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Vaccination against encapsulated bacteria in hereditary C2 deficiency results in antibody response and opsonization due to antibody-dependent complement activation

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Abstract Hereditary C2 deficiency (C2D) is an important susceptibility factor for invasive infections caused by encapsulated bacteria such as pneumococci and *Haemophilus influenzae* type b. The infections are mostly seen in childhood indicating that antibody-mediated acquired immunity is affected. C2D persons and healthy controls were vaccinated with ActHIB® and Pneumo23®. Analysis of specific antibodies to pneumococci serotype 6B, 7F, and 23F, and Hib was performed. Post-vaccination IgG antibodies against pneumococci serotype 6B and 23F at a concentration $\geq$1.0 mg/L was found in similar frequency in C2D persons and controls. Post-vaccination sera from C2D persons showed poor complement-mediated opsonization and phagocytosis of pneumococci by granulocytes when depending on classical and lectin pathway activation only, but increased ($p = 0.007$) and equaled that of the normal controls when also alternative pathway activation was allowed due to antibody-dependent C2 bypass activation. In conclusion, the C2D persons benefited from the vaccination and achieve an increased phagocytic capacity.

Abbreviations C2D, hereditary C2 deficiency or C2-deficient; C4b2a, classical pathway C3 convertase; GMC, geometric mean concentration; Hib, *Haemophilus influenzae* type b; MBL, mannan-binding lectin or mannose-binding lectin; *N. meningitidis*, *Neisseria meningitidis*; *S. pneumoniae*, *Streptococcus pneumoniae*.

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1. Introduction

Complement supports many immunological functions that contribute to protection from disease as well as to expression of disease manifestations. The complement system can be activated mainly through three pathways: the classical ($C1qr_2s_2$, C4 and C2), the alternative (C3, factor B, factor D and properdin), and the lectin pathway (MBL or ficolins / MASPs, C4 and C2) [1]. Each of the three activation pathways leads to the formation of a C3 convertase, C4b2a for the classical and the lectin pathway and C3bBb for the alternative pathway. The C3 convertases cleave C3 which leads to formation of the principal opsonins C3b and iC3b that stimulate phagocytosis. Complement activation subsequently continues with the terminal complement components (C5-C9) that assemble to form a cell lysing membrane attack complex which may kill gram-negative bacteria such as *Neisseria (N.) meningitidis* and *Haemophilus influenzae* type b (Hib). However, gram-positive bacteria as for example *Streptococcus (S.) pneumoniae* resist the bactericidal action of C5-C9.

Hereditary deficiency of the second component of complement (C2D) is one of the most common complement deficiency states in populations of Western descent and has an estimated prevalence of 1 in 20,000 [2,3]. Two principal variants of C2D have been described [4,5]. The predominant variant of C2D is type I (90%), which is caused by homozygosity for a 28-base pair deletion in the C2 gene resulting in a complete lack of C2 synthesis. This C2 deficiency gene is usually part of the major histocompatibility complex (MHC) haplotype *HLA-B18*, *S042*, *DR2* [4–6]. C2D is associated with autoimmune diseases such as systemic lupus erythematosus (SLE) and with an increased susceptibility to infections caused by encapsulated bacteria such as *S. pneumoniae* and Hib [2,3,7–9]. C2D may also be a risk factor for development of atherosclerosis [7]. However, many persons with C2D are apparently healthy [2,7,8].

Vaccination may be beneficial in complement-deficient patients [9]. For instance, favorable *in vitro* responses to tetravalent meningococcal vaccination have been demonstrated in properdin deficiency and in late-complement component deficiencies [10–12]. Vaccination of C2D patients has been considered to be advisable despite a lack of supportive data [2,13]. In C2D, generation of C3 fragments by action of the classical pathway C3 convertase C4b2a, which is an important mechanism for recruitment of complement-mediated defense by specific antibodies, will not work [14,15]. Also, the classical pathway is known to promote antibody responses to thymus-dependent antigens [16] and might influence responses to thymus-independent antigens [17] such as polysaccharides [18]. These circumstances suggest that vaccination responses in C2D are uncertain particularly against polysaccharide antigens. Nevertheless, we have reported for two C2D patients that anticapsular antibodies supported serum bactericidal reactions against *N. meningitidis* and Hib [19]. This raises the question if immunization with pneumococcal polysaccharides would promote opsonophagocytic killing of *S. pneumoniae* in C2D.

We have described a cohort of 40 C2D patients with a high frequency of severe infections (57%) mainly caused by encapsulated bacteria [7]. In a follow-up study of 44 C2D persons, we found that the *G2M*n/G2M*n* genotype was associated with protection against severe infections suggesting the involvement of an immunoglobulin(Ig)-dependent defense mechanism [20].

In the present study we have measured antibody responses following vaccination with the 23-valent pneumococcal vaccine Pneumo23® and Hib conjugate vaccine ActHIB® in C2D. The antibody responses were compared with those in a control group. We also investigated if immunization could promote opsonophagocytosis of *S. pneumoniae* in C2D. Issues regarding the clinical effect of vaccination were addressed in the C2D patients through review of medical records and a mailed questionnaire.

2. Materials and methods

2.1. Patients and controls

Between 1977 and 2007, 49 persons with C2D were identified in clinical routine analysis at the Clinical Immunology unit, University Hospital of Lund, Sweden. Since the initiation of the present study in 1993, 25 C2D persons were enrolled and a written informed consent was obtained from each person. None of the C2D persons or controls was vaccinated with any pneumococcal vaccine or Hib vaccine before inclusion in the present study. Demographics and clinical manifestations of the vaccinated persons are shown in Table 1. The distribution of gender was equal between the C2D persons (F: M, 16:9) and controls (F: M, 39:12). However, the C2D persons (median 41 years, range of 2–63 years) were older than the control group (median 27 years, 16–61 years, $p=0.02$, Mann–Whitney *U* test).

The participants received the 23-valent pneumococcal vaccine, which contains 25 μg of the following type-specific capsular polysaccharide: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F (Pneumo23®, Sanofi Pasteur MSD, S.N.C., Paris, France). The C2D persons were also vaccinated with Haemophilus type b conjugate vaccine, tetanus toxoid conjugate (ActHIB®, Sanofi Pasteur MSD). In 4 of the C2D persons vaccinated with Haemophilus type b conjugate vaccine, the pre-or post-vaccination blood samples were not technically handled in accord with the study protocol and therefore excluded from further analysis. A control group consisting of 51 healthy persons was also vaccinated with Pneumo23® and ActHIB®. The investigation was approved by the Lund University Ethics Committee (protocol LU 350–93).

2.2. Follow-up of adverse reactions and side effects to vaccination

Adverse reactions were followed among the C2D persons and controls. All vaccinated persons were asked to inform about any complaints by phone or by visiting the clinic during the first week after vaccination. A standardized form was filled out at three follow-up visits to the clinic (one month, 6 months and one year, respectively). No short-term adverse reactions as well as late adverse reactions and side effect were documented in the C2D persons. No SLE flare was triggered by the vaccination. Among controls, side effects were observed in one of the vaccinated. The person that experienced side effects to Pneumo23® in the control group