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Immune responses following meningococcal serogroups A, C, Y and W polysaccharide vaccination in C2-deficient persons: Evidence for increased levels of serum bactericidal antibodies

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ABSTRACT

Complement C2 deficiency (C2D) is associated with immunological diseases and increased susceptibility to invasive infections caused by encapsulated bacteria such as *Neisseria menigitidis*. In this study we evaluate the immunogenicity of vaccination against *N. menigitidis* in C2D.

C2D patients ($n=22$) and controls ($n=52$) were given a tetravalent meningococcal polysaccharide vaccine. Serum bactericidal antibody (SBA) titres (serogroups A, C, Y and W) were analysed using a rabbit complement source. Levels of IgG, IgM, and IgA, factor B, and factor H, polymorphisms of MBL and Fc-gamma receptors were determined.

The C2D patients responded with an increased SBA titre to all four serogroups ($p<0.001$). The response rates define as SBA titres ≥ 8 were found to be between 85.7% and 92.5%. The post-vaccination titres for serogroups C, Y and W were equal to healthy controls. C2D patients with a history of invasive infection had a lower post-vaccination SBA titres both compared to healthy C2D persons ($p=0.03$) and compared to controls ($p<0.0001$). We found that the *G2M*n/G2M*n* genotype were associated with a higher SBA titres after immunization ($p=0.03$). None of the other investigated immunological factors appear to be important in influencing the vaccine responses. Autoimmune diseases in C2D did not affect the vaccine response.

In general, vaccination against meningococci gave rise to antibody responses in the C2D patients that equal healthy controls. The response rate was lower to serogroup A and among C2D patients with history of invasive infections. The presence of *G2M*n/G2M*n* genotype was associated with higher SBA titres after immunization.

1. Introduction

Deficiency of C2, the second component of complement (C2D), has an estimated prevalence of 1 in 20,000, and is the most common homozygous complement deficiency state in populations of European descent [1,2]. Two types of the defect have been recognized [3,4]. C2 deficiency type I is the predominant variant, and originates from a 28-base pair deletion usually within the major histocompatibility complex haplotype *HLA-B18*, *SB42*, *DR2*, which results in a complete lack of C2 synthesis [3–5]. C2 deficiency is associated with immunological diseases such as systemic lupus erythematosus, severe infections, and with atherosclerosis [1,2,6–8]. Invasive infections caused by encapsulated bacteria as *Streptococcus pneumoniae* (*S. pneumoniae*), *Neisseria meningitidis* (*N. meningitidis*), and *Haemophilus influenzae* (*H. influenzae*) type b have been documented in about 25–30% of the reported C2D patients [2,7]. Similar infections are found in C3 deficiency syndromes. Late complement component deficiencies (LCCD) or defects of the alternative pathway are strongly associated with an increased incidence of invasive meningococcal infections [1,2,9]. While the role of the lectin pathway, especially mannose-binding lectin (MBL), in protection against meningococcal disease remains unclear, the lectin pathway may be important for protection in early childhood prior to maturation of the adaptive immune system [10].

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The classical activation pathway of complement involves the $C1qC1r_2C1s_2$ complex, C4 and C2. It has a major role for recruitment of opsonic C3 and the bactericidal late complement components (C5–C9) by IgM and IgG antibodies [2,11]. Complement-mediated defence can also be recruited through the alternative activation pathway with participation of C3, factor B, factor D and properdin [12]. It has been well shown that antibodies supporting alternative pathway activation [13,14] are of particular importance in deficiency states of the classical pathway.

Specific antibody that mediated immunity in C2D might involve the C2-bypass mechanism, which is known to require high concentrations of antibodies, intact C1, C4, and alternative pathway function [15,16]. Therefore, vaccination may be a useful way to improve immunity in patients with defects of the classical pathway. Vaccination with meningococcal polysaccharide in other complement defects such as properdin deficiency [17–19] and in LCCD has shown a bactericidal and/or opsonic response [18,20]. Thus far, no large study has shown similar beneficial vaccine effects against meningococcal disease in C2D.

N. meningitidis is a gram-negative diplococcus that, in order to invade, expresses a capsule polysaccharide. Based on the chemical composition of its capsule, meningococci are classified into 12 serogroups (A, B, C, E [formerly called 29E], H, I, J, L, W [formerly W135], X, Y, and Z). The majority of invasive infections are caused by six of these serogroups—A, B, C, W, X, and Y [21]. Today, there are in principal two types of polysaccharide-based vaccines against *N. meningitidis* available, a plain polysaccharide vaccine and a polysaccharide-protein conjugate vaccine. Tetravalent forms are available to protect against serogroups A, C, Y, and W.

In the present study, 22 C2-deficient persons were immunized with tetravalent meningococcal polysaccharide vaccine. A standardized assay for measuring serum bactericidal antibody (SBA) titres was used to evaluate the functional activity of the subject's serum [22]. Immunological factors with a potential to influence on vaccination responses in C2D were analysed, including immunoglobulins (Ig), IgG subclasses and their GM allotypes [23,24], concentrations of the alternative pathway proteins factor B, properdin, and factor H, and polymorphisms of MBL and the Fc receptors FcγRIIa and FcγRIIIb [25].

2. Methods and materials

2.1. Patients and controls

The 22 C2D persons that were enrolled in the present study were identified in clinical routine analysis at the Clinical Immunology Unit, Division of Laboratory Medicine, Region Skåne, Lund, Sweden. A written informed consent was obtained from each person or from custodians. None of the C2D persons or controls ($n = 52$) had been vaccinated with any meningococcal vaccine before or during the follow-up time. The study was approved by the Lund University Research Ethics Committee (protocol LU 350-93).

2.2. Serum samples

All participants were vaccinated with tetravalent meningococcal vaccine (Mencevax ACWY, SmithKleine Beecham, Brentford, England). Sera were obtained before and 4–6 weeks after vaccination. The sera were stored in aliquots at −70° C. All persons included in the study were followed on regular basis (one month and 6 months) with regard to adverse event and side effects. The C2D persons and controls were vaccinated between 2003 and 2004.

2.3. Complement proteins and immunoglobulins

Screening for complement deficiency was performed with haemolytic gel assays using sensitized sheep erythrocytes for classical pathway and guinea pig erythrocytes for the alternative pathway [26]. Individual complement proteins C2, factor B, factor D and properdin were determined by electroimmunoassay [27]. 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 [28]. MBL concentrations were determined by sandwich ELISA (mAb 131-1; Immunolex) [29]. IgG, IgA, and IgM were determined by turbidometry. Age related immunoglobulin reference intervals were used [30,31]. IgG-subclasses were determined by single immunodiffusion (IgG1–IgG3) and a commercial ELISA (IgG4) assuming age-related 2.5–97.5 percentile reference intervals.

2.4. Serum bactericidal antibodies

The vaccinees sera were analysed for each meningococcal serogroup (A, C, Y, and W) by the serum bactericidal antibody (SBA) assay as previously described [22]; the complement source was baby rabbit sera (rSBA; Pel-Freez Incorporated, Rodgers, AR). The SBA target strains were F8238 (A:4,21P1.20,9), C11 (C:16:P1.7-1,1), M03.0 241125 (Y:2a:P1.5,2), and M01 240070 W:NT:P1.18-1,3). SBA titres were expressed as the reciprocal of the final serum dilution resulting in 50% killing after 60 min of incubation. For computational purposes, SBA titres of <4 were assigned a value of 2.

2.5. DNA analysis

DNA was obtained from whole blood in both C2D persons and controls. MBL genotypes were analysed as previously described [29,32]. The polymorphism of the Fc-gamma receptors FcγRIIa and FcγRIIIb were in principal investigated in accord to Edberg et al. [33]. 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 [34–36].

2.6. Statistical analysis

Most of the data were analysed using the computer program SPSS version 22.0. Fisher's exact test and Mann–Whitney *U* test were used for analysis of statistical relations between C2D persons, controls, and immunological markers. Distributions were compared with the CHI^2 test. Wilcoxon signed rank test was used in conjunction with analysis of pre- and post-vaccination responses. All *p*-values were two-tailed and considered significant at $p < 0.05$.

3. Results

3.1. Clinical findings in the C2D cohort

Demographics and clinical data of manifestations documented in the C2D cohort are shown in Table 1. Due to lack of serum volume in a few C2-deficient persons, the number of analysed serum for each serogroup were for A: 21 samples, C: 21 samples, Y: 20 samples and for W: 21 samples. Results were obtained for all 52 controls for the four meningococcal serogroups. The distribution of gender was equal between C2D persons (F:M, 14:8) and controls (F:M, 39:13, $p = 0.32$, Chi-square test). The control group (median 28 years, 16–63 years) was significantly younger than the C2D persons at the time of vaccination (median 41 years, range 0–63 years, $p = 0.02$, Mann–Whitney *U* test). The meningococcal polysaccharide vaccine was well tolerated and no adverse events or side effects were reported.

Medical records were reviewed covering about 970 person-years including laboratory results, radiological findings, clinical physiology investigations, and autopsy reports. The follow-up observation period after vaccination was 215 person-years. The