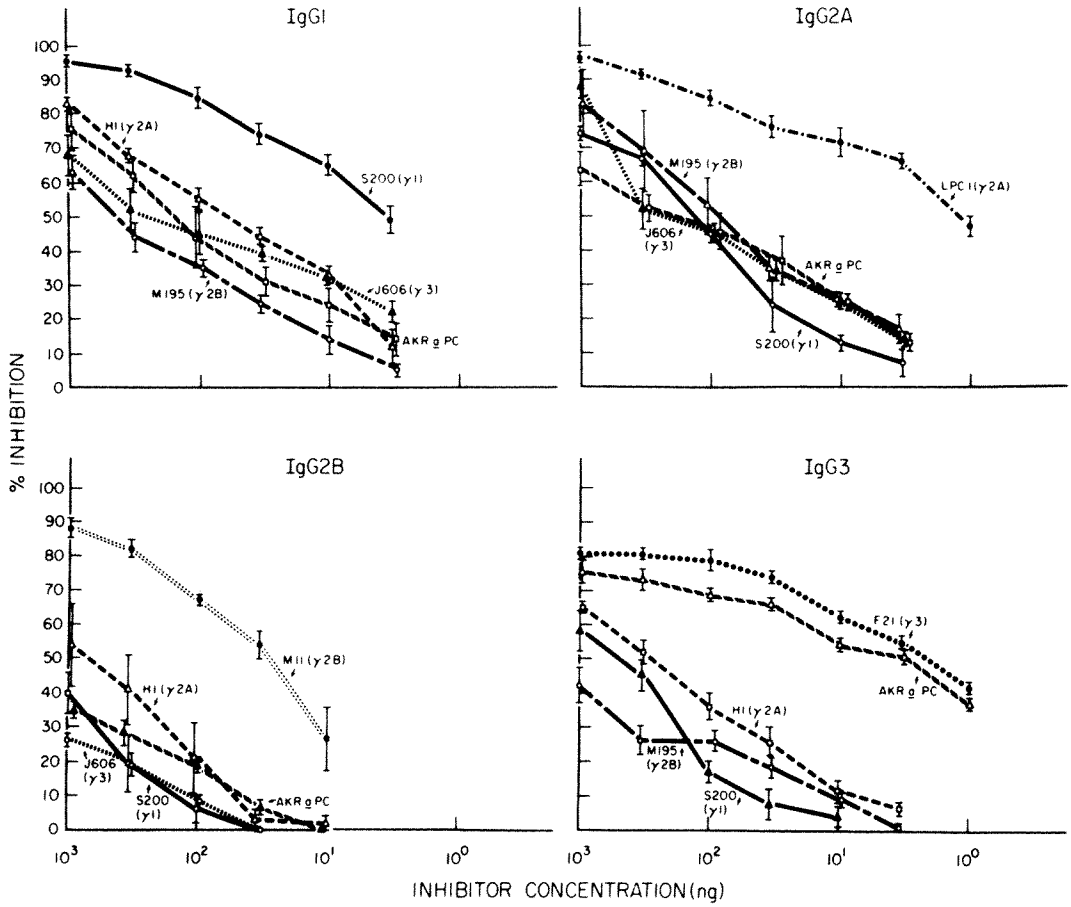

Figure 1. Radioimmunoassay for antibody subclass. The ability of serially diluted myeloma proteins or purified antibodies to inhibit the subclass-specific reaction of antiserum-coated plastic with ^{125}I-labeled myeloma proteins is analyzed. Myeloma proteins were derived from the 50% $(NH_4)_2SO_4$ precipitate of serum from tumor-bearing animals. Anti-PC antibodies were purified by passage of hyperimmune serum pooled from eight AKR mice over a PC-Sepharose immunoadsorbent. Each panel shows geometric means and S.E. for three separate determinations at each point. *Top left,* IgG1-specific antiserum with ^{125}I-S200 as proband; *top right,* IgG2a-specific antiserum with ^{125}I-H1 as proband; *bottom left,* IgG2b-specific antiserum with ^{125}I-M195 as proband; *bottom right,* IgG3-specific antiserum with ^{125}I-J606 as proband.

distributions of various antigen-specific IgG preparations and of normal BALB/cByJ sera are indicated in Figure 2. Antibodies to OVA and BGG are restricted primarily to the IgG1 subclass, a major constituent of nonimmune mouse IgG. These results are consistent with previous studies of mouse antibodies to these antigens (13). In contrast, antibodies to PC and GAC are almost entirely restricted to the IgG3 subclass. This result is particularly striking in light of the low circulating levels of IgG3 in normal mouse serum as shown and as has been previously reported (15). Group C streptococcal vaccine and α-(1→3) dextrans stimulate IgG responses that while not absolutely restricted to the IgG3 subclass, nevertheless contain significantly more of this isotype than of any other subclass. Note that in all cases the total amount of IgG detected in the isotype assays closely approximates the amount of protein titrated as measured by $O.D._{280}$.

Regulation of IgG subclass selection. The specific selection of IgG3 antibodies by bacterial carbohydrate antigens in the mouse suggests that some regulatory mechanism must act to recruit antibody-forming cell precursors in an isotype-specific fashion. In order to examine this question in more detail, we derivatized BGG with GAC and used the resulting GAC-BGG as an immunogen in A/J mice. In addition, we tested the subclass distributions of antibodies to DNP-KLH, DNP-OVA, and DNP-DEX. Figure 3 shows that the isotypic profiles of antibodies raised against the three DNP conjugates are essentially indistinguishable. DNP-specific antibodies raised by immunization with DNP-KLH or DNP-OVA contain significant amounts of IgG1 and IgG2a isotypes, consistent with previously published results (32). Anti-DNP antibodies induced in response to DNP-DEX are also primarily of the IgG1 and IgG2a subclasses. There is a slight increase in the IgG3 in this antibody population, but the increase is not significant.

Figure 3 also shows the subclass distributions of antibodies elicited by immunization with GAC-BGG as compared with those produced in response to group-A streptococcal vaccine or BGG alone. Coupling of GAC to BGG yields an immunogen that stimulates considerably more IgG1 anti-GAC antibodies in A/J mice than does streptococcal vaccine (26% IgG1 with GAC-BGG immunization as compared with 5% IgG1 in response to streptococcal vaccine). This effect may reflect the action of a subclass-specific regulatory mechanism or may result from the presentation of new antigenic determinants generated by the coupling of carbohydrate to BGG. In order to investigate whether these GAC-BGG-induced antibodies contained typical A/J anti-GAC V_H structures linked to IgG1 C_H domains, we examined the idiotypic characteristics of the NAG-purified anti-GAC-BGG antibodies. Figure 4 shows that eight A/J individuals immunized with group-A streptococcal vaccine all produced comparable levels of idiotypically-reactive material. This is typical of the response of A/J mice to GAC (33). Immunization with GAC-BGG, however, elicits a population of GAC-specific antibodies that do not bear the common A/J anti-GAC idiotypic determinants. Examination of the L chains of anti-

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GAC-BGG antibodies by isoelectric focusing reveals loss of numerous focusing bands that are characteristic of A/J anti-GAC L chains (R. M. Perlmutter, unpublished results). Thus it is likely that the shift in isotype expression resulting from use of GAC coupled to BGG as an immunogen represents the stimulation of new antibody clones differing in V_H as well as C_H structure. Despite the introduction of these new IgG molecules, the majority of anti-GAC antibody induced by GAC-BGG is of the IgG3 isotype.

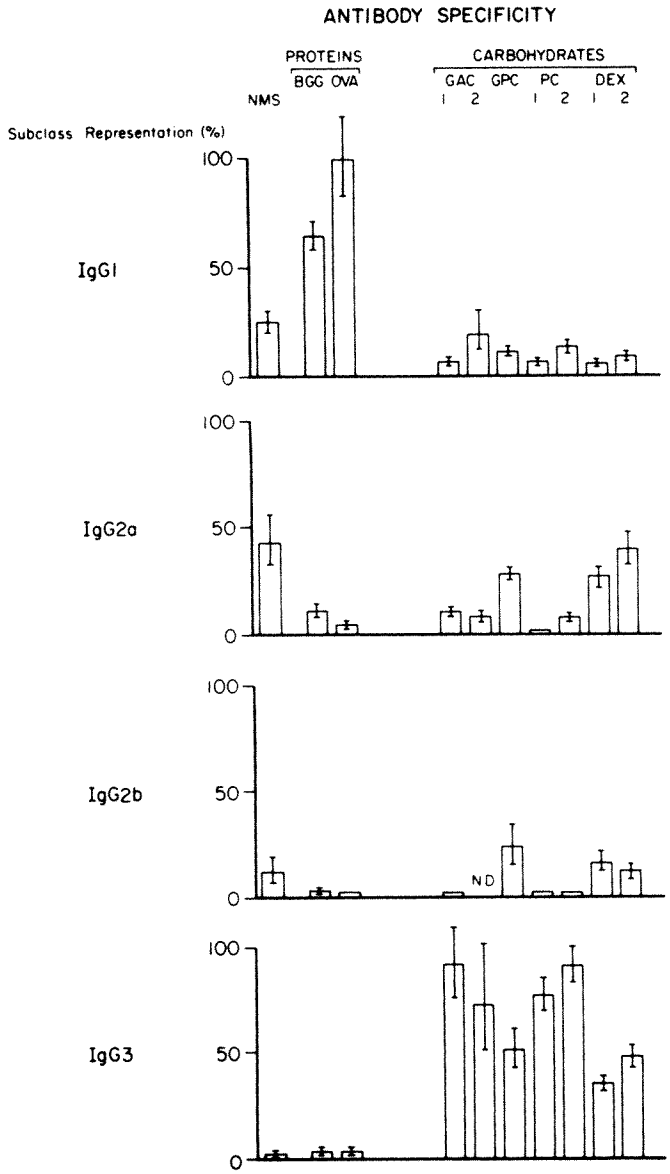

Figure 2. Subclass restriction of carbohydrate antibodies. The percentage of total protein in immunoadsorbent-purified IgG-enriched samples (as measured by optical density at 280 nm) which can be attributed to each mouse IgG subclass as determined by radioimmunoassay is indicated. Levels shown represent geometric means and S.E. for at least three determinations of each sample. NMS, pooled BALB/cJ nonimmune IgG; BGG, geometric mean of values obtained by using six individual A/J anti-BGG sera; OVA, geometric mean of values obtained by using five individual A/J anti-OVA sera; GAC 1, geometric mean of values obtained by using 12 individual A/J anti-GAC sera; GAC 2, geometric mean of values obtained by using seven individual BALB/c anti-GAC sera; GPC, anti-group C streptococcal IgG was purified from pooled antisera raised in six SWR mice; PC 1, anti-PC IgG was purified from pooled antisera raised in eight AKR mice; PC 2, anti-PC IgG was purified from pooled antisera raised in 12 A/J mice; Dex 1, geometric means of values obtained by using six individual BALB/c anti-α-(1→3) dextran sera; Dex 2, geometric means of values obtained by using six individual 129/J anti-α-(1→3) dextran sera.

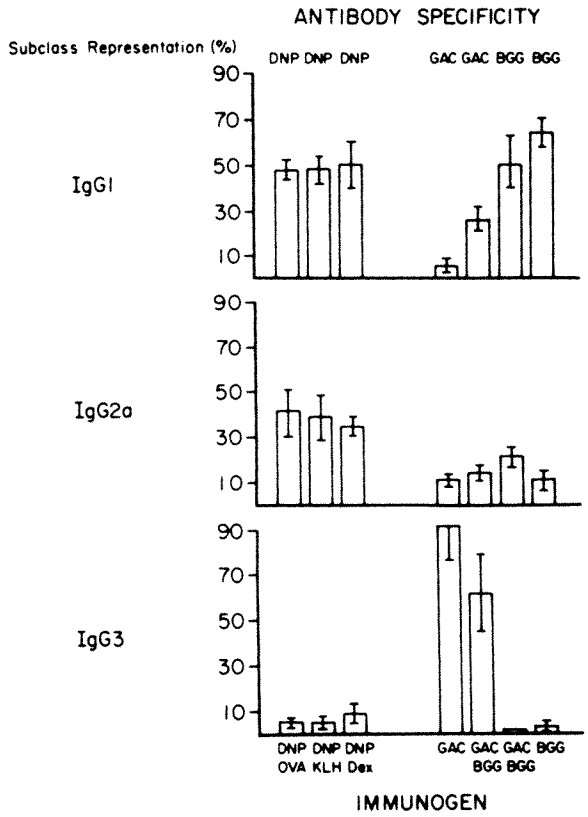

Figure 3. Effect of carrier substitution on subclass selection. Shown for each antigen-specific antibody fraction is the percentage of immunoreactive material which is attributable to IgG1, IgG2a or IgG3 isotypes. IgG2b levels were less than 10% in all cases. Each bar represents the geometric mean and S.E. for at least three determinations of each sample. DNP-OVA, purified anti-DNP antibodies were obtained from pooled antisera raised in five BALB/cByJ mice immunized with DNP-OVA. DNP-KLH, purified anti-DNP antibodies were obtained from pooled antisera raised in five BALB/cByJ mice immunized with DNP-KLH. DNP-DEX, purified anti-DNP antibodies were obtained from pooled antisera raised in five BALB/cByJ mice immunized with DNP-α-(1→3) dextran. GAC, geometric mean of values obtained by using 12 individual A/J anti-GAC sera. GAC-BGG, geometric mean of values obtained by using GAC-specific IgG from five individual A/J mice immunized with GAC-BGG. BGG-GAC, geometric mean of values obtained by using BGG-specific antibodies from five individual A/J mice immunized with GAC-BGG; BGG, geometric mean of values obtained by using six individual A/J anti-BGG sera.

We have also examined the effect of coupling GAC to BGG on the isotype distributions of anti-BGG antibodies in A/J mice. Figure 3 compares subclass compositions of A/J anti-BGG antibodies and A/J antibodies with specificity for BGG induced in this case with GAC-BGG. The majority of these antibodies belong to the IgG1 subclass and linkage of streptococcal carbohydrate to BGG does not appear to favor the stimulation of IgG3 BGG-specific antibody-forming cells.

DISCUSSION

Selective expression of individual IgG subclasses in response to a number of antigens including proteins, carbohydrates, membranes, and viruses (4–7, 16) is a well documented feature of human immunity. This is perhaps surprising in that considerable serologic (9) and structural (8, 10) evidence has been

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presented in support of the proposition that multiple C_H genes share a common V_H gene pool. Thus the regulation of subclass expression has been attributed to T cell function (11) or some aspect of antigen presentation (12, 13).

In this paper we report that mouse IgG antibodies directed against groups A and C streptococci, α-(1→3) dextrans, and phosphocholine are in the main restricted to the mouse IgG3 subclass. This finding is of particular interest in light of the low concentration, less than 0.1 mg per ml, of IgG3 in normal mouse serum (15). Recruitment of the rare IgG3 subclass by these antigens suggests that this constant region may possess unique biologic properties perhaps relevant to bacterial immunity. Consistent with this speculation, IgG3 has been reported to cross the mouse placenta six times more readily than other mouse subclasses (15). Although there exist reports of murine anti-streptococcal antibodies of the IgG2a subclass (34, 35), and of anti-pneumococcal antibodies of the IgG1 subclass (36), the presence of IgG3 was not specifically excluded in these studies.

Interestingly, mice are not the sole species in which carbohydrate antigens appear to elicit an IgG response restricted to a single subclass. Human antibodies to carbohydrates (4, 16) have been reported to be restricted mainly to the human IgG2 subclass. Leslie (17) observed that rat anti-GAC antibodies are confined predominantly to the rat IgG2c subclass and we have found (manuscript in preparation) that rat anti-PC antibodies are also restricted to the IgG2c subclass. Restriction of equine anti-pneumococcal antibodies to a single subclass of immunoglobulin has also been reported (18).

In each of these species, carbohydrate antigens appear to stimulate an IgG response largely confined to a single isotype. It is conceivable that this subclass selection reflects constant region similarities that transcend species differences. Equine-aggregating immunoglobulin (18), rat IgG2c (37), and mouse IgG3 (15) share in common the tendency to form noncovalent aggregates. Although IgG3 Fc fragments do not appear to bind complement (C) (15), IgG3 anti-GAC antibody does fix C poorly (38), as do human IgG2 (39) and rat IgG2c (unpublished observation). Neither human IgG2 nor mouse IgG3 is cytophilic and both bind staphylococcal protein A (3).

Amino acid sequence analysis of immunoglobulin constant regions has revealed that human subclasses diverged relatively recently in evolutionary time, perhaps since speciation (40). Mouse subclasses, however, are less closely related (41). In particular, mouse IgG1 appears to share more sequence homology with human subclasses than with mouse IgG2a (42). Thus it remains plausible that the primordial IgG3-like sequence arose relatively early in evolution and has persisted in diverse phylogenetic backgrounds. Thus far no sequence studies of mouse IgG3 constant regions have been reported, but it is interesting to note that human IgG2, which we propose as a functional analogue of mouse IgG3, appears by antigenic criteria to be the most primitive human subclass (43).

In Table I we list the known 7S immunoglobulin subclasses in horse, man, mouse, and rat and the tentative IgG3 analogue in each species. It is of interest that guinea pigs, which respond poorly if at all to group A streptococci, dextrans, and pneumococcal polysaccharide SIII (44, and unpublished observations) and which are especially susceptible to streptococcal infections (45), appear to lack an IgG3-like subclass.

Recruitment of a minor immunoglobulin isotype by carbohydrate antigens in four widely divergent species supports the concept of immunoregulation at the level of antibody subclass. In an attempt to analyze the mechanism underlying this subclass selection we compared the isotype distributions of anti-hapten antibodies induced with hapten-protein and hapten-carbohydrate conjugates; and we also examined the isotype restriction of anti-carbohydrate antibodies induced by immunization with a carbohydrate-protein conjugate. As shown in Figure 3, anti-DNP antibodies are primarily composed of IgG1 and IgG2a subclasses even if the DNP is presented on an α-(1→3) dextran carrier known to induce a predominantly IgG3 anti-carbohydrate response. Similarly, coupling of GAC to BGG did not in any way alter the subclass distribution of anti-BGG antibodies. Immunization of A/J mice with GAC-BGG did, however, result in the production of an increased proportion of IgG1 anti-GAC antibodies (Fig. 3) although the majority of anti-GAC antibodies induced by this immunization protocol are IgG3. Examination of the variable region repertoire of anti-GAC antibodies induced by GAC-BGG with an antiserum that detects variable region determinants common to most A/J anti-GAC (vaccine) antibodies showed that the carbohydrate-pro-

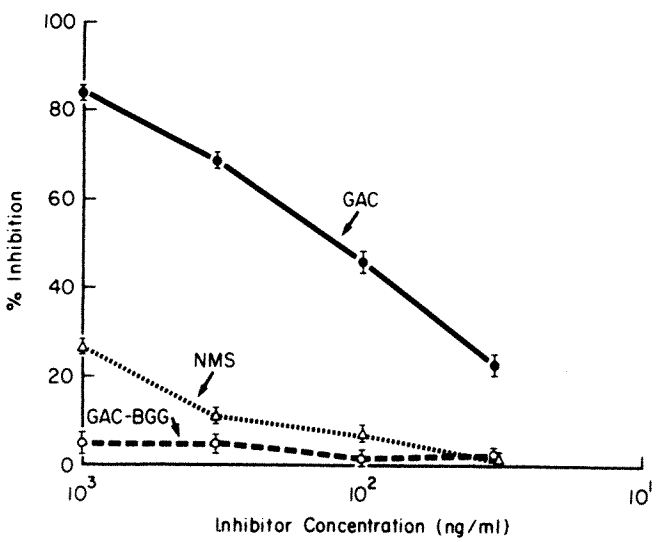

Figure 4. Idiotypic nonidentity of NAG-specific antibodies raised by immunization of A/J mice with group A streptococcal vaccine or GAC-BGG. Shown are the inhibition profiles for NAG-specific antibodies by using a guinea pig anti-idiotypic serum with purified A/J anti-GAC ^{125}I-antibody as proband. GAC, geometric means of values obtained by using eight individual A/J anti-group A streptococcal vaccine antibody preparations; GAC-BGG, geometric means of values obtained by using five individual A/J anti-GAC-BGG antibody preparations; NMS, non-immune A/J IgG from a 50% $(NH_4)_2SO_4$ precipitate of serum.

TABLE I

Species comparison of subclass preference of anti-carbohydrate antibodies

Species	IgG Subclasses	Predominant Subclass of Anti-CHO Antibody	% of Normal IgG	Reference
Man	IgG1, IgG2, IgG3, IgG4	IgG2	23	4, 16
Mouse	IgG1, IgG2a, IgG2b, IgG3	IgG3	2	This paper
Rat	IgG1, IgG2a, IgG2b, IgG2c	IgG2c	16	17, 37
Horse	IgGa, IgGb, IgGc, IgG1 and IgG(T)	IgG1 (?)	? small	18

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tein conjugate apparently recruits a distinct set of antibody-combining sites. This phenomenon may reflect modification of the group-A carbohydrate determinants resulting from the harsh extraction procedure (which includes formamide treatment, 46) or the coupling of the cyanogen bromide-modified carbohydrate to BGG. Experiments designed to elucidate the differences between group-A streptococcal vaccine and GAC-BGG antigens are currently underway.

The major conclusion from our investigations thus far is that subclass-restriction appears to be mediated by the specific moiety that interacts directly with the antibody-combining site. We must therefore consider the possibility that the V_H gene repertoire is not shared equally by all IgG subclasses (47). Although considerable evidence exists that IgM, IgG, and IgA classes frequently share V_H region structure (8, 10) and that the switch from IgM to IgG synthesis in normal immune response ontogeny solely involves alteration of C_H expression (9, 19), few studies of shared V_H determinants associated with multiple IgG subclasses have been reported. Production of IgG1 and IgG2a anti-D-alanine antibodies by x-irradiated mice repopulated with limiting dilutions of immunocompetent cells has suggested a monoclonal origin for these two IgG subclasses (48), but the IgG2a levels were exceedingly low, infrequently above background and possibly due to the presence of a contaminating second clone. Anti-DNP antibodies secreted in a monoclonal splenic-focus assay system and composed of IgG1 and IgG2 subclasses have also been observed (49). Considerably more information will be required to correctly assess the sharing of V_H regions by multiple IgG subclasses; however, we have thus far been unable to elicit the production of IgG1, IgG2a, or IgG2b antibodies with antigens that commonly elicit IgG3 responses without altering the V_H expression as well.

In considering the problem of antigen-directed IgG subclass selection we were impressed by recent reports characterizing the immune defect of CBA/N mice (50–52). These animals display an X-linked abnormality resulting in decreased or absent responses to many, but not all, thymus-independent antigens. In particular CBA/N mice fail to respond to a variety of carbohydrate antigens including LPS (50), SIII pneumococcal polysaccharide (50), dextrans (52) and PC regardless of carrier association (51, 52). Murine responses to LPS have previously been reported to be associated with IgG3 (53) and we have presented data indicating that dextrans and PC also elicit IgG responses restricted to the IgG3 subclass. Failure of PC to stimulate an immune response in the CBA/N mouse even when coupled to an otherwise immunogenic carrier is consistent with our notion of subclass selection determined by the haptenic moiety at the level of the antibody-forming cell precursor. CBA/N mice also apparently lack a mature subset of B lymphocytes as judged by the density of surface immunoglobulin (54) or the presence of cell-surface determinants defined by alloantisera (55, 56). This is of interest particularly since IgG3 is the major IgG subclass represented on the splenic B cell surface (57) just as IgG2 is the major IgG subclass present on human peripheral blood lymphocytes (58). It would be interesting to examine CBA/N mice for the presence of IgG3 in normal serum and for the ability of other carbohydrate antigens, particularly group-A streptococcal vaccine, to elicit an antibody response in defective individuals.

We have presented data that support the existence of a subclass-specific regulatory mechanism in the mouse activated by bacterial carbohydrate antigens. These data are in concert with previously reported examples of subclass-restricted antibodies to carbohydrates in humans (4, 16), rats (17), and horses (18). These observations may reflect the existence of a structurally similar subclass distributed across diverse phylogenetic backgrounds. Preliminary data suggest that subclass-selection is determined at the level of the antibody-forming cell precursor. These results imply that the variable region repertoires of different IgG subclasses in the mouse may be disjoint.

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