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Homozygosity for the IgG2 Subclass Allotype G2M(n) Protects against Severe Infection in Hereditary C2 Deficiency[1]

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Homozygous C2 deficiency (C2D) is the most common deficiency of the classical complement pathway in Western countries. It is mostly found in patients with autoimmune disease or susceptibility to bacterial infections and in healthy persons. We wished to assess to what extent other immunological factors might explain differences of susceptibility to infections in C2D. For this reason, 44 Swedish patients with C2D were stratified with regard to the severity of documented infections. Investigations of IgG subclass levels, IgG subclass-specific GM allotypes, concentrations of factor B, properdin, and factor H, and polymorphisms of mannan-binding lectin and the Fc receptors FcγRIIa and FcγRIIIb were performed. Homozygosity for the *G2M*n* allele, which is known to promote Ab responses to polysaccharide Ags, was strongly associated with the absence of severe infections ($p < 0.001$) in the patients, suggesting a major protective role. The combination of mannan (or mannose)-binding lectin and C2 deficiency was found to be a minor susceptibility factor for invasive infection ($p = 0.03$). Low concentrations of IgG2 and factor B might sometimes contribute to susceptibility to infection. Other factors investigated did not appear to be important. In conclusion, the findings indicated that efficient Ab responses to polysaccharides are protective against severe infection in C2D. Implications with regard to vaccination should be considered. *The Journal of Immunology,* **2006, 177: 722–728.**

Studies of inherited immunodeficiency states have strongly contributed to the current knowledge of immunological in vivo functions (1). An aspect that has only been partly explored is the influence of the modifying effects that coincident genetic factors may have on disease expression. Homozygous C2 deficiency (C2D)[3] is a well-defined deficiency of the complement system, with an estimated prevalence of ~1:20,000 in Western countries (2). C2D is associated with the susceptibility to infection caused by encapsulated bacteria and with the development of autoimmune conditions such as systemic lupus erythematosus, and it may also be a risk factor for atherosclerosis (2–4). Moreover, many persons with C2D are healthy (2–4). The phenotypic heterogeneity encountered in C2D probably indicates that other genes influence disease expression in the patients.

C2 supplies the catalytic moiety of the C3 convertase C4b2a, which can be generated through activation of the classical pathway, the principal mechanism for Ab-dependent recruitment of complement (5), or through the lectin pathway, which is an important constituent of innate immunity (6). The lectin pathway involves the recognition molecules mannan-binding lectin (MBL), L-ficolin, and H-ficolin, which form complexes with MBL-associated serine proteases and bind to microbial carbohydrates and other targets. Impaired functions of the classical pathway and the lectin pathway could both account for the clinical consequences of C2D. Complement-mediated defense in C2D mainly relies on the alternative pathway, C3 convertase C3bBb (5), the recruitment of which is usually intact in C2D.

In mice with experimental C1q deficiency, another classical pathway deficiency state, the expression of autoimmune disease, is strongly influenced by the genetic background (7). Most likely, the genetic background also influences susceptibility to infection. In patients with C2D and infections, individual case reports have described coincident findings of common variable immunodeficiency (8), low IgG2 concentrations combined with lack of the G2M(n) allotype (9, 10), and impaired alternative pathway function due to low factor B concentrations (11, 12) or properdin deficiency (13). Among the background genes in C2D, it is noteworthy that >90% of the cases are caused by the homozygous presence of a 28-bp deletion of the C2 gene in the MHC haplotype *HLA-B*18,S042,DRB1*15* and closely related haplotypes (2, 4). This implies that immune functions determined by the MHC might be expected to be unusually uniform in C2D as compared with many other immunodeficiencies.

We recently described a cohort of 40 Swedish patients with C2D in which invasive infection was the predominant manifestation (4). To date, this is the largest comprehensive study of C2-deficient patients reported by a single center. In the present investigation, 44 patients with C2D were stratified with regard to severity of infections. Selected immunological factors with potential influence on susceptibility to infection in C2D were analyzed, including IgG subclasses and their GM allotypes, concentrations of the alternative pathway proteins factor B, properdin, and factor H, and polymorphisms of MBL and the Fc receptors FcγRIIa (CD32) and FcγRIIIb (CD16).

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[3] Abbreviations used in this paper: C2D, homozygous C2 deficiency; IGHG, immunoglobulin constant heavy G chain; MBL, mannan (or mannose)-binding lectin.

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GM allotypes are markers of the Ig constant heavy G chain (IGHG) (14, 15). The IgG subclass-specific GM allotypes of IGHG1, IGHG2, and IGHG3 are well characterized and have important immunological functions (14–17). The homozygous presence of the IgG2 allotype G2M(n) is known to be associated with efficient IgG2 Ab responses to polysaccharide Ags both in adults and young children (18, 19). G2M(n) differs from G2M(n−), the alternative immunochemically distinguished IgG2 allotype, by the presence of methionine instead of a valine residue at CH2 position 52 in the Fc part of the IgG2 molecule (20). Furthermore, the two IgG2 allotypes differ with regard to physicochemical properties, maturation during childhood, and catabolic rate (21–23).

Among IgG subclass-specific GM allotypes, G3M(b) and G3M(g) are alternative markers for IgG3, whereas G1M(f)/G1M(a) and G2M(n)/G2M(n−) are alternative markers for IgG1 and IgG2, respectively. The alleles are inherited as haplotypes in fixed combinations, of which there are four principal variants in northwestern Europe: *GM*b;f;n*, *GM*b;f;n−*, *GM*g;a;n*, and *GM*g;a;n−*. Due to allelic exclusion, each B cell line only expresses genes from one haplotype (14, 15).

MBL polymorphism was examined on the assumption that combined C2 and MBL deficiency might be associated with increased susceptibility to infection (24, 25).

IgG receptors represent another group of factors involved in the defense against encapsulated bacteria. Homozygosity for the FcγRIIa-R131 allotype has been suggested to be a risk factor for pneumococcal infections in children and adults (26). Moreover, combined effects of FcγRIIa-R131/R131 and FcγRIIIb-NA2/NA2 have been shown to influence susceptibility to *Neisseria meningitidis* in patients with terminal complement component deficiencies (27).

Among the immunological factors investigated, we found that homozygosity for G2M(n) is protective against severe infection in C2D, indicating that efficient Ab responses to polysaccharides is of crucial importance in the patients. The impact of IgG2 levels, MBL deficiency, and components of the alternative pathway was less pronounced. There was no evidence for correlation between FcγRIIa or FcγRIIIb polymorphisms and susceptibility to infection in C2D.

Materials and Methods

Patients

Between 1977 and 2002, 40 Swedish patients with C2D were identified. Demographics and clinical manifestations have been previously described (4). A history of invasive infection, mainly septicemia and meningitis, was obtained in 57% of the patients. The predominant pathogen was *Streptococcus pneumoniae*. A diagnosis of systemic lupus erythematosus was made in 25% of the patients, and another 18% had undifferentiated connective tissue disease or vasculitis. An increased rate of atherosclerotic disease was also found. Another four patients, an essentially healthy 49-year-old male, a 36-year-old woman with undifferentiated connective tissue disease and invasive infection, a 63-year-old man with systemic lupus erythematosus, and a 12-year-old boy with ethmoiditis and an intracranial epidural abscess, were added to the study. A summary of data with stratification of the patients into four groups with regard to severity of infections is given (Tables I and II). The investigation was approved by the Lund University Research Ethics Committee (protocol LU 513-01). Written informed consent was obtained for each patient.

Igs and complement proteins

Serum and EDTA plasma were stored in aliquots at −80°C. Analysis of GM allotypes and IgG subclasses was performed as described in detail elsewhere (22, 28). In short, the IgG subclass allotypes G1M(f), G1M(a), G2M(n), and G3M(b) were quantified by a sensitive competitive indirect ELISA, whereas homozygosity and heterozygosity for G2M(n) and G2M(n−) were established by double immunodiffusion (29). Concentrations of the IgG subclasses IgG1, IgG2, and IgG3 were determined by single radial immunodiffusion using age-related reference intervals (22) expressed as 2.5–97.5 percentiles. IgG4 levels were measured with a commercial ELISA (Bindazyme; The Binding Site). IgE was determined by fluoroenzyme-immunometric assay (UniCAP; Phadia). IgM, IgG, and IgA were previously determined by turbidimetry (Cobas Mira; Roche Diagnostic Systems) in most of the patients (4), and the same method was used for the new patients included in the study. Factor B, properdin, and factor H were determined by electroimmunoassay (30). The pooled serum used for reference was assumed to contain factor B at 200 mg/L, factor H at 500 mg/L, and properdin at 25 mg/L (31). In four patients, concentrations of MBL were determined by sandwich ELISA (mAb 131-1; Immunolex) (32).

Ten patients were deceased and, in four of these, very limited amounts of serum were available for analysis. This explains why the number of patients varies somewhat for the parameters investigated.

Gene nomenclature

General guidelines were followed (33). For IGHG and the FcγRs FcγRIIa and FcγRIIIb, the HUGO Gene Nomenclature database was consulted (34), (www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl). For allotypes of FcγRIIa and FcγRIIIb, we adopted the designations used by van Sorge et al. (26). For the GM allotypes of IGHG1, IGHG2, and IGHG3 we used the International Immunogenetics Information System database (35) (http://imgt.cines.fr) using alphabetical designations. However, asterisks for indication of subspecificity groups of the IGHG3 allotypes G3M(b) and G3M(g) were omitted. Alleles, haplotypes, and genotypes were italicized with an asterisk between the gene symbol and the allele or haplotype designations (e.g., *G3M*b, G2M*n−*; *GM*b;f;n, GM*b;f;n−*; *G2M*n/G2M*n, G2M*n/G2M*n−*, etc.). For MBL deficiency and MBL sufficiency genotypes, we used the simplified designations suggested by Kronborg et al. (36). Thus, MBL sufficiency genotypes were homozygous for the wild-type structural gene A (*A/A*). Genotypes containing a high or medium expression promoter haplotype together with a wild-type structural gene (*YA*) and another haplotype with a structural mutation (0) were also classified as MBL sufficiency genotypes (*YA/0*). MBL deficiency genotypes were those that were homozygous for a structural mutation (0/0) or contained a low expression promoter haplotype with a wild-type structural gene (*XA*) and another promoter haplotype associated with a structural mutation (*XA*/0).

DNA analysis

DNA was obtained from whole blood of 40 persons with C2D (37) and was not available in four of the deceased patients. A reference population of healthy blood donors ($n = 200$) was used for the polymorphisms investigated. MBL genotypes were analyzed as previously described (32, 38). The polymorphisms of FcγRIIa and FcγRIIIb were investigated according to Edberg et al. (39) with minor modifications. Primers for the FcγRIIa and MBL variants were synthesized by MWG Biotec, and primers for FcγRIIIb were synthesized by biomers.net. *G2M*n* and *G2M*n−* alleles were identified by PCR analysis combined with pyrosequencing (20, 40), confirming the results obtained by allotyping of the proteins.

Statistical analysis

Most of the statistics were analyzed using the computer program SPSS, version 10.0. Fisher's exact test, Mann-Whitney *U* test, and the Jonckheere-Terpstra test were used for analysis of statistical relations between patient groups and immunological markers. Distributions were compared with the CHI^2 test. Binomial probability distribution was used to ascertain differences between medians of IgG subclass concentrations. Spearman rank correlation was used in conjunction with analysis of factor B, properdin, and factor H levels. All p values were two-tailed.

Results

Patients with C2D were stratified into four groups according to severity of infections (Table I). Patients with rheumatologic manifestations were fairly evenly distributed among the patient groups (Table II). The results of GM allotyping in relationship to severity of infections are given in Table III. The patients were classified with regard to homozygosity for G2M(n), heterozygosity for G2M(n) and G2M(n−), homozygosity for G2M(n−), and the associated GM haplotypes.

The *G2M*n/G2M*n* genotype was found in nine persons, seven of whom belonged to group 1, the patient group that only had minor infections (Table III). Two patients with this genotype had a history of repeated invasive infections. Statistical analysis with

Table I. *Stratification of C2D patients (n = 44) according to the severity of infections encountered during the observation periods available*[a]

	Group 1: Minor Infections	Group 2: Pneumonia, Minor Infections	Group 3: Invasive Infection (1 Episode), Pneumonia, Other Infections	Group 4: Invasive Infections (2 Episodes or More), Other Infections
No. of patients	12	7	12	13
Episodes of:				
Pneumonia		14	12	>20[b]
Septicemia[c]			8	15
Meningitis[d]			4	10
Other invasive Infections			3	17
Total no. of invasive infections			15[e]	42[f]

[a] Major infections are specified with regard to the number of documented episodes.
[b] One patient showed 57 episodes of pneumonia.
[c] Fifty-seven percent of the septicemia episodes occurred before the age of 13, and 35% occurred after the age of 40.
[d] Seventy-nine percent of the meningitis episodes occurred before the age of 13, and 14% occurred after the age of 40.
[e] Septicemia and meningitis were documented at the same time in three patients.
[f] One patient had two episodes of septicaemia. In conjunction with one of these episodes, epidural abscess and pyelonephritis were documented.

regard to the presence of *G2M*n/G2M*n* revealed a highly significant difference between group 1 and groups 2–4 (relative risk = 9.3; confidence interval (95%) = 2.2–38.8; $p < 0.001$; Fisher's exact test). Expression of *G2M*n* was consistently associated with the *GM*b;f;n* haplotype. The rare *GM*g;a;n* haplotype was not found in the cohort. A *G2M*n* dose-dependent trend from susceptibility to infection toward resistance to infection was demonstrated in the patient groups 1–4 ($p = 0.02$; Jonkheere-Terpstra test).

None of the patients in the cohort originally described had low levels of IgM, IgG, or IgA (4). The concentrations of these proteins were also normal in the new patients added to the study. A patient with urticaria was the only patient with clearly raised concentrations of IgE (410 IU/L; reference interval, <100 IU/L). IgG1 concentrations were slightly decreased in four patients. IgG3 levels were essentially normal. In accordance with previous studies (41, 42), the levels of IgG2 and IgG4 were found to be low. Thus, low IgG2 concentrations were present in 15 of the 44 patients investigated (Fig. 1). In adults, the range was 0.56–5.1 g/L (median, 2.3 g/L; reference interval, 1.7–6.1 g/L). The decrease of IgG4 concentrations was even more pronounced (range, <0.002–0.54 g/L in adults; median, 0.02 g/L; reference interval, 0.06–1.2 g/L). When distributed according to IgG2 allotypes, all medians for IgG2 concentrations were below the medians of the age-related reference interval (Fig. 1). In the largest group, adults with *G2M*n/G2M*n−*, the difference was statistically significant ($p < 0.001$). The results also indicated a *G2M*n* dose-dependent effect on IgG2 concentrations (Fig. 1), similar to that reported in complement-sufficient persons (18, 19).

Eight adults had low IgG2 levels, and five of these had a history of invasive infections (Fig. 1). Adults with invasive infections did not differ from the other patients with regard to median IgG2 levels ($p = 0.11$; Mann-Whitney *U* test). Among the children investigated ($n = 12$), seven had moderately or slightly low IgG2 levels as defined by age-related reference intervals (not shown). Seven of the children had invasive infections, and four of these showed normal IgG2 concentrations. In conclusion, no consistent correlation was found between severity of the infections and the concentrations of IgG2. Similar conclusions were drawn with regard to the other IgG subclass proteins.

Concentrations of factor B are known to be comparatively low in C2D (41), as was also found in the present study (Fig. 2, *A* and *B*). The concentrations of properdin and factor H showed normal distribution. Factor B levels were moderately decreased in four patients, three of whom had a history of repeated invasive infections. Moreover, patient groups 3–4 showed a median factor B level (158 mg/L) that was somewhat lower than the median level in groups 1 and 2 (190 mg/L; $p = 0.02$; Mann-Whitney *U* test). We also examined the relationship between factor B and factor H concentrations, considering that this might influence alternative

Table II. *Demographic data of the CD2 patients*[a]

No Invasive Infections	Invasive Infections
Group 1 $n = 12$ (27%) Age at diagnosis of C2D (median) = 23 Person-years = 485 Person-years (median) = 43 Patients that died during the observation period ($n = 2$) Rheumatologic disease ($n = 5$)	Group 3 $n = 12$ (27%) Age at diagnosis of C2D (median) = 40 Person-years = 520 Person-years (median) = 52 Patients that died during the observation period ($n = 1$) Rheumatologic disease ($n = 5$)
Group 2 $n = 7$ (16%) Age at diagnosis of C2D (median) = 41 Person-years = 333 Person-years (median) = 49 Patients that died during the observation period ($n = 5$) Rheumatologic disease ($n = 5$)	Group 4 $n = 13$ (30%) Age at diagnosis of C2D (median) = 11 Person-years = 383 Person-years (median) = 22 Patients that died during the observation period ($n = 2$) Rheumatologic disease ($n = 3$)

[a] The patient groups are the same as those described in Table I.

Table III. *Homozygosity for the G2M*n allele and the GM*b;f;n haplotype confers resistance to invasive infection in C2D*

G2M Allotype[b]	G2M(n,n)	G2M(n,n−)			G2M(n−,n−)		
GM Haplotype	*b;f;n/b;f;n	*b;f;n/g;a;n−	*b;f;n/*b;f;n−	*n/n−	*b;f;n−/g;a;n−	*g;a;n−/g;a;n−	*b;f;n −/*b;f;n−
Group 1 (n = 12)	7[c]	2[d]	0[d]	1[e]	0	1	1
Group 2 (n = 7)	0	6	0		1	0	0
Group 3 (n = 12)	0	4	5		3	0	0
Group 4 (n = 13)	2	4	2		4	0	1
Total (n = 44)	20.9%	37.2%	16.3%		18.6%	2.3%	4.7%
Controls (n = 430)[f]	19.3%	27.2%	17.4%		17.7%	9.8%	6.7%

[a] The G2M(n) and G2M(n−) allotypes and the associated GM haplotypes are given in the C2D patients, who were divided into groups according to the severity of the infections (see Table I).
[b] In 40 patients, confirmatory investigation of *G2Mn* and *G2Mn−* was performed by DNA analysis.
[c] $p < 0.001$ (Fisher's exact test) for the homozygous presence of G2M(n) in group 1 compared with groups 2–4.
[d] As compared with the *GMb;f;n−* haplotype, the increased prevalence of the *GMg;a;n−* haplotype among heterozygous patients with noninvasive infections was not statistically significant (p = 0.15, Mann-Whitney U test).
[e] One patient could only be analyzed for G2M(n) due to lack of serum.
[f] The distribution of GM haplotypes in the C2D cohort did not differ (p = 0.72, CHI^2 test) from that found in a previously described control population (22).

pathway function in C2-deficient serum (43). However, factor B and factor H concentrations were fairly closely correlated in the patients (r = 0.64; confidence interval (95%) = 0.40–0.80; $p <$ 0.0001) in accord with previous findings in complement-sufficient persons (44). No correlation was found between concentrations of factor B and properdin (p = 0.21; r = 0.20; Fig. 2*B*).

Based on MBL genotypes, 40 C2D patients were classified as being MBL-sufficient or MBL-deficient (36). The six patients with MBL deficiency genotypes all had a history of invasive infection (Table IV). However, the difference was not statistically significant (p = 0.06; Fisher's exact test). The investigation was supplemented by measurements of MBL concentration in the sera of four patients, assuming MBL sufficiency at MBL concentrations >0.5 mg/L (45). The patients were clearly MBL-sufficient (range, 2.4–10.5 mg/L). With the inclusion of the four additional patients in the statistical analysis, the association between MBL deficiency and invasive infection in patients with C2D was found to be statistically significant (relative risk = 1.3; confidence interval (95%) = 1.1–1.6; p = 0.03; Fisher's exact test). No patient with combined C2 and MBL deficiency had rheumatologic disease.

No correlation was found between FcγRIIa or FcγRIIIb allotypes and severity of infections (Table V). The distribution of FcγR allotypes in C2D resembled that found in healthy controls.

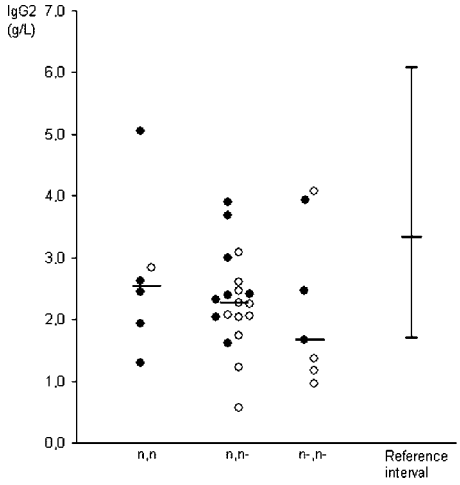

FIGURE 1. IgG2 levels in relationship to IgG2 allotypes (n,n, n,n−, n−,n−) and invasive infection in 32 C2-deficient adults. Open symbols indicate patients with invasive infections (patient groups 3 and 4, Table I). The children are divided into three age groups, each with its own symbol and reference interval. Medians are indicated with horizontal bars.

Combinations of FcγRIIa and FcγRIIIb allotypes were not informative. In a study of meningococcal disease, Platonov et al. (46) reported that FcγRIIa polymorphism influenced outcome, but not in patients below the age of 5 years. In our study, the exclusion of nine children with invasive infections that occurred below this age did not change the results.

Discussion

The *G2M*n/G2M*n* genotype was found to be protective against severe infection in C2D, suggesting the involvement of an Ig-dependent mechanism capable of compensating for the impaired immunity caused by the complement deficiency. Judging from the history of patients without severe infections, the protective function of *G2M*n/G2M*n* was already operative at early age, which implies that it did not require a mature immune system and was sustained during prolonged observation. Of note, two patients with the genotype had repeated invasive infections, which shows that the protective effect of *G2M*n/G2M*n* is sometimes insufficient. One of the patients was a child who was homozygous for the FcγRIIa-R131 and FcγRIIIb-NA2 allotypes, considered to be an unfavorable combination of FcγRs (27). However, the influence of FcγRIIa and FcγRIIIb polymorphisms was found to be low in C2D.

Basic defense mechanisms against *S. pneumoniae* are known to include specific Abs and complement. Experiments in genetically engineered mice suggest that innate immunity to *S. pneumoniae* involves natural Ab and a functional classical pathway of complement (47). Earlier animal studies have emphasized a role of the alternative pathway (48). Splenic marginal zone B cells are a likely source of natural Abs and can respond rapidly to thymus-independent Ags (49) such as capsular polysaccharides that can induce protective Ab responses (50). Furthermore, a subset of circulating $CD27^+$ memory B cells develops early in life and shares properties with splenic marginal zone B cells (51).

The strong impact of G2M(n) on immunity in C2D is difficult to fully understand. The most simple explanation is the established association between the homozygous presence of G2M(n) and the findings of quantitatively strong Ab responses to polysaccharide Ags (18, 19). Several mechanisms have been suggested through which *G2M*n* and the associated *GM*b;f;n* haplotype might promote Ab responses, including involvement of haplotype-linked genes and slow processing of Ag by macrophages (17, 18, 52). Circulating $CD27^+$ memory B cells account for Ab responses to polysaccharides and show evidence of Ab diversification at an early age before immune responses to Ag might be expected to

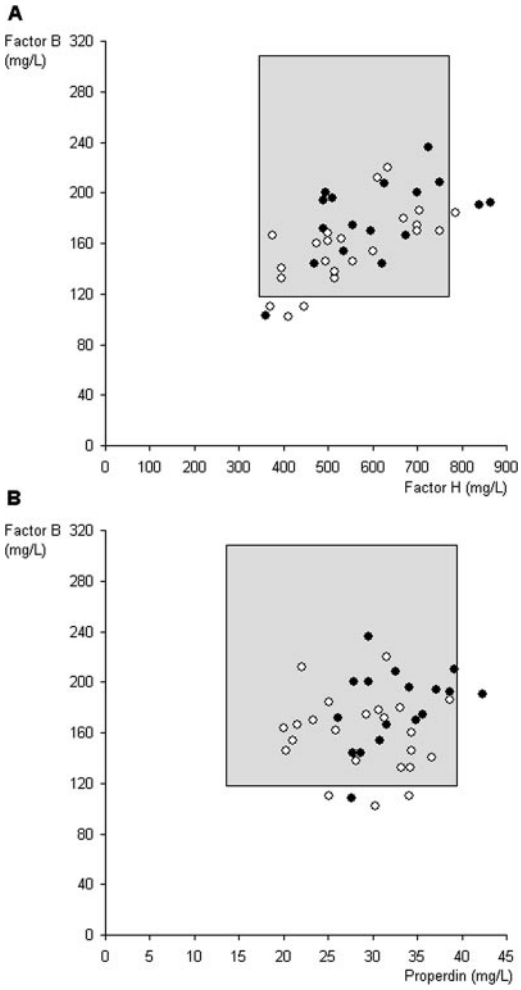

FIGURE 2. Concentrations of factor B vs concentrations of factor H (*A*) and concentrations of properdin (*B*) in 40 patients with C2D are depicted. The 95% reference areas are shaded. Open symbols indicate patients with invasive infections (patient groups 3 and 4, Table I). Factor B levels were correlated with the levels of factor H ($r = 0.64$; $p < 0.0001$), but not with those of properdin ($r = 0.20$). The median factor B concentration (158 mg/L) in patients with invasive infection was lower than the median concentration in the other patients (190 mg/L; $p = 0.02$, Mann-Whitney *U* test). Such statistically significant differences were not found for factor H and properdin.

Table V. *FcγRIIa and FcγRIIIb polymorphisms in 40 C2D patients*

Severity of Infections	FcγRIIa Allotypes[a]			FcγRIIIb Allotypes[a]		
	HH	HR	RR	NA1/1	NA1/2	NA2/2
Group 1 ($n = 10$)	3	5	2	2	1	7
Group 2 ($n = 6$)	2	3	1	0	2	4
Group 3 ($n = 11$)	2	7	2	0	3	8
Group 4 ($n = 13$)	3	7	3	1	9	3
Total ($n = 40$)	25.0%	55.0%	20.0%	7.5%	37.5%	55.0%
Controls ($n = 200$)[b]	22.5%	51.5%	26.0%	11.5%	47.0%	41.5%

[a] No correlation was found between severity of infections (see Table I) and FcγR polymorphisms.
[b] Healthy blood donors.

have occurred (51). There are four variants of B cells as determined by GM haplotypes (16). The possibility that the *GM*b;f;n* haplotype contributes to early Ab diversification in $CD27^+$ memory B cells might perhaps be considered.

Given the moderate size of the cohort investigated, statistically clear-cut results were expected for common variants of immunological factors with a strong impact on susceptibility to infection in C2D. The *G2M*n/G2M*n* genotype met these qualifications. We also assumed that the study would provide useful information concerning less frequent variants and factors with modest influence on disease expression. Based on previous reports (9, 10), we expected the *G2M*n−/G2M*n−* genotype to be associated with susceptibility to invasive infections (9, 10). A *G2M*n* dose-dependent trend from susceptibility to infection toward resistance to infection was found that supports this assumption to some extent.

Concentrations of IgG2 and IgG4 are low in deficiencies of the classical pathway, which probably reflects impaired maturation of Ig production (41, 42). Because the *GM*b;f;n* haplotype partly determines the concentrations of IgG2 (28), the question was asked of whether IgG2 levels might reflect susceptibility to infection in C2D. Indeed, low IgG2 levels were found in several patients with invasive infection, but correlations between IgG2 levels and patient groups were not statistically significant. In general accord with results of Alper et al. (41), IgG subclass concentrations did not predict the occurrence of infections in C2D.

With regard to other Igs, only one patient with C2D showed increased IgE concentrations. Considering the evidence for impaired isotype switching with very low IgG4 levels in C2D, it is conceivable that C2D might counteract development of atopic disease.

Table IV. *MBL polymorphisms in 40 C2D patients*[a]

Severity of infections	MBL Sufficiency Genotypes		MBL Deficiency Genotypes[b]		MBL Concentrations[c]	
	A/A	*YA*/0	*XA*/0	0/0	MBL-Sufficient	MBL-Deficient
Group 1 ($n = 12$)	6	4	0	0	2	0
Group 2 ($n = 7$)	5	1	0	0	1	0
Group 3 ($n = 12$)	4	3	4	0	1	0
Group 4 ($n = 13$)	8	3	0	2	0	0
Total ($n = 44$)[d]	57.5%	27.5%	10.0%	5.0%		
Controls ($n = 200$)[e]	58.0%	28.0%	7.0%	7.0%		

[a] Serum concentrations of MBL were determined by sandwich ELISA in four patients from whom DNA samples were not obtainable. The patients were divided into groups according to severity of infections (see Table I).
[b] All patients with MBL deficiency genotypes had invasive infections, but the difference was not statistically significant ($p = 0.06$; Fisher's exact test).
[c] The four patients showed MBL concentrations at 10.5, 10.0, 2.75, and 2.4 mg/L, respectively. Values >0.5 mg/L were considered to indicate MBL sufficiency (46).
[d] Combined analysis, using results of MBL genotyping and MBL measurements, indicated that the association between MBL deficiency and invasive infections in C2D was statistically significant (relative risk = 1.3, confidence interval (95%) = 1.1–1.6; $P = 0.03$, Fisher's exact test).
[e] Healthy blood donors.

Low factor B levels have been suggested to cause susceptibility to infection in C2D (11, 12). We found moderately low factor B concentrations in 13% of the patients with invasive infection and in 6% of the other patients, indicating that low factor B levels could be a minor susceptibility factor. Interestingly, a statistically significant association was found between combined C2 and MBL deficiency and the occurrence of invasive infections, suggesting that MBL has a C2-independent role in host defense (24, 25).

FcγRIIa-R131 and FcγRIIIb polymorphisms are associated with increased susceptibility to meningococcal disease in deficiencies of the terminal complement components (27). Perhaps surprisingly, no such effect was found in C2D. Phagocytosis with ligand binding to receptors for Fc and C3b/iC3b is considered to be a major defense mechanism against *S. pneumoniae* (50). Phagocytic killing of *N. meningitidis* involving Abs and the alternative pathway of complement has been described in experiments with C2-deficient sera (53). It is not known if FcγRs were required in the assay system. Antibody-dependent opsonophagocytosis of *S. pyogenes* was recently shown to require involvement of iC3b receptors (CD18/CD11b), but not FcγRs (54). Moreover, results of animal experiments indicate that FcγRs might not always be of critical importance in defense against *S. pneumoniae* (55, 56).

Abs might also mediate protective effects through other complement-dependent mechanisms in C2D. Anticapsular IgM and IgG Abs may trigger immune adherence of *S. pneumoniae* to CR1 by recruitment of C4 (57, 58). Repeated severe infections in children with C2D usually cease after adolescence (3, 4) indicating establishment of acquired immunity. Acquired immunity could involve IgA and IgG2 Abs capable of activating the alternative pathway (59). Alternative pathway-mediated serum bactericidal responses against *N. meningitidis* and *Haemophilus influenzae* type b have been documented in C2D following immunization with capsular polysaccharide vaccines (53). Janoff et al. (60) have emphasized the potential role of alternative pathway activation by anticapsular IgA Abs in defense against *S. pneumoniae*.

In a broad sense, our findings suggest that efficient Ab responses to polysaccharides are a principal cause for absence of severe infections in C2D. MBL deficiency and impaired alternative pathway function due to low factor B concentrations may increase susceptibility to infections, but they hardly have a major impact on disease expression. At the same time, the findings are consistent with the possibility that combinations of C2D with rare defects of immunity such as properdin deficiency (13) or common variable immunodeficiency (8) could be important in individual cases. Quite clearly, rheumatologic disease was not an important determinant of susceptibility to infection in the cohort investigated.

In conclusion, evidence was provided to suggest that the *G2M*n/G2M*n* genotype protects against severe infection in C2D. GM typing should be helpful for assessment of prognosis in C2D, including C2-deficient siblings of index patients presenting with infection. Vaccination with polysaccharide vaccines in C2D has been discussed (3), and it does promote bactericidal responses despite the absence of a functional classical pathway (53). The present findings indicate that Ab-dependent immunity can overcome susceptibility to infection in C2D and might thus contribute to the establishment of future rationales for vaccination in C2D and perhaps also in other complement deficiency states.

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Disclosures

The authors have no financial conflict of interest.

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