Characterization of Type I Complement C2 Deficiency MHC Haplotypes

Strong Conservation of the Complotype/HLA-B-Region and Absence of Disease Association Due to Linked Class II Genes

Lennart Truedsson, Chester A Alper, Zuheir I. Awdeh, Per Johansen, Anders G. Sjöholm, and Gunnar Sturfelt

*Department of Medical Microbiology, Clinical Immunology Section and *Department of Rheumatology, Lund University, Lund, Sweden; +The Center for Blood Research and Harvard Medical School, Boston, MA 02118

ABSTRACT. Fourteen individuals with complete C2 deficiency from 11 families and 3 heterozygous C2-deficient individuals from two families were investigated. In all the 24 independent C2-deficient haplotypes, the complotype S042 was present and the majority (21/24) was [HLA-B18, S042, DR2]. All carried the type I C2 deficiency C2 pseudogene with its characteristic 28 bp deletion. All but two haplotypes had 10 AC/GT repeats in the TNFα microsatellite polymorphism and all but one of the haplotypes were identical at or near HLA-B as assessed by RFLP using BstEII digestion and two genomic probes, R5A and M20A, located 100 and 38 kb centromeric to HLA-B, respectively. The exceptional haplotype was HLA-B40 with four AC/GT repeats at TNF-α. Three of the haplotypes were not DR2 based on generic and sequence-specific oligonucleotide typing. Another four haplotypes showed different DO-variants detected by RFLP analysis using BglII and MspI digestion. Thus, the [HLA-B18, S042, DR2] haplotype appears to be more fixed in the region between the complement genes and the HLA-B locus (96%) than in the region between the complement genes and DR (88%) and DO loci (71%). Of the 14 individuals studied, six had SLE or SLE-like syndromes and six had a history of severe infections although two were apparently healthy. Three of the six SLE patients and two individuals with repeated infections were homozygous for [HLA-B18, S042, DR2] and also homozygous for DQBl*0602 and the common DO variant. Thus, MHC class II genes linked to the C2 pseudogene do not appear to determine different clinical consequences of C2 deficiency. Journal of Immunology, 1993, 151: 5856.

Complete genetic deficiency of most serum complement proteins has been observed with the exceptions of factor B and C4-binding protein and are associated with disease (for review see Refs. 1 and 2).

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Deficiencies of the classical pathway proteins, the C1 subcomponents (C1q, C1r, and C1s), C4 and C2 are associated with SLE or SLE-like syndromes. The association between C2 deficiency and SLE appears to be least prominent and C2 deficiency is also associated with susceptibility to infections (3). C2 deficiency is the most common of complement protein deficiencies and the C2 null allele has been estimated to have a prevalence of about 1/100 in Caucasians (4).

The genes for C2, factor B and the C4 isotypes C4A and C4B are located close to each other within the MHC class
III region. Combinations of alleles of these genes are inherited together as stable units and are referred to as complotypes (5). The complotype associated with C2 deficiency is most often SO42 (6), shorthand notation for BF*S, C2*O0, C4A*4, C4B*2. This complotype is in linkage disequilibrium with HLA-B18 and HLA-DR2 and this combination of alleles form the conserved extended haplotype [HLA-B18, SO42, DR2]. Heterogeneity in the molecular basis for C2 deficiency has recently been found with a rare second type of genetic defect (7). The common form, caused by a 28 bp deletion in the C2 gene (8), has therefore been designated type I.

Many associations exist between specific MHC haplotypes and disease. If all C2-deficient MHC haplotypes carry the same alleles throughout, it might be worthwhile to analyze other polymorphic proteins within the immune system because the influence of MHC genes are presumably invariable among these individuals. However, it might be that specific MHC genes found in some but not all of the haplotypes are associated with specific disease conditions. To elucidate this question, it is necessary to characterize many genes on C2 deficiency haplotypes. In this study we have analyzed a number of independent C2 deficiency haplotypes for a variety of polymorphic DNA markers from close to the HLA-B locus in the class I region to HLA-DO in the class II region.

Materials and Methods

Subjects

Fourteen individuals with complete C2 deficiency from 10 Swedish and 1 Bostonian family and three individuals with heterozygous deficiency from two families in the Boston area, all Caucasians, were studied. To assess allele frequency 100 Swedish blood donors were investigated. Complete C2 deficiency was ascertained by hemolytic assay (9) and by measuring C2 concentration in serum by electroimmunoassay (10) and heterozygous C2 deficiency was ascertained by presence of low serum C2 concentration that segregated with MHC haplotypes in the families. The presence of the different genetic variants on C2 deficiency haplotypes was defined by analysis of 1st degree relatives. Of the individuals with homozygous C2 deficiency, six had a history of severe infections and six had SLE or an SLE-like syndrome, two of these were siblings. In two families, the C2-deficient patients had an apparently healthy C2-deficient sibling.

HLA-typing

HLA-A, -B, and -DR Ag were assigned by the standard National Institutes of Health lymphocyte microcytotoxicity assay (11). HLA-typing was performed with well-characterized alloantisera.

C4 and factor B typing

Typing of C4 was done on serum samples treated with carboxypeptidase B type I (Sigma Chemical Co., St. Louis, MO) 130 U/ml according to the method of Sim and Cross (12). The samples were then desialated by incubation with neuraminidase from Clostridium perfringens type VI (Sigma), at a concentration of 10 U/ml of serum for 15 h at 4°C. C4 variants were detected by gel electrophoresis in agarose gel and immunofixation with rabbit anti-human C4 antiserum (Dakopatts A/S, Glostrup, Denmark) (13). Factor B typing was performed by electrophoresis as previously described (14). Bands were visualized by immunoblotting using factor B-specific rabbit antiserum and swine anti-rabbit IgG (Dakopatts) conjugated with alkaline phosphatase from bovine intestine, type VII-T (Sigma). Complement genetic nomenclature is used according to that originally introduced for factor B (14) and C4 (13).

DNA isolation

DNA was extracted from peripheral blood leukocytes by phenol/chloroform according to standard procedures (15).

RFLP analysis

Separation of DNA fragments obtained by restriction enzyme digestion was performed by gel electrophoresis in 1% agarose at 4 V/cm for about 16 h at 20°C except for SstI-digested DNA that was separated in 2% agarose at 4°C and low voltage, 2 to 3 V/cm for 90 h (16). Restriction enzymes were purchased from BRL (Gaithersburg, MD) and digestion was performed according to the manufacturer’s instructions with 3 to 5 U/µg of DNA. After electrophoresis, DNA fragments were transferred to nylon membranes (Sureblot, Oncor, Gaithersburg, MD or Nytran, Schleicher & Schuell, Keene, NH) and processed according to the manufacturer’s instructions.

DNA probes

The following probes were used for RFLP analysis: 1) R5A, a 600-bp fragment and M20A, a 300-bp fragment about 110 and 38 kb centromeric from the HLA-B locus, respectively (17). 2) A 300-bp C2 gene fragment derived from the genomic clone pG850 (18). 3) A 600-bp B probe from the clone pFB3b obtained by Clal/BamHI digestion (19). 4) A 500-bp 5’ C4 fragment derived from the full length C4 cDNA clone pA7-T (20) by BamHI/KpnI double digestion. 5) A 3’ C4 cDNA probe pC4AL1 (21). 6) A 900-bp BglII fragment from the cosmid clone cos 1E3 for CYP21 genes (22). DRB and DOB probes were obtained from the 10th International Histocompatibility Workshop (23). The probes were labelled with α-[32P]JdCTP (Amersham Corp, Arlington Heights, IL) by random hexanucleotide priming.
PCR analysis

Detection of the 28-bp deletion specific for type I C2 deficiency was done by PCR amplification (24, 25) using the primers 5' AAAGCTGGGCGCTAAAATCCAGCG-3' and 5' GAGCACAGGAAGCCGCTCTCGAGG-3' (8). After initial denaturation for 10 min at 95°C, 2 to 2.5 U of the polymerase per 100 μl reaction volume was added and the DNA was amplified by melting at 95°C for 2 min, annealing at 60°C for 2 min, and polymerizing at 72°C for 2 min. Forty cycles were performed followed by a final elongation at 72°C for 7 min. Either Taq polymerase (Perkin Elmer Cetus, Norwalk, CT) or Vent-polymerase (New England Biolabs, Beverly, MA) were used with buffers containing 1.5 or 2 mM Mg2+, respectively, according to instructions provided by the manufacturers. The PCR products were analyzed by gel electrophoresis in 4% agarose (2% NuSieve, FMC Bioproducts, Rockland, ME and 2% agarose, Ultra Pure Reagent, IBI, New Haven, CT) and stained by ethidium bromide.

Analysis of the microsatellite TNF-α described by Nedospasov et al. (26) was done by initial PCR amplification using the primers 5'-GCCTCTAGATTTGATCCAGCG-3' and 5'-GCCCTCTAGATTTCATCCAGC-3' which amplifies a fragment of about 235 bp size. The following cycle was used 35 times: 94°C for 30 s, 60°C for 1 min and 72°C for 1 min. A second amplification with 10 cycles was done by the primers 5'-CCTCTCCCCTGCAACACA-3' and 5'-GCCTCTAGATTTCATCCAGC-3', which amplifies an approximately 110 bp 5' part of the fragment containing the TNF-α microsatellite (26). For this amplification, 20% of the dCTP was substituted with CY-[32P]CTP with a sp. act. of 3000 Ci/mmol (Amersham), giving rise to labeled fragments of AC/GT repeats; 2, 3, 3, 3, and 3, 10 were used as size markers. The labeled fragments were size separated on a 6% polyacrylamide gel using three samples with known numbers of AC/GT repeats; 2, 3, 3, 3, and 3, 10 were used as size markers. The bands were visualized by autoradiography.

DNA typing for DRB1 and DQB1 alleles was carried out by SSO probe hybridization according to the protocols of the 11th International HLA Workshop (27). Briefly, PCR amplification was carried out on 0.2-μg samples of genomic DNA in a 50-μl reaction containing 25 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 0.2 mM of each deoxynucleotide, 1 U of Taq polymerase (Promega, Madison, WI) and 1.5 mM MgCl2. The primers used were the ones recommended by the 11th International HLA Workshop (27).

The DNA was amplified in the DNA Thermal Cycler (Perkin Elmer Cetus) programmed appropriately for each target and checked by electrophoresis in a 3% agarose gel. About 50 ng of amplified DNA from each sample were spotted on a nylon membrane. Several replicate membranes were made and each of the membranes was prehybridized at 54°C with hybridization buffer (50 mM Tris-HCl, 3 M tetramethyl ammonium chloride, 2 mM EDTA, 0.1% SDS, 100 μg/ml heat denatured herring sperm DNA, and 5x Denhardt's solution) for at least 30 min. The allele-specific oligonucleotides for each of the three loci were labeled with gamma γ-[32P]ATP. HLA-DRB1, and DQB1 alleles were identified by autoradiography after hybridization with the labeled SSO probes.

Results

Complement protein typing

All the C2-deficient sera analyzed had only the C4A 4 and C4B 2 variants and the common BF S variant. One of the complotypes in all the three heterozygous individuals, based on family analysis, was SO42. Thus, all the 24 C2-deficient haplotypes studied contained the complotype SO42.

Deletion in C2 gene

The C2-deficient individuals were screened for the 28-bp deletion described for the common form of C2 deficiency (type I). All the C2-deficient persons had a deletion of this size in a small DNA-fragment obtained by PCR amplification using C2-specific primers and the three persons with heterozygous C2 deficiency all were heterozygous for the fragment with the deletion (Fig. 1). Of 100 Swedish blood donors, one individual was heterozygous for the 28-bp deletion thus giving an estimated allele frequency for the C2 deficiency gene of 1/200 (95% confidence interval: 0–3%).

HLA-typing

For 11 of the 14 individuals homozygous for C2 deficiency, serologic HLA types were known. All of these were HLA-B18 except one who was HLA-B18,40. The class II generic types were in complete concordance with the typings achieved by DNA analysis.

MHC class III

The complotypes were analyzed with a set of RFLP probes ranging from the C2 gene to the CYP21 gene centromeric of the C4B gene. Locations of the different markers used are shown in Figure 2. All the C2-deficient haplotypes displayed identical patterns with a 2.75-kb Smal fragment obtained with the C2 probe and presence of two C4 genes with a short C4B gene and no deletion of CYP21 genes (28). The RFLP constellation for the SO42 complotype is shown in Table I.

The most polymorphic of three recently described microsatellites in or near the TNF genes (TNF-α) was investigated (Fig. 2). Of the 11 samples from unrelated persons

3 Abbreviations used in this paper: PCR, polymerase chain reaction; SSO, sequence specific oligonucleotide.
a 3.0 kb-band corresponding to the combined pattern previously observed on the extended haplotype [HLA-B38, SC21, DR4] and assigned B by Egea et al. (29). In addition to the previously published polymorphic bands, we also observed bands of approximate sizes of 16 and 18 kb occurring together with the 6.9-kb band with the R5A probe and a 6.5-kb band together with the 3.0 and 5.2 bands with the M20A probe (data not shown). The exceptional sample with a 6.5 and a 6.9 band with R5A was from the same C2-deficient individual who was heterozygous at the HLA-B locus with HLA-B18,40. This person was also heterozygous in the TNF-α repeats, having 10 and 4 repeats. The other individual with heterozygosity in TNF-α repeats had the common RFLP markers with the two HLA-B-related probes.

MHC class II
The typing of DRB1 with PCR amplification followed by probing with SSO probes revealed that all but three haplotypes were DR2 or DRB1*1501. These results were in complete accord with RFLP analysis using BglII, MspI, or TaqI digestion and probing with a DRB probe. DNA typing of DQB1 showed that 18 of the 24 haplotypes were DQB1*0602. RFLP analysis using a DOB probe showed that of 7 of the 24 haplotypes at this location differed from the common C2 deficiency haplotype (Table III, Fig. 2).

Disease associations
The disease associations in relation to HLA-D variants are given in Table IV. Three of the five individuals with SLE or an SLE-like syndrome were homozygous for DR2, DQB1*0602, and the most common DO variant (assigned DO1, see Table III). Two of these SLE patients were siblings. These individuals were then among the total of six C2-deficient individuals who were homozygous at all polymorphic sites analyzed in this study. We found a total of 16 of the 24 independent C2 deficiency haplotypes to be identical from HLA-B to DR (Fig. 2). Two of the six individuals with infections were also homozygous for this haplotype. The individual with HLA-B18,40 was also DR1,2 and heterozygous at the DO locus with both DO variants differing from the one found on C2 deficiency haplotypes. This individual had a history of infections.

Discussion
All C2 deficiency haplotypes analyzed in our study had the recently described 28-bp deletion in the sixth exon (8), and all haplotypes contained the same complotype S042. Of another four unrelated Swedish C2-deficient individuals known, only one might have a non-type I C2 deficiency haplotype (C4 phenotype C4A 3, 4, C4B 1, 2, DNA not
FIGURE 2. Schematic map of the human MHC with approximate location of some of the genes shown as blocks. The location of polymorphic markers analyzed are indicated by arrows. Regions of the 24 independent MHC haplotypes carrying the type I C2 deficiency gene are shown as bars with the portion of identical regions given to the left. In one haplotype the number of AC/GT dinucleotide repeats in the TNF-α microsatellite was different but markers on both sides, i.e., Sst1/C2 and BstEII/RSA and M20A were the same as for the majority of haplotypes. In two other haplotypes with the common DO1 variant, from the same individual, the DQB1*0603 and not the DQB1*0602 allele was found, c.f., Table III.

Table I
RFLP constellation for type I C2 deficiency complotype SB42 found on 24 independent haplotypes

<table>
<thead>
<tr>
<th>Probe</th>
<th>Enzyme</th>
<th>C2</th>
<th>BF</th>
<th>5' C4</th>
<th>3' C4</th>
<th>CYP21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SstI</td>
<td>TaqI</td>
<td>BglII</td>
<td>TaqI</td>
<td>XbaI/BamHI</td>
</tr>
<tr>
<td>Fragment size (kb)b</td>
<td>2.40</td>
<td>2.65</td>
<td>2.70</td>
<td>2.75</td>
<td>4.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Patternc</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* The 5.4 kb fragment is correlated with a short C4B gene.
* Fragments observed in normal populations.
* X indicate presence of restriction fragment.

Table II
Number of AC/GT repeats in TNF-α microsatellite obtained in individuals carrying type I C2 deficiency haplotype

<table>
<thead>
<tr>
<th>No. or Repeats</th>
<th>No. of Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous</td>
<td></td>
</tr>
<tr>
<td>10, 10</td>
<td>9</td>
</tr>
<tr>
<td>10, 4</td>
<td>1</td>
</tr>
<tr>
<td>10, 0a</td>
<td>1</td>
</tr>
<tr>
<td>Heterozygous</td>
<td></td>
</tr>
<tr>
<td>10, 11</td>
<td>1</td>
</tr>
<tr>
<td>10, 2</td>
<td>1</td>
</tr>
</tbody>
</table>

a 0 indicate a smaller fragment corresponding to absence of repeats.

Thus, it can be concluded that type I C2 deficiency is by far the most common form in the Swedish population and probably in Caucasian populations as judged from earlier linkage analysis (6). All haplotypes analyzed also displayed the same complotype RFLP constellation (31) including the 2.75-kb Sst1/C2 band (Table I, Fig. 2). This band was recently demonstrated to be characteristic for complotypes with the C2*B or the C2*QO gene (32). Our data confirm that the 2.75-kb band is found in all C2*Q0 examined and support the hypothesis that the deletion in one C2*B gene being part of the complotype SB42 gave rise to the type I C2 deficiency complotype. Inasmuch as the majority of the haplotypes was [HLA-B18, S042, DR2], this confirms earlier observations (6) and makes it most likely that the deletion in the C2 gene occurred in the haplotype [HLA-B18, SB42, DR2].

The fixity of the [HLA-B18, S042, DR2] haplotype is more prominent toward HLA-B compared to HLA-DR, both loci were located approximately 500 kb from the C2 gene (Fig. 2). This is in accordance with results obtained by analysis of other polymorphic sites in this part of the MHC class III region (33). At the DO loci, nearly 1000 kb from the C2 gene, 7 of the 24 haplotypes differed from the common one, making 70.8% identical over this stretch of DNA (Fig. 2) with exception for two haplotypes carrying DQB1*0603 instead of DQB1*0602 (Table III).
Table III
HLA-DR types, DQB1 alleles, and DNA-variants of DOB gene obtained by RFLP analysis in individuals carrying type I C2 deficiency haplotypes

<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>DQB1</th>
<th>DOB</th>
<th>BglII/DOB</th>
<th>MspI/DOB</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.6</td>
<td>1.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Homozygous C2-deficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR2, 2</td>
<td>0602,0602</td>
<td>DO1, 1</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DR2, 2</td>
<td>0603,0603</td>
<td>DO1, 1</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DR2, 2</td>
<td>0602,0602</td>
<td>DO1, 2</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DR2, 2</td>
<td>0602,0603</td>
<td>DO1, 3</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DR1, 2</td>
<td>0602,0301</td>
<td>DO2, 3</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DR2, 4</td>
<td>0602,0301</td>
<td>DO1, 2</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DR2, 9</td>
<td>0602,0303.2</td>
<td>DO1, 2</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Heterozygous C2-deficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR2, 2</td>
<td>0602,0402</td>
<td>DO1, 3</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DR1, 2</td>
<td>0602,05var</td>
<td>DO1, 2</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>DR2, 3</td>
<td>0602,0201</td>
<td>DO1, 2</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* MHC-identical siblings are counted once.
* Defined from the observed RFLP patterns.
* These are from the same family and have the same DR2-bearing haplotype.
* 05 variant described by Erlich et al. (10).

Table IV
HLA-DR types, DQB1 alleles and DNA-variants of the DOB gene in 14 individuals homozygous for type I complement C2 deficiency in relation to disease associations

<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>DQB1</th>
<th>DO</th>
<th>SLE</th>
<th>Infections</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR2, 2</td>
<td>0602,0602</td>
<td>DO1, 1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>DR2, 2</td>
<td>0603,0603</td>
<td>DO1, 1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR2, 2</td>
<td>0602,0602</td>
<td>DO1, 2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR2, 2</td>
<td>0602,0602</td>
<td>DO1, 3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR2, 4</td>
<td>0602,0301</td>
<td>DO2, 3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR2, 4</td>
<td>0602,0301</td>
<td>DO1, 2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR2, 9</td>
<td>0602,0303.2</td>
<td>DO2, 2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Defined from the RFLP patterns given in Table III.
* Two of these patients were siblings.

An explanation is that the DQB1*0603 allele arose by mutation on one instance of the ancient haplotype. This departure from the complete haplotype can be used to make an estimate of the time that has passed since the deletion in the C2 gene occurred. Using the formula \((1-Q)^n = X\), where the recombination fraction \(Q\) is given the value 0.01, \(X\) or extent of linkage disequilibrium is 70.8%, and \(n\) is the number of generations. With 25 yr as a generation, the deletion should have occurred about 850 yr ago. If all the exceptional DQB1 are regarded as resulting from recombination, the corresponding period of time will be about 1200 yr. Previous estimates based on serologic typing data have been similar (14). However, it must be noted that this calculation could be partly based on incorrect assumptions. If the haplotype is more strongly conserved the deletion would have occurred much earlier.

Inasmuch as all the 14 individuals with complete C2 deficiency studied were homozygous for the same deletion in the C2 gene, it is clear that the C2 deficiency alone did not give rise to a specific disease, i.e., lupus or susceptibility to infection. The observation that three individuals with SLE and two with increased susceptibility to infections in the studied group were homozygous for HLA-DR2 or DRB1*1501, DQB1*0602 and also homozygous at the DO locus indicates that MHC class II genes are not decisive for any of these disease associations. The frequency of homozygous C4A deficiency is increased in SLE (35) but whether heterozygous C2 deficiency also is more common in SLE than in the normal population is not clear but this has been suggested from previous studies (36).

Two of the 14 C2-deficient individuals studied were healthy siblings to C2-deficient patients. It can be estimated from the C2 deficiency allele frequency reported here (1/200) that 1 in 40,000 Swedes should be homozygous for the defect, bearing in mind that this figure is based on analysis of 100 blood donors. In our experience the laboratory diagnosis of complete C2 deficiency is rare indicating that many C2-deficient individuals are healthy and escape detection. This is further supported by the notion that from an ongoing epidemiologic study of SLE in Southern Sweden (37), none of 86 patients recruited from an adult population of about 160,000 was homozygous for C2 deficiency. However, symptoms in C2-deficient SLE patients are usually mild, often confined to skin and mucus membranes and the patients often lack antinuclear antibodies, so that the disease frequency might well be underestimated.

The reason that C2 deficiency appears to be less disease-associated than deficiencies in other complement proteins early in the classical pathway activation is not known. However, it could be due to the fact that activation of the complement cascades may occur without participation of C2. It has been shown that erythrocytes bearing a high density of specific antibodies activate complement in the absence of C2 (38). Also, activation of C1 followed by C4
cleavage results in C4b fragments that, bound to immune complexes, could be of importance in the elimination of noxious immune complexes—an important function of the complement system.

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