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REVIEW

Selective IgG subclass deficiency: quantification and clinical relevance

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SUMMARY

Each of the four human IgG subclasses exhibits a unique profile of effector functions relevant to the clearance and elimination of infecting microorganisms. The quantitative response within each IgG subclass varies with the nature of the antigen, its route of entry and, presumably, the form in which it is presented to the immune system. This results in antibody responses to certain antigens being predominantly or exclusively of a single IgG subclass. An inability to produce antibody of the optimally protective isotype can result in a selective immunodeficiency state. This is particularly apparent for responses to certain bacterial carbohydrate antigens that are normally of IgG2 isotype. A failure to produce the appropriate specific antibody response may result in recurrent upper and/or lower respiratory tract infection. Careful patient investigation can identify such deficiencies and suggest appropriate clinical management. In this review we outline the biology and clinical relevance of the IgG subclasses and summarize current rational treatment approaches.

Keywords IgG subclasses antibody deficiency capsulate bacterial infections

INTRODUCTION

The five human immunoglobulin classes, and subsequently the subclasses of IgG and IgA, were originally defined by the antigenic uniqueness of their heavy chains (Ballieux *et al.*, 1964; Terry & Fahey, 1964; Grey & Kunkel, 1964; World Health Organization, 1966). It is now established that the nine heavy chain isotypes are products of discrete constant region genes encoded within the heavy chain locus of chromosome 14 and that each may be expressed with rearranged variable region genes to yield a single polypeptide chain (Honjo, 1983; Rathbun *et al.*, 1989). The basic immunoglobulin molecule is a four-chain structure composed of two identical heavy chains and two identical light chains. This is a consequence of the fact that each individual plasma cell makes antibody molecules of a single specificity. The light chains may be of kappa or lambda type (encoded on chromosomes 2 and 22, respectively) but an individual immunoglobulin molecule has either two kappa chains of identical amino acid sequence or two lambda chains each of identical sequence.

Each immunoglobulin isotype exhibits a unique profile of effector functions (Table 1) and a given isotype may predominate in an antibody response to a given antigen within a defined local environment, e.g. IgM responses to blood group antigens and IgA responses at mucosal surfaces. IgG provides comprehensive systemic immune protection since it is the predominant isotype present in blood and it readily equilibrates with extravascular fluids. An inability to produce antibodies within any given isotype may result in immunodeficiency with characteristic clinical manifestations. The consequences of selective IgG subclass deficiency has only recently been fully appreciated, as commercial reagents have become available to the clinical laboratory. It is our purpose to review briefly our understanding of the functional role of the IgG subclasses, and the possible clinical consequences of selective IgG subclass deficiencies. Comprehensive cover of this field is provided by Shakib (1986), Hanson, Soderstrom & Oxelius (1986), Skvaril, Morell & Perret (1988). Other brief reviews of value are provided by Hamilton (1987) and Madassery *et al.* (1988).

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NORMAL DEVELOPMENT AND EXPRESSION OF IgG SUBCLASSES

Total serum IgG levels may vary considerably between healthy adults, but the proportion of each subclass is maintained within a relatively narrow range: IgG1, 60–65%; IgG2, 20–25%; IgG3, 5–10%; IgG4, 3–6% (French & Harrison, 1984; French, 1986a, 1986b). The incidence of paraproteins of each subclass in multiple myeloma is similar to that observed for serum, suggesting that IgG in the blood reflects antibody synthesis in the bone marrow (Jefferis, 1988). However, there is evidence to suggest that the proportions of each subclass produced following antigenic stimulation at local sites may differ, due to selective

Table 1. Biological properties of human immunoglobulins

Isotype	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM	IgD	IgE
C1 activation	++	+	+++	−	−	−	+++	−	−
Placental transfer	+	+	+	+	−	−	−	−	−
Mucosal transfer	−	−	−	−	+	+	+	−	−
Staphylococcus Protein A binding	+	+	−*	+	−	−	−	−	−
Steptococcus Protein G binding	+	+	+	+	−	−	−	−	−
Fc receptor binding									
FcγRI (monocytes)	+++	−	+++	++	−	−	−	−	−
FcγRII (monocytes, neutrophils, eosinophils, platelets, B lymphocytes)	+	−	+	−	−	−	−	−	−
FcγRIII (neutrophils, eosinophils, macrophages, LGL, NK, T lymphocytes)	+	−	+	−	−	−	−	−	−
FcεRI (mast cells, basophils)	−	−	−	−	−	−	−	−	+++
FcεRII (monocytes, platelets, neutrophils, B & T lymphocytes, eosinophils)	−	−	−	−	−	−	−	−	++
FcαR (monocytes, neutrophils, eosinophils, T & B lymphocytes)	−	−	−	−	+	+	−	−	−
FcμR (T lymphocytes, macrophages)	−	−	−	−	−	−	+	−	−
FcδR (T & B lymphocytes)	−	−	−	−	−	−	−	+	−

* Dependent on IgG3 allotype.
LGL, large granular lymphocytes; NK, natural killer.

localization of B lymphocyte subsets and/or selective stimulation of B cell subsets by factors present in the local environment. Thus, while IgG1- and IgG2-bearing lymphocytes predominate in the blood, IgG1 and IgG3 lymphocytes predominate within tonsil lymphocytes (Mayumi, Kuritani & Cooper, 1983; Partridge *et al.*, 1984). Studies *in vitro* show that interleukins can modulate or regulate IgG subclass production (Flores-Romo *et al.*, 1990; Ishizaka *et al.*, 1990).

The human infant is not immunocompetent at birth and is protected from infection by maternal IgG transferred across the placenta by an active transport mechanism. This can result in total IgG levels in fetal and cord blood being higher than in the maternal circulation, in large part due to selective transfer of IgG1 yielding a fetal/maternal ratio >1; the corresponding ratio for IgG2 is 0·7 (many authors quote an early review that claimed IgG2 does not cross the placenta; this ignores many more recent reports demonstrating IgG2 in cord and new-born blood). However, these ratios may be significantly different for premature infants of low birth weight (low-weight-for-date) (Chandra, 1986). Expansion of infant vascular and extravascular fluid volumes, with growth, and catabolism of maternal IgG results in a steady decline in serum IgG levels over the first 6 months of life; therafter, a slow increase in serum IgG levels is observed with 70% of adult levels being reached by the end of the second year of life; adult levels are achieved by 10–12 years of age (Oxelius, 1979; French 1986a, 1986b). Each IgG subclass has an individual pattern of development with IgG1 and IgG3 attaining adult levels at an earlier age than IgG2 and IgG4. It is essential, therefore, that when investigating children for possible IgG subclass deficiencies the quantitative values obtained are evaluated against the normal range for a cohort of children within the appropriate age range. Several groups of investigators have published tables of values; however, there is a lack of concordance because of differences in reagents and assay protocols used (Morell *et al.*, 1972; Oxelius, 1979; Schur, Rosen & Norman, 1979a; Lee, Heiner & Wara, 1986; Black *et al.*, 1988). National reference levels for the UK are published by the Supraregional Protein Reference Unit in their handbook titled PRU Handbook of Clinical Immunochemistry (Milford-Ward, 1988).

A further parameter of significance is the alleles of immunoglobulin genes that are present and expressed by an individual (de Lange, 1989). Thus, individuals homozygous for the G2m(n) allele have higher levels of IgG2 than do homozygous G2m(n)-negative individuals (Granoff *et al.*, 1988a); similarly, individuals homozygous for the G3m(b) allele have higher levels of IgG3 than do individuals homozygous for the G3m(g) allele. Heterozygous individuals have intermediate levels of these subclasses (Morell *et al.*, 1972). Since the frequency of immunoglobulin alleles varies between population groups, normal ranges will similarly vary. This may be true for specific antibody responses as well as total IgG subclass level (Whittingham & Probert, 1986).

SPECIFIC ANTIBODY RESPONSES

The proportions of each subclass (subclass profile) present within antigen specific IgG may differ substantially from the mean values for total IgG (Table 2). The broad generalization may be made that IgG responses to protein antigens are produced predominantly within IgG1 and IgG3 (Skvaril, 1986). Early reports claimed that human antibody responses to bacterial polysaccharide antigens comprised predominantly or exclusively of the IgG2 isotype (summarized by Hammarstrom & Smith, 1986a). However, more recent studies using highly purified polysaccharide antigens and improved assay protocols have revealed considerable heterogeneity in the subclass composition of the antibodies to *Haemophilus* b polyribose phosphate

Table 2. Sublcass profile of specific antibody responses

Antigen	IgG1	IgG2	IgG3	IgG4
Tetanus toxoid	+++	+	+	++
Polysaccharides	++	+++	+	(+)
Rhesus-D	+++	−	+++	−
Factor VIII	−	−	−	+++
Phospholipase A2	+++	+	+	+
Phospholipase A2*	+	+	+	+++

* Response in bee-keepers.

Table 3. Expression of human FcγR on effector cells

	FcγRI	FcγRII	FcγRIII
Monocytes	+	+	−
Macrophages	+	+	+
Neutrophils	−	+	+
Eosinophils	−	+	−
B cells	−	+	−
Platelets	−	+	−
NK, K cells	−	−	+
T cells	−	−	+

(Shackleford *et al.*, 1987; Makela *et al.*, 1987) and meningococcal (types A and C) capsular polysaccharides (Rautonen *et al.*, 1986) in healthy adults, considerable amounts of IgG1 antibodies are also produced against these antigens. Similarly, *Klebsiella* capsular polysaccharides (Skvaril, Cryz & Furer, 1985) and *Salmonella* lipopolysaccharides (Persson *et al.*, 1986) primarily induce IgG1 antibodies. In addition, there is evidence that chronic antigenic stimulation with protein antigens can lead to progressively increased proportions of IgG4 antibody. Since each IgG subclass has a distinct range of potential effector functions it will be apparent that the IgG subclass profile of a specific IgG antibody response will determine the clearance mechanisms activated and hence the efficacy with which an infection is controlled. The anti-Rhesus D response is revealing since it is restricted to IgG1 and IgG3 with the ratio between these two isotypes varying with the individual. The severity of haemolytic disease in the newborn is also variable and has been correlated with a predominance of IgG3 anti-Rhesus D antibody and an aggressive antibody-dependent cellular cytotoxicity (ADCC) (Urbaniak & Greiss, 1980). This illustrates what may become a more general finding, i.e. that although IgG1 and IgG3 have the same apparent range of effector functions there may be significant quantitative differences in their activities under a given set of conditions *in vitro* or *in vivo*. Thus, IgG3 is more efficient than IgG1, on a weight-for-weight basis, at activating complement. Additionally, there is recent evidence demonstrating quantitative differences in effector function between IgG molecules of the same subclass but of differing allotype (Bruggemann *et al.*, 1987).

An example of isotype switch following chronic antigenic stimulation is the response to phospholipase A2 present in the sting of a bee. Persons who have been occasionally stung produce predominantly IgG1 antibodies to this protein antigen, whereas beekeepers, who are habitually stung, may produce predominantly IgG4 antibodies. It will be apparent, therefore, that the IgG subclass profile of a specific antibody response may determine its effectiveness and that selective IgG subclass deficiency states may compromise the host.

Considerable progress has been made in recent years in defining the molecular structure, subclass specificity and tissue distribution of human Fcγ receptors (FcγR) (Table 1 and Table 3; reviewed by Pound & Walker, 1990). Appropriate activation of effector cells can result in the generation and/or release of reactive oxygen species, phagocytosis or killing through ADCC mechanisms. These studies have generally used antibody populations of a single isotype but are now being extended to mixtures of antibody isotypes observed in natural antibody responses (Walker *et al.*, 1988, 1989; Hadley & Kumpel, 1989). Complement-mediated inflammatory reactions have been widely studied, and the general conclusion has been that IgG1 and IgG3 activate C1 efficiently while IgG2 does so only weakly and IgG4 is inactive. Unfortunately we cannot necessarily extrapolate from these *in vitro* studies to *in vivo* protection, because most experimental systems have employed guinea pig or rabbit C1. Recent studies have provided further evidence that IgG2 and IgG4 do not activate human C1 (Bruggemann *et al.*, 1987; Dangl *et al.*, 1988). Hence the paradox: what are the properties of IgG2 that make it the protective antibody isotype for certain pathogenic organisms?

SELECTIVE IgG SUBCLASS DEFICIENCY

This field originates from the report of Schur *et al.* (1970) of an association between selective IgG2 deficiency and susceptibility to recurrent pyogenic infection. Subsequently it was documented that patients with selective IgA deficiency, who were also IgG2 deficient, developed recurrent respiratory infections and lung damage (Oxelius *et al.*, 1981). Since then, large numbers of patients with selective IgG subclass deficiencies have been reported (reviewed by Oxelius, 1984; Hammarstrom & Smith, 1986b; Schur, 1988). It has been suggested that IgG subclass deficiency may be one of the commonest forms of antibody deficiency; however, precise estimates of the prevalence and clinical significance are unavailable (Hanson *et al.*, 1986; Goldblatt *et al.*, 1989). Many clinical associations of IgG subclass deficiencies have been claimed (Table 4); however, the association of selective IgG2 deficiency with increased susceptibility to infection with capsulated bacterial pathogens (e.g. *Streptococcus pneumoniae*) is the only clearly defined clinical entity (Second IUIS/WHO Report, 1988). Presently we may define these deficiencies according to three quantitative criteria: (i) the complete absence of one or more subclasses; (ii) levels for one or more subclasses that are below the normal range for age matched controls. Since immunoglobulin levels do not conform to a Gaussian distribution, the use of log-transformed data or 5th percentiles for establishing the limits of normal ranges are to be preferred (Soderstrom *et al.*, 1989); and (iii) an inability to produce specific antibodies within a given subclass; although the total level for the subclass may be within the normal range.

The definition of a quantitative defect and its correlation with clinical status is often problematic but considerable clinical experience is now reported (see below); in addition, it must be remembered that Gm haplotypes influence IgG subclass levels (Morell *et al.*, 1972; de Lange, 1989). For example, individuals

Table 4. Association of abnormal serum IgG subclass levels and disease

Infections	Change in IgG subclass	Reference
Recurrent infections with capsulated bacteria	↓IgG2/IgG4	Oxelius (1984); Hammarstrom & Smith (1986a); Shackelford *et al.* (1990b)
Recurrent sinopulmonary infections, Bronchiectasis	↓IgG2/IgG3/IgG4	Bjorkander *et al.* (1985); Umetsu, Ambrosino & Geha (1985)
Otitis media in children	↓IgG2 antibodies to pneumococcus	Freijd *et al.* (1984); Goldblatt *et al.* (1989)
Cystic fibrosis with chronic *P. aeruginosa* infection	↑IgG2	Fick *et al.* (1986)
Mothers of children with group B streptococcal sepsis	↓IgG1,2,3	Oxelius *et al.* (1983)
AIDS	Variable IgG subclass deficiences	Aucouturier *et al.* (1986) Parkin (1988)
Gastro-intestinal symptoms		
Diarrhoea, failure to thrive, food intolerance	Variable subclass deficiencies	Bremard-Oury *et al.* (1986)
Allergic disease		
Bronchial asthma	↓IgG2,3,4	Oxelius (1984); Page *et al.* (1988)
Atopic eczema, dermatitis	↑IgG4	Merrett *et al.* (1984)
Vasculitis, including Henoch–Schönlein purpura	Variable subclass deficiencies	Bremaar-Oury *et al.* (1986)
Autoimmune diseases	↓IgG2	Oxelius (1984);
(diabetes melitus type I)	↓IgG3	Oxelius *et al.* (1986)
Neurological disorders		
Treatment refractory epilepsy	↓IgG2	Arizumi Shiihara & Hibio (1983); Duse *et al.* (1986)
Friedreich's ataxia	↓IgG3	Oxelius *et al.* (1986)
Ataxia telangiectasia	↓IgG2/IgG4	Revat-Peran, Buriot & Salier (1981)
Miscellaneous immunodeficiencies		
Inherited deficiency of classical complement, pathway components	↓IgG4	Bird & Lachmann (1987)
C3 deficiency	↓IgG2	Bird & Lachmann (1987)
IgA deficiency	↓IgG2/IgG4/IgG3	Oxelius *et al.* (1981)
Post bone marrow transplantation	variable IgG subclass deficiencies	Preud'homme *et al.* (1988)
Adenosine deaminase defiency	↓IgG2	Levy *et al.* (1988)

positive for the IgG3 allotype G3m(b) have higher serum IgG3 levels than those positive for the G3m(g) allotypic marker (Morell *et al.*, 1972). It is important to appreciate that isolated and combined IgG subclass deficiencies, defined by the above criteria, may be revealed among healthy individuals attending blood donor clinics. This underlines the fact that there are many overlapping components to immune protection and a partial deficit in one limb may be compensated for in another; however, when multiple partial defects occur the individual may be compromised and disease susceptibility may manifest itself in an episodic manner.

While quantification of IgG subclasses may reveal deficiencies not apparent when total IgG levels are evaluated, it has also been shown that individuals with normal levels of a given IgG subclass may at the same time lack the ability to produce a specific antibody response within that subclass. Such deficiencies have been well documented (Geha, 1988), particularly for IgG2 subclass deficient responses to capsular polysaccharides of organisms commonly causing respiratory tract infections. Selective deficiencies of IgG2 antibody responses to pneumococcal polysaccharide has been reported in otitis-prone children (Freijd *et al.*, 1984). Investigation of patients with recurrent infections might include, therefore, a semi-quantitative determination of the IgG subclass distribution of antibodies specific for the causative microorganisms.

CLINICAL SIGNIFICANCE OF IgG SUBCLASS DEFICIENCY

The precise significance attached to a demonstration of low serum IgG subclass levels depends on the criteria used for establishing normal bounds. For example, in a large Swedish study examining the serum IgG subclass levels of 6580 patients with increased susceptibility to infection, the normal ranges were derived from a group of 40 adults and 182 children stratified by age (Oxelius *et al.*, 1986). Individuals who had IgG subclass levels 2 s.d. below age group mean were classified as deficient. On a statistical basis, this would mean that one in 20 normal individuals would be outside the normal bounds of this small sample of normal subjects. Using these criteria, 313 patients were classified as IgG3 deficient; a ratio of 0·95: 20, which is almost exactly the number of individuals who would be expected to be 2 s.d. below the control group mean, merely by chance. A history of recurrent upper and lower respiratory tract infections, bronchial asthma or obstructive airways disease was reported in most of these IgG3-deficient patients. However,

Table 5. Suggested modified Koch postulates for determining causal link between IgG subclass deficiencies and associated clinico-pathological changes

1. Deficiency must be consistently associated with given pathological condition
2. Clinical problem should be resolved by correcting defect or replacement therapy
3. It should be possible to explain clinical association according to the known or experimentally determined physiological properties of the relevant IgG isotype
4. Induction of similar deficiencies in animal experimental models should result in analogous pathological changes

* Editorial (1884)

since the patient group was selected for a history of recurrent infection, the exact significance of the association reported in this study is difficult to determine. Furthermore, association does not necessarily imply a causal link.

A similar situation exists in relation to IgG4 deficiency; a substantial minority of normal persons have no serum IgG4 demonstrable by radial immunodiffusion (RID) (French & Harrison, 1984) or even solid-phase competitive ELISA (Papadea, Check & Reimer, 1985; Hamilton, 1987). Hence, in spite of claims to the contrary (Heiner, Myers & Beck, 1983), selective deficiency of IgG4 alone is difficult to interpret as abnormal. Heiner, Lee & Short (1986) claim that using a sensitive radioimmunoassay, patients with selective IgG4 deficiency can be differentiated; this is said to correlate with occurrence of severe bacterial infections and limited clinical improvement was reported after immunoglobulin or plasma replacement therapy.

The above uncertainties will only be resolved by: (i) relating IgG subclass estimation in patients to age-stratified normal ranges (3rd or 5th percentiles) derived from large population-based studies carried out by standardized methodology (Hamilton, 1987; French, 1986b). Age-stratified normal ranges for the UK have been published by the National Supraregional Protein Reference Unit using the 5th percentile as the lower limit (Milford-Ward, 1988); and (ii) application of modified Koch Postulates (Koch, 1884) to clarify a casual link between subclass deficiencies and associated conditions (Table 5).

IgG1 is the main IgG isotype in serum; hence IgG1 deficiency is usually reflected in a reduction in the total level of serum IgG. Low IgG1 levels (often in association with reduction in other immunoglobulin isotypes) is seen in a variety of primary and secondary antibody deficiencies (Aucouturier *et al.*, 1986; Wedgewood, Ochs & Oxelius, 1986). IgG3 deficiency occurs most often in adults, and IgG2 deficiency is commonest in children. IgG2 deficiency is often associated with low IgG4 levels and selective IgA deficiency, probably reflecting the close linkage of these heavy chain genes. The clinical associations of IgG2 deficiency are discussed in detail below.

Observations in support of a special role of IgG2 antibodies in providing protection from bacterial infections

IgG2 is one of the main immunoglobulin isotypes induced by carbohydrate antigens like pneumococcal polysaccharide in adults, while protein antigens induce predominantly IgG1 and/or IgG3 antibodies (Hammarstrom & Smith, 1986a).

IgG2 levels are slow to mature during childhood (Schur *et al.*, 1979a); adult levels are reached only by 10–12 years of age (van der Giessen *et al.*, 1975; French, 1986b). This corresponds with the slow ontogeny of anti-polysaccharide antibody responses in children (Peltola *et al.*, 1977; Borgano *et al.*, 1978; Cowan *et al.*, 1978; Schur, Rosen & Norman, 1979b; Granoff, 1980; Freijd *et al.*, 1984; Austrian, 1989) and their susceptibility to invasive disease caused by high-grade capsulated pathogens like *Haemophilus influenzae* type b (Gold, 1985; Austrian, 1989).

Patients who are selectively deficient in IgA and IgG2 appear to be especially prone to recurrent sepsis caused by capsulated bacteria, while IgA-deficient individuals who are asymptomatic have normal serum IgG2 levels (Oxelius *et al.*, 1981; Stanley, Corbo & Cole, 1984; Hammarstrom, Persson & Smith, 1985). Furthermore, a subset of IgA-deficient patients with normal serum IgG2 levels but deficient IgG2 responses to pneumococcal polysaccharides appears to be highly susceptible to pneumococcal sepsis (Lane & MacLennan, 1986). This association of low serum IgG2 levels with recurrent upper and lower respiratory tract infection, otitis media, impaired lung functions and invasive diseases caused by *S. pneumoniae* and *H. influenzae* type b has been documented extensively and independently in children and adults (Siber *et al.*, 1980; Frejid *et al.*, 1984; Matter *et al.*, 1985; Bjorkander *et al.*, 1985; Shackelford *et al.*, 1985).

Of Caucasians who are positive for the G2m(n) allotypic marker, 60–70% have higher serum IgG2 levels and higher antibody responses to *H. influenzae* type b polyribose phosphate (Hib-prp) and pneumococcal and meningococcal capsular polysaccharides, respectively, especially of the IgG2 subclass (Ambrosino *et al.*, 1985; Granoff *et al.*, 1988a). G2m(n)-negative individuals have a seven-fold increased risk of vaccine failure (Granoff & Munson, 1986) following administration of Hib-prp vaccine. In some (Ambrosino *et al.*, 1985), but not all (Granoff & Munson, 1986) studies the possession of the G2m(n) phenotype in Caucasians was associated with a decreased risk of invasive *H. influenzae* type b disease in infancy. Black children positive for the Km1 allotypic marker of the kappa light chain have higher concentration of IgG2 isotype in their serum, as well as higher IgG antibody to Hib-prp vaccine. This observation has been related to the decreased risk of invasive *H. influenzae* type b disease in Black subjects but not in Caucasians (Granoff *et al.*, 1984).

Taken together, the above data provide circumstantial evidence that IgG2 anti-capsular antibodies are pre-eminent in protecting against bacterial sepsis.

Observations contradicting this hypothesis

Healthy individuals with inherited selective IgG2 deficiency due to deletions of the $\gamma 2$ heavy chain constant region gene have been described (LeFranc, LeFranc & Rabbits, 1982; Mignone *et al.*, 1984; Hammarstrom & Smith, 1984).

A recent report documents 11 children out of a cohort of 575 healthy children with serum IgG2 concentration, 2 s.d. lower than the mean for age, who showed no evidence of increased susceptibility to infection during a follow-up period ranging from 1 to 5 years. The degree of IgG2 subclass deficiency in these asymptomatic children was as great as that observed in infection-prone children with IgG2 deficiency associated with poor specific antibody responses (Shackleford *et al.*, 1990a).

Impaired ability to respond to polysaccharide antigens, associated with increased susceptibility to bacterial sepsis, has

been described in several patients with normal serum concentrations of all immunoglobulin isotypes (Ambrosino *et al.*, 1987, 1988; Geha, 1988; Knutsen, 1989). A particularly interesting study was that documenting children who developed invasive *H. influenzae* type b disease despite vaccination with Hib-prp. All but three of 34 children had normal serum immunoglobulin levels (including IgG2) and were otherwise healthy but had defective antibody responses to Hib-prp during convalescence and following re-immunization with Hib-prp vaccine (Granoff *et al.*, 1986b, 1986c).

Many studies have revealed considerable heterogeneity in the subclass composition of anti-polysaccharide antibodies in subclass composition of anti-polysaccharide antibodies in healthy adults; considerable amounts of IgG1 antibodies are produced against antigens like Hib-prp and meningococcal A and C polysaccharides (Shackleford *et al.*, 1987; Makela *et al.*, 1987; Rynnel-Dagoo, Freijd & Prellner, 1985; Rautonen *et al.*, 1986).

Available data (see above) do not suggest that the IgG2 isotype is more efficient in inducing complement-mediated killing or phagocyte mediated clearance of capsulated bacteria. IgG1 is more efficient on a molar basis at initiating the generation of the classical pathway C3 convertase, and binds more efficiently to Fc receptors on mononuclear phagocytes and neutrophils. Recent data show that IgG1 antibodies are 100–1000-fold more efficient than the IgG2 isotype at inducing human complement-mediated killing of *H. influenzae* type b (Amir, Liang & Granoff, 1990). However, sera from normal individuals producing predominantly IgG2 anti Hib-prp antibodies can induce effective complement-mediated or phagocyte-mediated bacterial killing, suggesting that factors other than isotype composition (e.g. avidity) may influence the functional efficacy of anti-capsular antibodies (Weinberg *et al.*, 1986; Amir *et al.*, 1990).

Taken together, the above observations make it unlikely that IgG2 anti-carbohydrate antibodies possess unique *in vivo* bactericidal potential due to preferential utilization of V region genes endowing higher affinity for antigen, as postulated by Hammarstrom *et al.* (1986). The association of susceptibility to infection and IgG subclass deficiency is also unlikely to be entirely explained by an inability to produce antibodies of the IgG2 isotype. Hence the primary defect may be an inability to respond to carbohydrate antigens (cf. the defect of CBA/n mice) and a low serum IgG2 level is a secondary concomitant serving as an indicator of such an abnormality.

THE MANAGEMENT OF PATIENTS WITH IgG SUBCLASS DEFICIENCY

Who should be investigated?

Serum IgG subclasses should be measured in patients developing recurrent pyogenic infection especially with high-grade capsulated bacteria, even if total immunoglobulin levels are normal. Recurrent sinopulmonary infections with or without airway obstruction appear to be the commonest mode of presentation (Oxelius, 1984; Second IUIS/WHO report, 1988; Bird, 1989; Goldblatt *et al.*, 1989). Other clinical manifestations are summarized in Table 4.

Interpretation of low IgG subclass levels

This can be difficult, due to variations of IgG subclass concentrations with age and lack of universally standardized methods of assay. Most patients with a history of recurrent infection should have specific antibody levels to a panel of protein (e.g. tetanus and diphtheria toxoids) and polysaccharide antigens (e.g. pneumococcal and *H. influenzae* type b capsular polysaccharides) evaluated. If these levels are low, patients should be immunized and serological responses compared with those from age-matched control subjects. Since normal individuals, especially children, may have low natural antibody levels to Hib-prp, test immunization with the Hib-prp vaccine should be employed in all subjects above 2 years of age. Recent published data (Shackelford *et al.*, 1990a) and our own experience suggest that asymptomatic individuals with low IgG subclass levels have normal antibody responses to Hib-prp vaccine. More reports now suggest that specific antibody deficiency despite normal serum immunoglobulin and IgG subclass levels may define a substantial subset of patients with increased susceptibility to bacterial sepsis (see above).

Further management

This depends on the individual patient and extent of established organ damage. Conservative treatment comprising of early antibiotic treatment during infections, prophylactic antibiotic treatment in selected individuals and supportive symptomatic therapy (e.g. treatment of bronchial asthma, chest physiotherapy, middle ear drainage in patients with otitis media) should be the first line of treatment. In those individuals presenting primarily with invasive *H. influenzae* type b disease with no respiratory symptoms, immunization with Hib-prp protein conjugate vaccines may induce protective antibody levels (Granoff *et al.*, 1989). Upper and lower respiratory infections are due to non-typable strains of *H. influenzae* and will not be helped by immunization with Hib-prp vaccines. Subclass-deficient patients may require more than one dose of Hib-prp conjugate vaccines before they produce an adequate antibody response; this pattern of seroconversion is characteristic of young infants aged 6 months or less (Granoff *et al.*, 1989). Hence, protection following Hib-prp conjugate vaccines should not be assumed and serial antibody measurements to monitor adequacy of and persistence of humoral response would be prudent. Protein-conjugated vaccines to the *Pneumococcus* and *Neisseria meningitidis* types A, C, Y and W135 (Jordan, 1989; Anderson & Betts, 1989) are being evaluated and may become available within the next 5 years.

Several studies have reported symptomatic improvement of patients with IgG subclass deficiencies following immunoglobulin replacement therapy (Shackeford *et al.*, 1990b; Silk & Geha, 1987). However, additional data are required before immunoglobulin replacement therapy can be generally recommended for patients with selective subclass deficiency. Our own practice is to reserve immunoglobulin replacement therapy for patients with recurrent infection, low IgG subclass levels and failure of specific antibody responses, who have failed to improve following an energetic trial of conventional therapy including antibiotic prophylaxis as set out above. The results of a multi-centre, randomized, placebo-controlled, double-blind trial underway in the USA is awaited with much interest (Knutsen, 1989).

QUANTIFICATION OF IgG SUBCLASS LEVELS AND SPECIFIC ANTIBODY RESPONSES

The human IgG subclasses have been intensively studied in specialist laboratories for more than two decades, during which time their biological functions and clinical relevance has been established. Application of this knowledge to routine clinical investigations has only recently been possible, with the availability of IgG subclass-specific monoclonal antibodies. Previously, polyclonal reagents were produced following immunization of non-human mammalian species with purified IgG subclass protein. This results in the production of antibodies reactive with all or most subclass proteins and a minority population of antibodies specific for the subclass of the immunizing protein. This is an unavoidable consequence of the high sequence homology between the IgG subclass proteins. Such antisera require extensive absorption with other subclass paraproteins to render them specific for the target subclass. The resulting polyclonal reagents are usually of low titre and are not specific enough for application in very sensitive assay protocols; however, they have been widely and successfully applied to quantification using the single RID technique (the Mancini method) (Djurup & Weeke, 1986).

Hybridoma technology has been applied to the production of monoclonal antibodies specific for each of the IgG subclasses, and more recently most of the IgG allotypes. However, monoclonal antibodies are rarely universal reagents giving optimal titres and specificity in all assay protocols. It is essential, therefore, to take account of the assay restriction that has been documented (Jefferis *et al.*, 1985; Jefferis, 1986a, 1986b). The most available and reliable assay protocol for both polyclonal and monoclonal reagents remains the Mancini method. The standard protocol allows severe or total deficiency of IgG1, IgG2 and IgG3 to be revealed, but may not be sensitive enough to detect IgG4 levels at the lower end of the normal range. It is possible to increase the sensitivity of the technique but the extra steps necessary do not commend themselves to the routine laboratory. Many investigators have reported sensitive ELISA protocols employing monoclonal reagents (Papadea *et al.*, 1985; Hussain *et al.*, 1986; Bird *et al.*, 1987) and several commercial companies now market kits. A recent collaborative study demonstrate widely discrepant results when expert laboratories quantified subclass levels of a common panel of sera (M. Kemeny, personal communication).

This introduces the problem of reference sera for IgG subclass quantification. WHO reference sera for the quantification of total IgG levels have been available for more than two decades (World Health Organisation, 1966); however, values for the subclass levels have not been established in WHO-approved studies. An informal three-centre study has published consensus values for the WHO reference serum 67/97 and this is the best basis for standardization currently available (Klein *et al.*, 1985). Ampoules of 67/97 for calibration of 'in-house' reference sera are available on application to WHO Collatorating Centre for Human Immunoglobulin Subclasses, Building 1, Room 1354 DO3, CDC, Atlanta, GA 30333, USA. (Within the UK, reference serum SpS-O1, calibrated against 67/97, is available from PRU Central Antiserum Purchasing Unit, Royal Hallamshire Hospital, Sheffield, S10 2JF).

Investigations of specific antibody responses mostly employ capture assay protocols. Commonly the antigen is absorbed to the solid phase, exposed to the serum under investigation and the antibody isotypes revealed with enzyme labelled second antibody and substrate (Hammarstrom & Smith, 1986c; Skvaril, 1986; Hammarstrom & Smith, 1987). Absence of a response within a given subclass may be effectively revealed. However, estimates of the relative concentrations of each IgG subclass are too inexact for our needs in advancing understanding of IgG subclass production and regulation.

New vaccines and immunization protocols are being developed but their evaluation depends critically on our ability to quantify accurately the responses provoked (Granoff, Weinberg & Shackelford, 1988). Attempts at standardization depend on the availability of affinity-purified antibodies, of each of the four subclasses, specific for the antigen under investigation. This has been achieved for certain specificities (Seppala *et al.*, 1984; Parkes *et al.*, 1990) and protocols of general applicability have been developed (Bird *et al.*, 1984; Persson, 1987; Parkes *et al.*, 1990). These approaches are currently being applied to antibody responses to *H. influenzae*, *S. pneumoniae* and *N. meningitidis* in our laboratories.

CONCLUSION

During the last two decades a much better understanding has been achieved of the physical, chemical and effector functions of human immunoglobulin isotypes, including IgG subclasses. The molecular and genetic basis of the above differences have been largely characterized. The heterogeneity of isotype responses based on the type and duration of antigenic stimulus and host characteristics like age and genetic factors have been defined. Development of monoclonal antibody reagents and methods that allow precise quantitative analysis have been paralleled by identification of patients with selective subclass deficiencies, in some of whom the deficiency was masked by a normal IgG level. A further level of complexity is emerging with the twin realization that normal serum immunoglobulin isotype levels (including IgG subclass levels) may mask clinically significant deficiencies of specific antibody levels especially against carbohydrate antigens; and that individuals with IgG subclass deficiencies may have quantitatively normal specific antibody responses, and remain in good health.

The precise biological role of immunoglobulin isotypes *in vivo* and the evolutionary advantages of the quantitative and qualitative heterogeneity of antibody responses remain to be clarified. For example, we cannot currently explain why anti-carbohydrate antibodies of the IgG2 subclass are important in protecting the human host against capsulated bacterial infection. What is clearly required is a systematic correlation of the quantitative and qualitative heterogeneity of human antibody responses against a broad spectrum of antigens, with clinical and microbiological data in patients with infections.

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