Molecular aspects of hereditary complement component C5 deficiency in humans

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Abstract

Introduction: The complement system plays a key role in the host defense against pathogens. The deficiency of complement components predisposes the system to recurrent infections and autoimmune diseases. In particular, serum C5 deficiency (C5D) may be serious for human health, because this protein plays a key role in controlling infections, mainly with Neisseria spp.

Aim: The aim of this article is to present the structure and function of the human C5 gene encoding complement component C5, with particular regard to the molecular characteristics of the mutations causing hereditary complement C5 deficiency.

Material and methods: This article is based on the available literature. A total of 35 articles were included in the study.

Results and discussion: Based on the literature review, it was shown that C5 mediates inflammatory processes and bacterial cytolysis. The cause of hereditary C5 deficiency in humans is inefficient or reduced serum C5 biosynthesis, due to mutations in the C5 gene. This quantitative and functional C5 deficiency is associated with recurrent Neisseria spp. infections, the lack of bactericidal activity and an impaired ability of serum to induce chemotaxis. The molecular characterization of previously described C5D-related mutations in the human C5 gene has been performed, and the clinical presentation of some molecularly examined C5D probands has also been discussed.

Conclusions: The deficiency of C5 protein, which bridges innate and adaptive immunity, is related with 18 different mutations in the C5 gene found in over 30 families of various origins. Screening for complement defects seems particularly important, especially in asymptomatic relatives of probands.
1. INTRODUCTION

The complement system, as one of the innate immune mechanisms, plays a key role in the host defense against pathogens. It is comprised of more than 30 plasma and membrane proteins, many of which are zymogens and are activated through a triggered-enzyme cascade. The main physiological activities of complement include opsonization, chemotaxis, bacterial lysis, modulation of adaptive immunity and clearance of immune complexes and apoptotic cells. Its activation occurs by three pathways: the classical, the lectin and the alternative pathway. All of these pathways lead to the common final pathway through the production of C3 convertase, which releases C3a and C3b, and then the production of C5 convertase, which cleaves the C5 protein into C5a and C5b peptides. On the surface of a bacterial cell, C5b, after binding C6, C7, C8 and C9, forms a membrane attack complex (C5b-9, MAC), which leads to cytolysis and death. Therefore, a properly functioning complement system plays a crucial role in maintaining host homeostasis. In turn, the deficiency of complement components predisposes the host to recurrent infections and autoimmune diseases. In particular, the deficiency of complement C5, due to its function, can be serious for human health, as this protein plays a key role in controlling infections with Gram-negative bacteria, mainly Neisseria spp.2–7

2. AIM

The aim of this article is to present the structure and function of the human C5 gene encoding complement component C5, with particular regard to the molecular characteristics of the mutations causing hereditary complement C5 deficiency, including their location, type and importance, as well as the clinical presentation associated with Neisseria spp. infections.

3. MATERIAL AND METHODS

This article is based on the available literature. The search for publications was performed using the PubMed, Google Scholar and OMIM databases. The following keywords were used in the search: complement component system, complement component C5, C5 gene and C5 deficiency. Ultimately, a total of 35 articles published in peer-reviewed journals were included in the study.

4. RESULTS AND DISCUSSION

4.1. Complement component C5

Human complement component C5 is a plasma glycoprotein (190 kDa) composed of two polypeptide chains: α (115 kDa) and β (75 kDa) linked by a disulfide bond. It is mainly synthesized by hepatocytes, monocytes and lymphocytes as a precursor of 1976 amino acids, containing an 18-amino acid leader peptide and a 4-amino acid arginine-rich linker (RPRR) connecting the α and β chains. The activation of C5 by C5 convertase involves cleaving the α chain between Arg275 and Leu276 and generating C5a and C5b fragments. C5a (11 kDa) is a cationic peptide containing 4 α-helices stabilized by three internal disulfide bridges. As an anaphylatoxin, it causes smooth muscle contraction, increased vascular permeability, degranulation of mast cells and basophils, and an oxygen burst in neutrophils, macrophages and monocytes. It is also a strong chemoattractant for macrophages, monocytes, neutrophils, and lymphocytes which recruits them to the site of infection and induces the release of pro-inflammatory cytokines (interleukins IL-1β, IL-6, IL-8, and tumor necrosis factor TNF-α). It also increases the expression of adhesion molecules on endothelium and in immune cells. C5a enhances the expression of Fc gamma receptors I and III (FcγRI and FcγRIII) and stimulates the clearance of immune complexes, thereby bridging humoral and cellular immunity. In turn, C5b (180 kDa) on the target membrane is involved in the formation of MAC due to the presence of the C345C domain, which is the binding site for the C6 and C7 components. Only the attachment of C8αβ disrupts membrane integrity, because the β chain binds to C5b-7 and the α chain penetrates the lipid bilayer. C9 combines with C8a and begins to polymerize, enlarging the pore size and forming the final C5b-9, MAC complex. This is a channel responsible for the outflow of ions and macromolecules from bacterial cells and the inflow of water and lysozyme into them, causing osmotic lysis. This mechanism is not well understood, but multiple MACs must be incorporated into the cell membrane together, which is crucial to maintain the bactericidal properties of human serum.

4.2. C5 gene

The human C5 gene is located on chromosome 9q33.2, covers 79 kb and consists of 41 exons, encoding α (exons 1–16) and β (exons 17–41) chains. The 6-kb mRNA is translated into pre-C5 protein (in β-α orientation), which is post-translationally processed to the mature, two-chain C5 form by the removal of the RPRR region. The differentially spliced transcripts containing alternative exons (16 or 21a) and polyadenylation signals have been reported in fibroblast and liver cells from healthy humans. In addition, the 5′ flanking region of the gene contains sequences homologous to the responsive elements with known functions in inflammatory processes, such as interferon, IL-6 and nuclear factor kappa β (NF-κB). Gene function studies have shown that a blockade of the C5a receptor in human monocytes inhibits the production of IL-12 and other pro-inflammatory cytokines and leads to an anti-inflammatory phenotype. Some polymorphic variants of the C5 gene have been shown to be associated with rheumatoid arthritis, age-related macular degeneration, paroxysmal nocturnal haemoglobinuria, atypical uremic syndrome and asthma.
4.3. Complement component C5 deficiency (C5D)

Hereditary C5D (OMIM #609536) is a rare genetic disorder with an autosomal recessive mode of inheritance leading to inefficient or reduced biosynthesis of human C5. The quantitative analysis of C5 in serum has shown that its deficiency is associated with recurrent bacterial infections, mainly bacteria of the genus Neisseria, as well as the lack of bactericidal effect and impaired ability of serum to induce chemotaxis. Neisseria meningitidis, an encapsulated Gram-negative bacterium, is a common worldwide cause of invasive meningococcal disease (IMD), which clinically manifests as meningococcal meningitis (MM) and meningococcal septicaemia (MS). In healthy adults, the plasma C5 levels are approximately 75 µg/mL. This protein has been shown to play a central and coordinating role in inflammation and bacterial cell elimination. Therefore, C5D patients have opsonically active C3b, that is released upon activation of the classical or alternative pathway, while they are unable to generate C5b-C9. As a consequence, they are more susceptible to systemic infections, primarily bacteria of the genus Neisseria, the elimination of which requires intact bactericidal activity of the serum. Obviously, patients with C5D are also unable to produce normal C5a, which increases the risk of complications in infectious diseases. This has been clarified in more detail in vitro studies using fresh whole blood from people with C5D. Lappegård et al. showed that C5, not C3, was mainly responsible for the expression of N. meningitidis-induced tissue factor, which has been implicated in the development of disseminated intravascular coagulation, a fatal complication of sepsis. The authors also confirmed that phagocytosis, oxidative burst and the induction of cytokine and adhesion molecule expression are essentially C5a-dependent processes. In turn, Hellerud et al. found the lack of meningococcal killing, and bactericidal and opsonophagocytic activity in C5-deficient blood, suggesting that C5 induces efficient elimination by opsonophagocytosis only through the formation of C5a. In a population-based study conducted in a French cohort of 154 patients with terminal complement deficiencies, 21 were diagnosed with C5D.

The history of research on functional complement C5 deficiency dates back to the early 1970s. The C5D was characterized as one of the first complement defects associated with N. meningitidis infections in family studies based on quantitative and qualitative protein analysis. Miller and Nilson were the first to describe C5 dysfunction resulting in the lack of phagocytosis-enhancing serum activity in a Caucasian proband, her mother, and 15 relatives. The proband was a seven-month-old infant with a failure to thrive, severe eczematosid dermatitis and repeated local and systemic infections from a variety of Gram-negative bacteria and coagulase-positive staphylococci. In the second family, two brothers were affected, with symptoms previously described as Leiner’s disease; such as seborrheic dermatitis, diarrhea, recurrent infections of Gram-negative etiology and severe wasting. These studies also suggested an autosomal recessive inheritance of C5D. The subsequent authors mentioned that C5D was accompanied by other autoimmune diseases: systemic lupus erythematosus, discoid lupus erythematosus, Kernig’s syndrome and Sjögren’s syndrome, although the relationship between C5D and these diseases has not yet been clarified. Snyderman et al. were the first to demonstrate that C5D could be associated with repeated disseminated gonococcal infection caused by N. gonorrhoeae. However, in a Turkish family, two members with C5D had recurrent MM and MS, two had recurrent respiratory tract infection and purulent otitis media and one was healthy and did not exhibit any increased susceptibility to infections. Moreover, such cases of C5D without any pathological symptoms have also been noted. Overall, citing Delgado-Cerviño et al., these quantitative and qualitative studies of serum C5 included a total of 33 C5D patients from 17 unrelated families.

Whereas the molecular studies at the DNA level to identify the mutations underlying these deficiencies began later in the 1990s. The genetically determined immune response to pathogens is known to be altered by various nucleotide variants in immune-related genes. Because N. meningitidis is a human-restricted pathogen, all of its evolutionary adaptations must be specific to human responses, making the molecular genetics studies in this area in humans particularly important. Wang et al. were the first to detect two nonsense mutations in exons 1 and 36 of the C5 gene in members of three unrelated African-American families. All affected individuals were compound heterozygotes, prompting researchers to look for further inherited defects causing C5D. To date, the 18 different mutations in the human C5 gene have been reported in the literature to cause C5D in over 30 families from various geographic regions and ethnic groups. The molecular characteristics of these mutations, including their location, type and effect at the protein level, as well as familial origin, are presented chronologically in the Table 1. The location of mutations in the C5 gene in Table 1 was given according to Colobran et al. These C5 gene variants result from missense, nonsense, insertion-deletion and splicing mutations; most of which have been identified in only one family. The only exception is the A252T mutation. In the Western Cape region of South Africa, of 65 Black African meningococcal disease patients, five (7.7%) were homozygous for this mutation and had very low serum C5 levels, ranging from 0.1% to 4.0%. In the sub-Saharan African population, this mutation was estimated at a frequency of 3.0% and was previously thought to occur only in this region. Recently, individual cases of A252T mutation have also been discovered in the Middle East and South Asia. Based on a review of the literature on molecular studies of the C5 gene, Table 2 presents the characteristics of selected C5D probands and their clinical and genetic features. Overall, the C5D probands showed repeated episodes of IMD, MM, MS, and in individual cases also pneumonia and otitis media (Table 2). In the blood serum of all cases, extremely low or absent C5 concentrations and negligible haemolytic activity of the complement system in the classical (CH50) and/or alternative (AH50) pathways were found. Mostly, the affected members were from con-
Table 1. The C5 gene mutations identified in C5D family studies.

<table>
<thead>
<tr>
<th>Mutation (cDNA, protein)</th>
<th>Location</th>
<th>Type of mutation</th>
<th>Effect of mutation</th>
<th>Number</th>
<th>Families</th>
<th>Origin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.55C&gt;T, p.Q19X</td>
<td>exon 1</td>
<td>SNV, transition</td>
<td>a premature stop in codon that encodes the first aa of the C5 β-chain, truncated protein</td>
<td>2</td>
<td></td>
<td>African-American, USA</td>
<td>32</td>
</tr>
<tr>
<td>c.4426C&gt;T, p.R1476X</td>
<td>exon 36</td>
<td>SNV, transition</td>
<td>a premature stop in codon that encodes the 1476 aa of the C5 α-chain, truncated protein</td>
<td>1</td>
<td></td>
<td>African-American, USA</td>
<td>32</td>
</tr>
<tr>
<td>c.48871_73CCC&gt;GC, p.A1624fsX1645</td>
<td>exon 40</td>
<td>indel</td>
<td>frameshift, stop codon, the lack of 50 aa in the C-terminal region covering the NTR/C345C domain</td>
<td>1</td>
<td>Spain</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>c.1115A&gt;G, p.G335AfsX337</td>
<td>exon 10</td>
<td>SNV, transition</td>
<td>mutation in ESE, the lack of exon 10, frameshift, stop codon in exon 11</td>
<td>1</td>
<td>Japan</td>
<td>Turkey</td>
<td>2</td>
</tr>
<tr>
<td>c.4017G&gt;A, p.1289-1339del</td>
<td>exon 30</td>
<td>SNV, transition</td>
<td>splicing mutation, the lack of exon 30, in-frame deletion of 51 aa</td>
<td>1</td>
<td>Brazil</td>
<td>Brazilian</td>
<td>9</td>
</tr>
<tr>
<td>c.892C&gt;T, p.Q298X</td>
<td>exon 9</td>
<td>SNV, transition</td>
<td>stop codon, truncated protein</td>
<td>1</td>
<td>Italy</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>c.1883_84AG&gt;CTCT, p.E628fsX649</td>
<td>exon 15</td>
<td>indel</td>
<td>mutation in ESE, the lack of exon 15, frameshift, stop codon in exon 16</td>
<td>1</td>
<td>Spain</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>c.2536T&gt;C, p.Y846H</td>
<td>exon 20</td>
<td>SNV, transition</td>
<td>amino acid substitution</td>
<td>1</td>
<td>Spain</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>c.1178_81delAAAC, p.T394fsX396</td>
<td>exon 11</td>
<td>indel</td>
<td>frameshift, a premature stop codon in Asp589</td>
<td>1</td>
<td>Netherlands</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>c.4972C&gt;T, p.Q1658X</td>
<td>exon 41</td>
<td>SNV, transition</td>
<td>stop codon, truncated protein</td>
<td>1</td>
<td>USA</td>
<td>African-American, USA</td>
<td>32</td>
</tr>
<tr>
<td>c.3486+1G&gt;T, p.1113-1163del</td>
<td>intron 27</td>
<td>SNV, transversion</td>
<td>splicing mutation, the lack of exon 27, in-frame deletion of 32 aa</td>
<td>1</td>
<td>South Africa</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>c.2348+1G&gt;A, p.Q785YfsX789</td>
<td>exon 18</td>
<td>SNV, transversion</td>
<td>splicing mutation, the lack of exon 19, frameshift, a premature STOP in codon 789</td>
<td>1</td>
<td>Denmark</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>c.1775T&gt;G, p.M592R</td>
<td>exon 14</td>
<td>SNV, transversion</td>
<td>amino acid substitution</td>
<td>1</td>
<td>Norway</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>c.754G&gt;A, p.A252T</td>
<td>exon 7</td>
<td>SNV, transition</td>
<td>amino acid substitution</td>
<td>14</td>
<td>Morocco</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>c.960_962del, p.N320del</td>
<td>exon 9</td>
<td>indel</td>
<td>in-frame deletion of Asp320</td>
<td>2</td>
<td>Morocco</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>c.2607_2608del, p.Ser-870ProfsX3</td>
<td>exon 21</td>
<td>indel</td>
<td>deletion of two adenines, frameshift, stop codon</td>
<td>1</td>
<td>Portugal</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>c.1055A&gt;G, p.Tyr352Cys</td>
<td>unknown</td>
<td>SNV, transition</td>
<td>amino acid substitution</td>
<td>1</td>
<td>Portugal</td>
<td></td>
<td>31</td>
</tr>
</tbody>
</table>

Comments: SNV – single nucleotide variant; aa – amino acid.

Table 2. The clinical and genetic features of selected probands based on the molecular studies of the C5 gene.

<table>
<thead>
<tr>
<th>Mutation (cDNA, protein)</th>
<th>Parental consanguinity</th>
<th>Proband age of onset (years), sex, ethnicity</th>
<th>Infection phenotype</th>
<th>Other</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.48871_73CCC&gt;GC, p.A1624fsX1645</td>
<td>no</td>
<td>4, unknown, Spanish</td>
<td>MS</td>
<td>pneumonia episodes</td>
<td>1</td>
</tr>
<tr>
<td>c.1115A&gt;G, p.G335AfsX337</td>
<td>yes</td>
<td>3, M, Turkish</td>
<td>recurrent MM</td>
<td>headache, fever, vomiting, abdominal purpura</td>
<td>2</td>
</tr>
<tr>
<td>c.4017G&gt;A, p.1289-1339del</td>
<td>yes</td>
<td>3 months, M, Brazilian</td>
<td>recurrent MM</td>
<td>recurrent MM</td>
<td>2</td>
</tr>
<tr>
<td>c.1883_84AG&gt;CTCT, p.E628fsX649</td>
<td>yes</td>
<td>unknown, M, Spanish</td>
<td>recurrent meningococcal infections, (N. meningitidis serogroup B)</td>
<td>unknown</td>
<td>3</td>
</tr>
<tr>
<td>c.2536T&gt;C, p.Y846H</td>
<td>no</td>
<td>12, F, Norwegian</td>
<td>recurrent meningococcal infections from infancy</td>
<td>MM (4 episodes: 1- N. meningitidis, serogroup unk., serogroup Y, serogroup C)</td>
<td>18</td>
</tr>
<tr>
<td>c.3486+1G&gt;T, p.1113-1163del</td>
<td>no</td>
<td>18, F, Danish</td>
<td>MM (2 episodes: 1- N. meningitidis, serogroup B; 2- serogroup unk.)</td>
<td>otitis media</td>
<td>18</td>
</tr>
<tr>
<td>c.2348+1G&gt;A, p.Q785YfsX789</td>
<td>yes</td>
<td>3, M, Saudi Arabian</td>
<td>MS</td>
<td>fever, thrombocytopenia, hypotension, purpuric skin rashes</td>
<td>4</td>
</tr>
<tr>
<td>c.1775T&gt;G, p.M592R</td>
<td>no</td>
<td>7, F, Moroccan</td>
<td>(N. meningitidis, serogroup A)</td>
<td>recurrent IMD</td>
<td>55</td>
</tr>
<tr>
<td>c.754G&gt;A, p.A252T</td>
<td>yes</td>
<td>17 months, F, Moroccan</td>
<td>(N. meningitidis, serogroup E29)</td>
<td>IMD</td>
<td>55</td>
</tr>
<tr>
<td>c.960_962del, p.N320del</td>
<td>no</td>
<td>6, F, Portuguese</td>
<td>(N. meningitidis, serogroup B)</td>
<td>hypotension, generalized purpura fulminans, septic shock</td>
<td>31</td>
</tr>
</tbody>
</table>

sanguineous families. It was also observed that most of the heterozygous relatives of the probands had half the normal serum C5 level, which was sufficient for proper functioning because they were healthy and did not show increased susceptibility to infections.\textsuperscript{4,18,31}

According to the literature, the preventive measures in patients with MAC deficiencies, including C5, are vaccinations against the most common encapsulated bacteria, such as \textit{N. meningitidis}, \textit{Streptococcus pneumonia} and \textit{Haemophilus influenzae}. In turn, the treatment of these patients involves antibiotic prophylaxis, and during active infection it is also possible to administer fresh frozen plasma to replenish the missing C5 component of the complement cascade.\textsuperscript{3,4,31}

5. CONCLUSIONS

(1) The complement component C5, as an element of innate immunity, is not only responsible for MAC assembly, but also has a number of chemotactic, anaphylactic and pro-inflammatory properties, ensuring an appropriate adaptive immune response.

(2) Complement C5 deficiency is a disorder with a diverse molecular basis, and to date, the 18 different mutations in the human C5 gene have been reported to cause hereditary C5D in over 30 families from various geographic regions and ethnic groups.

(3) Molecular diagnostic methods for identifying complement disorders in patients susceptible to \textit{Neisseria} spp. infection appear important not only to prevent recurrence of infection, but also to screen asymptomatic family members and obtain genetic counseling, especially when parental consanguinity is present in the family history.

Conflict of interest
None declared.

Funding
None declared.

References
\textsuperscript{12} Futoma-Kołoch B, Bugla-Płoskońska G. Efektywność bakteriobójczego działania surowicy wynikająca z obecności układu dopełniacza i lizozymu wobec bakterii, które unikają odpowiedzi immunologicznej organizmu [The efficiency of the bactericidal action of serum raised by complement and lysozyme against bacteria which avoid the immunological response of higher organisms]. \textit{Postepy Hig Med Dosw (Online)}. 2009;63:471–484.


