

Immunologic compensation in a patient with a large IgH constant region deletion

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Background: Deficiencies of serum Ig of the IgG isotype typically predispose individuals to recurrent infections in some but not all cases. Patients with large deletions of the Ig heavy chain genes are free of recurrent and severe infections.

Objective: We sought to determine a mechanism of immunologic compensation that would possibly explain the reason for this patient's paucity of infection despite lacking several classes of serum Ig.

Methods: The patient is a 50-year-old white man. Serum Ig levels and specific antibody titers were measured by using various methods, including nephelometry, enzyme immunoassay, and radial immunodiffusion. The status of the Ig heavy chain genes was examined by means of Southern blotting of genomic DNA isolated from EBV-transformed B cells.

Results: The patient's serum lacked detectable IgG1, IgG2, IgG4, and IgA1 levels. Southern blot analysis demonstrated a large heavy chain constant (C) region gene deletion that included C γ 1, C α 1, ψ C γ , C γ 2, and C γ 4. Antibody responses to capsular pneumococcal and hemophilus polysaccharide antigens were essentially absent. However, IgG3 antibodies against the protein antigen tetanus toxoid were present. Relatively high antibody titers were found against pneumococcal surface proteins as well.

Conclusion: We conclude that our patient's relative freedom from serious infection may be as a result of production of IgG3 antibodies to pneumococcal capsular proteins. (J Allergy Clin Immunol 2001;107:1051-5.)

Key words: Immunoglobulin, gene, deletion, compensation, pneumococcus

Primary immunodeficiencies are a diverse group of disorders involving defects of the cellular, complement, phagocytic, and humoral host-defense limbs.¹⁻⁷ The most common defects involve the humoral limb^{8,9} and range from the complete absence of B cells (eg, X-linked agammaglobulinemia) to normal serum antibodies with

Abbreviations used

PsaA: Pneumococcal surface antigen A
PspA: Pneumococcal surface protein A
TPBS: Tween-PBS

poor qualitative responses to polysaccharides.^{10,11} Inadequate antibody titer or function, particularly involving the IgG1 and IgG2 subclasses, typically predisposes the host to recurrent and potentially life-threatening infections with polysaccharide-encapsulated organisms, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*.¹² In contrast to the usual clinical features of IgG subclass deficiencies are the unexpected reports of individuals with large deletions of their Ig heavy chain genes who experience no serious infection despite the absence of serum IgG1, IgG2, IgG4, and IgA1.¹³⁻¹⁷ A handful of these patients with this deletion have been described, and their lack of serious infections are only explained by undefined immunologic compensation.¹³⁻¹⁷ We describe one such patient whose IgG3 responses to *S pneumoniae* capsular proteins may provide an explanation for the general good health of this group of patients.

METHODS

Serum Ig

Serum IgG, IgM, and IgA levels were measured with the QM300 rate nephelometer (Kallestad Laboratories, Chaska, Minn), and serum IgE levels were measured with the Immulite Analyzer (Diagnostic Products Corporation, Los Angeles, Calif). The IgG subclasses were quantified with an mAb-based enzyme immunoassay by using standardized mAbs. The IgA subclasses were determined by means of radial immunodiffusion (The Binding Site, San Diego, Calif).

Serum antibody responses

The patient's responses to 12 pneumococcal capsular polysaccharide serotypes were determined with a microplate enzyme immunoassay by using capsular polysaccharide preparations obtained from the American Type Culture Collection (Rockville, Md). IgG antibodies to tetanus toxoid, diphtheria toxin, and *H influenzae* type b polysaccharide were determined by using microplate enzyme immunoassay methods.

Serum was stored at -80°C before use in direct ELISA. Microtiter 96-well plates (NUNC, Wiesbaden, Germany) were coated overnight at 4°C with 1 μ g/mL recombinant pneumococcal surface protein A (PspA)/Rx1 (amino acids 1-302, a gift from Dr Susan Hollingshead, University of Alabama at Birmingham, Ala), 1

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TABLE I. Serum Ig levels

Ig isotype	Patient	Control subject
IgG (mg/dL)	437	625-1319
IgA (mg/dL)	317	72-321
IgM (mg/dL)	216	44-247
IgE (IU/mL)	158	5-79
IgG1 (mg/dL)	<1	271-1010
IgG2 (mg/dL)	<1	104-570
IgG3 (mg/dL)	522	14-133
IgG4 (mg/dL)	<1	4-71
IgA1 (mg/dL)	1.2	57-321
IgA2 (mg/dL)	650	5-82

TABLE II. Specific antibody responses

Antibody specificity	Concentration	Reference range	Heavy chain isotype
Diphtheria toxin (IU/mL)	<0.2	>0.1	?
Tetanus toxoid (IU/mL)	1.2	>0.1	IgG3
<i>Haemophilus influenzae</i> b (μg/mL)	<0.4	>1.0	?
<i>Pneumococcus</i> (μg/mL)*			
1	<0.3	>1.8	ND
3	<0.3	>1.3	ND
4	0.3	>1.3	ND
6	0.3	>1.8	ND
8	4.3	>1.8	IgG3
9	0.5	>1.8	ND
12	<0.3	>1.3	ND
14	<0.3	>1.8	ND
19	<0.3	>1.3	ND
23	<0.3	>1.3	ND
51	1.3	>1.8	IgG3
56	<0.3	>1.8	ND

ND, Not detectable.

*Pneumococcal polysaccharide capsule serotypes.

TABLE III. Antibodies against pneumococcal cell surface proteins

	Anti-PspA, family 1	Anti-PsaA	Anti-pneumolysin
IgG	54.1 μg/mL (6.97× HSP5)	3.7× HSP5	7.8× HSP5
IgM	16.70× HSP5	16.1× HSP5	1.60× HSP5
IgA	0.12× HSP5	0.05× HSP5	0.17× HSP5

Assays of antibody activity against pneumococcal proteins were done in reference to a pool of human sera (HSP5). Until the serum pool is standardized, the antibody titer is defined relative to the concentration of antibody to each protein in HSP5. The HSP5 standard serum pool has been calibrated to contain 7.76 μg/mL anti-PspA IgG.

μg/mL recombinant pneumococcal surface antigen A (PsaA; a gift from Dr E. Ades, CDC, Atlanta, Ga), or 1 μg/mL detoxified pneumolysin (a gift from James Paton, Adelaide University, Australia). All immune sera were also tested on a control plate to which no antigen was bound. Coated plates and noncoated control plates were rinsed with Tween-PBS (TPBS; 1.05% Tween-20, 100 mmol/L NaCl, 0.5 mmol/L NaH₂PO₄, and 1.5 mmol/L Na₂HPO₄) and blocked with 1% BSA in PBS for 1 hour. After blocking the plates, the sera to be tested were titrated by using a 1:3 dilution series in 100 μL of 1% BSA in PBS and incubated overnight at 4°C. The plates were then washed with TPBS and incubated with biotin-

conjugated goat anti-human Ig antiserum (anti-IgG [H+L]), goat anti-human IgM, or goat anti-human IgA for 2 hours at room temperature. Plates were then washed with Tween-BSA and incubated with streptavidin-alkaline phosphatase for 1 hour at room temperature. Antisera and streptavidin-alkaline phosphatase were obtained from Southern Biotechnology Associates (Birmingham, Ala). Plates were then washed again with TPBS and developed with *p*-nitrophenyl phosphate (Sigma, St Louis, Mo), and absorbency was read at 405 nm. Data for antibody responses to proteins are reported in micrograms per milliliter of antibody to PspA, as the fold increase in antibody levels of the patient compared with that in a pool of normal human sera, or both.¹⁸⁻²⁰

Southern blot analysis

Ten micrograms of genomic DNA from the patient's EBV-transformed B cells or a control human monocyte cell line (THP-1) was digested with *Bam*HI, *Eco*RI, *Hind*III, or *Pst*I and electrophoresed on 0.8% agarose gels in 1× TRIS-acetate-EDTA buffer or 0.5× TRIS-borate-EDTA buffer at 40 to 60 V for 15 to 42 hours. DNA was then transferred to nylon membranes, which were prehybridized for 2 hours at 65°C.

Twenty nanograms of the human Cγ4 or Cα2 double-stranded DNA probes was labeled with phosphorous 32-labeled deoxycytosine triphosphate by means of random priming (Stratagene). Probes were developed in Dr Burrows' laboratory, as previously described.²¹ Labeled probes were denatured and hybridized to the membranes in a solution of 10× Denhardt's solution, 5 mmol/L EDTA, 0.1 μg/μL boiled salmon sperm DNA, 0.1% SDS, 3× saline sodium citrate, and 10% dextran sulfate at 65°C for 24 hours. Hybridized membranes were washed in 1 mmol/L EDTA in 0.1% SDS and decreasing concentrations of saline sodium citrate (3×-0.2×) and exposed to x-ray film.²¹

RESULTS

Case description

The 50-year-old male patient was analyzed by means of routine medical examination. The physical examination and medical history were normal, notable for only 1 to 2 episodes of sinusitis per year. Because of the history of myeloma in the patient's family, his physician performed a serum protein electrophoresis test. The results showed a decrease in the gammaglobulin region, which prompted a referral to our clinic and further laboratory testing.

The serum IgG measurement revealed a value below the normal range for this isotype (Table I). IgA and IgM levels were both within normal limits, although the IgA level was in the upper range of normal. The IgE level was elevated. The IgG subclass analysis indicated that the patient could only produce IgG3, with undetectable levels of the IgG1, IgG2, and IgG4 subclasses. Analysis of IgA indicated that virtually all of the IgA was of the IgA2 subclass. The patient responded poorly to immunization with the 23-valent pneumococcal vaccine, with only minimal responses to serotypes 8, 9, and 51 (Table II). Responses to the other serotypes were undetectable. The antibodies to serotypes 8 and 51 were of the IgG3 subclass.

Antibody responses

To further evaluate the possible basis for this patient's good health despite the loss of a significant part of his humoral immune system, we determined antibody titers to 3 pneumococcal virulence proteins to which antibody

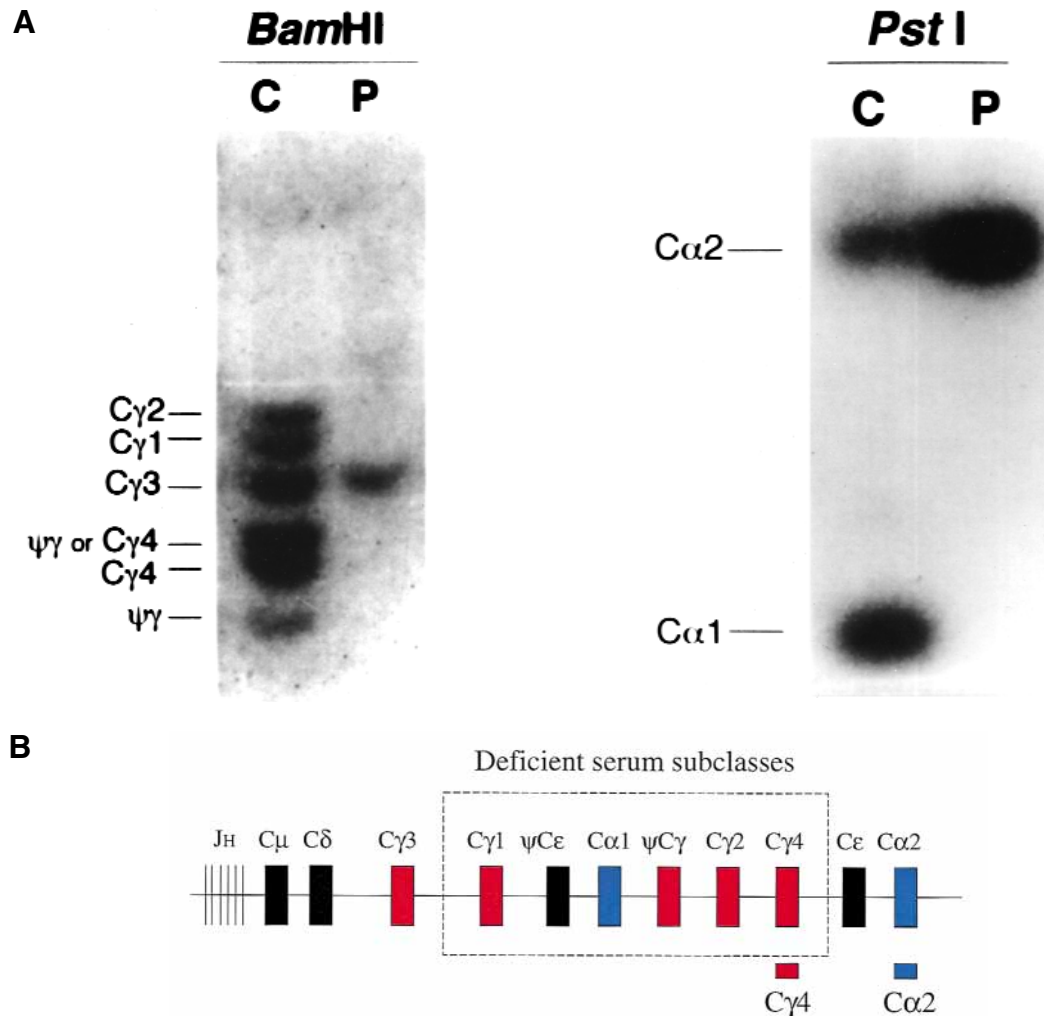


FIG 1. Status of the Ig constant region genes. **A**, Southern blot analysis of genomic DNA from a control subject (*C*) and the patient (*P*). DNA was digested with *Bam*HI and hybridized with a *C*γ4 probe (*left panel*) or with *Pst*II and hybridized with a *C*α probe (*right panel*). The bands corresponding to each constant region gene are indicated to the left of each panel. The duplicate *C*γ4/*ψC*γ bands in the control sample correspond to alleles indistinguishable because of *Bam*HI restriction site polymorphisms.²¹ **B**, Map of the human Ig heavy chain locus in germline configuration. The constant region genes deleted from both alleles of the patient are indicated in the *dotted box*. The regions where the probes bound are indicated and color coded.

has been shown to be protective in mice.^{18,20,22} Table III shows the preexisting IgG antibody response to the pneumococcal cell surface proteins PspA, PsaA, and pneumolysin. For 2 of the antigens, the level of serum IgM antibodies was much in excess of that found in the normal serum pool, whereas IgA antibody levels to all 3 antigens were far below those in the serum pool. These data suggest that the patient may be able to mount protective immunity to a potentially serious infection by forming IgG3 antibodies against 3 pneumococcal antigens known to elicit protective antibodies in mice.

Genetic basis of the antibody deficiency

Fig 1 shows a Southern blot analysis of the status of the genes encoding *C*γ and *C*α in DNA isolated from the

patient and a control individual. The *C*γ4 probe crosshybridizes with all genes encoding *C*γ in the Ig heavy chain locus, which is illustrated in Fig 1, *B*. Hybridizing bands corresponding to the genes encoding *C*γ1 to *C*γ4 and *ψC*γ are clearly visible in the control sample (Fig 1, *A*, left panel).²¹ By contrast, only the *C*γ3 band is detectable in the DNA from the patient. By using a probe that hybridizes to both *C*α1 and *C*α2, only the gene encoding *C*α2 is present in the patient's DNA (Fig 1, *A*, right panel). These data indicate that there has been a large deletion (approximately 180 kb) of constant region genes from both of the Ig heavy chain alleles. The deletion spans *C*γ1, *C*α1, *ψC*γ, *C*γ2, and *C*γ4 (Fig 1, *B*). Although the status of the genes encoding *C*ε was not examined, the gene encoding *ψC*ε is also likely deleted. As expect-

ed, the deleted constant region genes correspond precisely to the missing serum Ig subclasses.

DISCUSSION

Congenital defects of the humoral limb of the immune system represent at least 50% of all primary immunodeficiencies.^{8,9} The pathophysiology of this heterogeneous group of defects includes the following: (1) the inability to increase antibody affinity during an immune response; (2) the inability to produce non-IgM isotypes; or (3) a complete absence of B cells.^{3-6,10,11,23-25} Clinical manifestations of humoral immune deficiencies may be quite striking and typically include recurrent infection. The infections occur throughout the respiratory tract (ie, sinus, ears, bronchus, and lungs) and commonly involve polysaccharide-encapsulated organisms, such as the pneumococci.

Antibody deficiency may also result from gene deletions in the Ig heavy chain locus. Such deletions may be large, spanning multiple constant region genes and resulting in decreased efficiency or inability to produce serum Ig.¹³⁻¹⁷ A cadre of these patients lacking all serum Igs except IgM, IgG3, and IgA2 have been characterized in reference to their gene deletion.¹³⁻¹⁷ Undefined immunologic compensation was used as an explanation for these patients' relatively infection-free lives. Our patient had such a deletion, and similarly, despite these serologic deficits, he was free of serious infection. Although he did not respond to immunization with the pneumococcal polysaccharide vaccine, the patient had high antibody levels to 3 pneumococcal proteins, as well as tetanus protein antigen. The antibodies to tetanus toxoid are the result of prior vaccination.

Antibodies to PspA, pneumolysin, and PsaA, which are able to prevent carriage and invasion of pneumococci in mice,²² are found in most human sera^{19,26} and are well represented in the human serum pool.^{18,20} Our patient had high levels of antibody to each of these antigens. The amount of antibody to PspA exceeded that of 80 random adult sera²⁰ and was about 7-fold greater than the geometric mean of the 80 sera.

There is much evidence that antibodies to capsular polysaccharides provide protection from invasive pneumococcal infection, and it has long been assumed that anticapsular antibodies are the main protective antibodies against pneumococci in human subjects.²⁷⁻²⁹ Human IgG antibodies to polysaccharide antigens are largely restricted to the IgG2 subclass. The good health of our patient and of other patients with Ig deletions, including the IgG2 isotype, suggests that their high levels of antibodies to one or more pneumococcal proteins may account for their general freedom from pneumococcal disease.

Although there is considerable evidence that antibodies of different IgG subclasses are augmented through different kinds of antigenic stimulation, there are no conclusive data showing that different subclasses play markedly different roles in protection. In the case of the mouse, it has been previously reported that all 4 murine IgG subclasses offer similar levels of protection against

pneumococci.^{19,30} Thus the observation reported here that IgG3 antibodies to pneumococci may be protective is not entirely unexpected.

Several pneumococcal protein antigens, including PspA, PsaA, and pneumolysin, have been shown to elicit protective antibodies in mice against otherwise lethal pneumococcal infection.³¹⁻³⁵ These protein antigens also elicit antibodies in human subjects, but it is not known whether they can provide protection against *S pneumoniae* infection.¹⁸⁻²⁰ Our patient generated high titers of IgG3 to these antigens, suggesting that his lack of apparent infection is due in part to the immunologic response to these proteins.

In conclusion, we report a mechanism by which patients with large-spanning deletions of the Ig heavy chain loci may be protected from pneumococcal infections through antibody formation to pneumococcal capsular proteins.

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