Polymorphism of the Human Complement Component C3- Genetic and Immunological Aspects

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by

Rolf Brönnestam

Umeå 1973
To HELGA, MONICA and CHRISTINA
This dissertation is based on the following publications, which will be referred to in the text by the Roman numerals given below:


PREFACE

Genetic polymorphism is by definition the occurrence in the same population of two or more alleles at one locus, each with a frequency high enough not to be maintained by recurrent mutation only (1). Among the human plasma proteins two major categories of polymorphism have been described, allotypic and electrophoretic heterogeneity. Allotypy is defined by Oudin as individual antigenic differences among proteins within a species (2). The first discovered polymorphism of this category was the Gm system of immunoglobulin G by Grubb (3). The first described electrophoretic heterogeneity in plasma proteins was the Hp (haptoglobin) system discovered by Smithies (4). Since then genetic variants of several other human plasma proteins have been found. This dissertation is concerned with the genetic and immunological aspects of the polymorphism of the third component of human complement, C3.

NOMENCLATURE

In the present study the nomenclature recommended by the World Health Organization Committee on Complement Nomenclature, Boston, 1968, (5) is used. The component C3 is identical to C'3c, C'3 or β1C-globulin in previous nomenclature systems.

The nomenclature for the C3 polymorphism proposed by Azen and Smithies (6) was used in the first two publications (I, II). At the First International Symposium and Workshop on the Polymorphism of the third Component of the Human Complement System, Bonn, June 26-28, 1972, a new nomenclature was recommended which has been used in the subsequent publications. The common phenotypes are called F, FS and S and the corresponding genes C3^F and C3^S. Further details of the nomenclature are given in publication V.
INTRODUCTION

Properties and functions of C3.

The third component of human complement, C3, is a protein with a molecular weight of 185,000. It has the highest serum concentration of the complement components. Most authors have found the level in human serum to be 0.9 - 1.5 mg/ml (cf. 7). The synthetic rate is calculated to be about 30 mg/kg body weight/day (8). C3 is identical to the electrophoretically characterized serum protein $\beta_1$C-globulin (9).

At complement activation a small fragment, C3a, is split off from C3 leaving C3b. This larger fragment, C3b, can be attached to immune complexes as an activated complement component or remain in the fluid phase in a loose complex with C3a as the inactive product C3i (10, 11). Serum enzymes, among them the C3 inactivator, split C3b into two further conversion products, C3c and C3d, electrophoretically known as $\beta_1$A-globulin and $\alpha_2$D-globulin (11, 12). C3 is unstable upon storage and at 37°C it is rapidly converted to C3c and C3d (13, 14). The conversion of C3 is slower at 4°C and at this temperature another conversion product is seen, designated the $\gamma_1$-product by Alper and Johnson (15).

In the "classic" complement reaction C3 is activated by immunoglobulin aggregates through the enzyme C3 convertase (C42). This reaction involves the activation of the earlier reacting complement components C1, C4 and C2 (16). An alternative complement reaction is activation of C3 without involvement of C1, C4 and C2. This is accomplished through the activation of another serum protein, the C3 proactivator (properdin factor B) (17, 18). This mode of activation can be induced by several substances, including endotoxin of Gram-negative bacteria, a protein in cobra venom and yeast cell walls.
A biological effect of C3 activation, by the classical or the alternative pathway, is the generation of anaphylatoxin and chemotactic activity through the fragment C3a (11, 19). A consequence of C3b fixation is further activation of the complement components in the cytolytic reaction through C5 (20). For the immune adherence reaction only fixation of C3 is required (21). An analogous phenomenon is the attachment of immune complexes to phagocytosing cells. Fixation of C3 to these complexes is required for attachment and phagocytosis to occur (22, 23). The attachment is mediated by specific C3 receptors on the phagocytosing cells (24, 25). Other C3 receptors have been demonstrated on B-lymphocytes (24, 26). The biological importance of these is unknown at present.

Hidden antigenic determinants of C3 are exposed upon fixation to immune complexes. Immunoconglutinins are autoantibodies formed in response to these antigens during the course of various diseases (27).

The importance of C3 for the immune defence is illustrated by the two reported cases of C3 deficiency. Both of the patients suffered from repeated pyogenic infections. One of the individuals was homozygous for a "silent" gene at the C3 locus (28). In the other case the C3 deficiency was due to an abnormal metabolism of C3 (29). The serum of this patient showed impaired bactericidal activity for Gram-negative organisms and impaired enhancement of phagocytosis of pneumococci by normal leukocytes.

Molecular variations of C3.

An allotypic variation of C3 was reported in 1965 by Ropartz et al. (30). No further investigations of this system have been published. Electrophoretic variants of C3 were first described by Wieme in 1965 (31). He found some rare cases of sera with protein bands which had either faster or slower mobility than the common C3 component on agar gel electrophoresis. The proteins were labile on storage and they were probably C3. No genetic studies of
these variants were made. Genetically determined variants with a slower electrophoretic mobility than that of the common C3 variant were later reported by Wieme and Demeulenaere (32) and by Wieme et al. (33). Wieme and Segers (34) also observed a genetically determined C3 variant with a faster mobility than that of the common C3 variant. Alper and Propp (35) described a slow migrating variant of C3 using agarose gel electrophoresis.

A genetic polymorphism in C3 was found independently in 1968 by Alper and Propp (36) using agarose gel electrophoresis and by Azen and Smithies (6) using starch gel electrophoresis. Rose and Geserick (37) described in 1969 a polymorphism of stored sera, called the Pt system which was detectable by starch gel electrophoresis. The Pt system was later identified as C3c, the main conversion product of C3 (38). In most Caucasian populations examined so far, two common variants and a number of rare variants of C3 have been found (cf. V). There is good agreement between the results obtained by the methods of Alper and Propp and Azen and Smithies (39). The polymorphism of the conversion product C3c seems to be identical to that of native C3. Agreement in typing results for the common phenotypes between the method of Rose and Geserick and the other methods has also been reported (40). It is, however, not yet settled whether the rare variants can also be identified in the converted form.

Family and mother/child studies performed by several investigators have suggested an autosomal codominant inheritance of the C3 genes (36, 39, 40, 41, 42, 43, 44, 45). No linkage between the C3 system and any other genetic system in human blood cells or plasma proteins has so far been discovered. Association studies have been performed with the ABO, Rh and MN blood group systems as well as the haptoglobin and Gc systems (40, 42), the transferrin groups (40) and the PGM1 system (42). The negative results of the association studies of the C3 system with the ABO, Rh, Hp and Gc systems have been confirmed by unpublished observations from this laboratory.
AIMS OF THE INVESTIGATION

In the present thesis the following major problems were studied:
1. The distribution of C3 phenotypes in different Swedish population groups.
2. The relationship between C3 phenotypes and C3 serum levels.
3. The relationship between C3 phenotypes and stability of C3 upon storage.
4. The relationship between C3 phenotypes and rheumatoid arthritis.
5. The relationship between C3 phenotypes and the titres of antibodies against A and B blood group antigens.
The last two problems were studied in order to elucidate a possible immunological significance of the C3 polymorphism.

MATERIALS AND METHODS

The following series of serum samples have been used:
1. 213 samples of apparently healthy individuals, laboratory personnel or students in Umeå (I, IV).
2. 221 samples from Lappish children attending the nomad schools. Most individuals in this series were genuine nomad Lapps (II). 148 of the samples could be classified. In the rest the C3 zones were not detectable due to conversion.
3. 1196 samples from blood donors living in the areas of Gällivare, Umeå, Gothenburg, Linköping and Lund (III, V, VI, VII).
4. 545 samples of sera sent to the Department of Clinical Bacteriology in Umeå for serological tests of rheumatoid serum factors. Medical records of the patients were collected, the individuals scored according to the CIOMS (Rome) criteria for rheumatoid arthritis, (46) and grouped into three categories: 180 patients with probable, definite or classic rheumatoid arthritis, 24 patients that could not be classified, 341 patients with a lower score than
"probable rheumatoid arthritis", non-rheumatoid patients (VI).

5. 251 samples from newly delivered mothers. This material was selected because of previously recorded hemolysis at the ABO-grouping, Rh-negativity, suspected or proved immunization in any blood group system (VII).

The following methods have been used:
1. C3 phenotyping by high voltage agarose gel electrophoresis according to Berg et al. (47) and Teisberg (48).
2. Immunochemical quantitation of C3 by electrophoresis in agarose gel containing antibodies according to Laurell (49) (III).
3. Determination of the conversion rate of C3 by storage at 4°C followed by antigen-antibody crossed electrophoresis according to Laurell (50) (IV).
4. Production of rabbit antiserum to human C3 using a modified form of the technique described by Mardiney and Müller-Eberhard (51) (III, IV).
5. Demonstration of rheumatoid serum factors by the Waaler-Rose test (52) (VI).
6. Determination of the antibody titres against A or B blood group antigens by agglutination at different temperatures (VII).

RESULTS AND DISCUSSION


The results of the C3 typing of the different series of serum samples from Sweden are summarized in the table together with the results of the studies so far published from the other Nordic countries. Among the non-Lappish population the frequency of the C3F gene varied between 0.14 and 0.23. The observed numbers of phenotypes in all series investigated are in good agreement with the expected ones assuming a Hardy-Weinberg equilibrium. Within Sweden there are small differences in the frequency of the gene C3F in the non-Lappish population from different areas (V).
<table>
<thead>
<tr>
<th>Population</th>
<th>Number of individuals</th>
<th>C3S</th>
<th>C3F</th>
<th>Rare genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Lappish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>406</td>
<td>0.815</td>
<td>0.182</td>
<td>0.001</td>
<td>(43)</td>
</tr>
<tr>
<td>Finland</td>
<td>1034</td>
<td>0.8298</td>
<td>0.1702</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>136</td>
<td>0.8419</td>
<td>0.1581</td>
<td>0.0184</td>
<td>(47)</td>
</tr>
<tr>
<td>Norway</td>
<td>2454</td>
<td>0.7865</td>
<td>0.2135</td>
<td>0.0053</td>
<td>(53)</td>
</tr>
<tr>
<td>Sweden</td>
<td>213</td>
<td>0.770</td>
<td>0.230</td>
<td>0.0084</td>
<td>(1)</td>
</tr>
<tr>
<td>Sweden</td>
<td>1196</td>
<td>0.7972</td>
<td>0.2028</td>
<td>0.0084</td>
<td>(V)</td>
</tr>
<tr>
<td>Lappish</td>
<td>576</td>
<td>0.9505</td>
<td>0.0495</td>
<td>0.0084</td>
<td>(54)</td>
</tr>
<tr>
<td>Finland</td>
<td>198</td>
<td>0.9369</td>
<td>0.0631</td>
<td>0.0084</td>
<td>(55)</td>
</tr>
<tr>
<td>Norway</td>
<td>148</td>
<td>0.973</td>
<td>0.027</td>
<td></td>
<td>(11)</td>
</tr>
</tbody>
</table>
Also in other countries in Europe and among Caucasians in North-America the estimated frequencies of the gene \( C^3 \) are very similar, around 0.20 (cf. V).

In the Lappish population of Finland, Norway and Sweden the gene \( C^3 \) is rare with a frequency varying between 0.03 and 0.06. The results given in the table show a highly significant difference \((P<0.0005)\) between the Lappish and non-Lappish population. This result adds to the picture of the genetic uniqueness of the Lapps.

A series of studies on non-Caucasian populations (cf. V) have so far indicated that the gene \( C^3 \) may be polymorphic only among Caucasians.

A number of rare variants of C3 have also been discovered in Sweden (V). As in Norway (53) the most common of these was the variant F 0.5 with a mobility slightly faster than that of the common variant F.

2. Relationship between C3 phenotypes and C3 levels (III).

Genetically controlled variations of the C3 concentration in serum caused by a "silent" allele at the C3 locus have been described previously (28, 56). In an early report on the C3 polymorphism Alper and Propp (36) studied the C3 level in relation to the common phenotypes. They found large numerical differences between the means of the serum concentrations. The differences were not statistically significant but the material was small and the standard deviations large. In the present study the range of the C3 concentration level was 52-147 mg/100 ml and the mean value 87.8 mg/100 ml with a standard deviation of 16.1 mg/100 ml. No differences between the three common phenotypes were found. The results are in agreement with a study performed independently by Agarwal et al. (57). A number of rare C3 phenotypes have also been reported to have normal concentrations of C3 (36, 58, 59, 60).
The studies referred to above were performed employing quantitation by immunochemical methods. Such methods are, however, not always sufficient in studies of complement components. An example is given by Miller and Nilsson (61) who found a hereditary defective function of C5 in spite of normal serum levels as determined by quantitative immunodiffusion and normal immunoelectrophoresis and Ouchterlony analysis. Functional studies of the C3 variants have so far showed no differences concerning the hemolytic activity (62). A small number of sera have also been studied by immune adherence titration (36) which showed no differences between the C3 variants. The studies of C3 activity so far published are, however, too limited to exclude the possibility of functional differences between the C3 variants.

3. Relationship between C3 phenotypes and C3 stability (IV).

A characteristic property of the protein C3 is its instability upon storage. Unpublished observations in this laboratory have revealed great differences in the conversion rate in vitro between sera from different individuals.

The present study was performed to investigate the relationship between C3 stability and C3 phenotypes. 56 sera were studied after storage at 4°C, no significant differences in the conversion rate between different C3 phenotypes were found.

The results of the present study are of practical importance when related to the method of C3 phenotyping described by Rose and Geserick (37). In this method the C3 types are determined after storage and conversion of C3. A difference in the conversion rate between the variants F and S could lead to erroneous results in cases of incomplete conversion.

4. Relationship between C3 phenotypes and rheumatoid arthritis (VI).

Among the rheumatoid arthritis patients studied the frequency of the gene C3^F was 0.250, among the non-rheuma-
toid patients 0.170 and among healthy blood donors from the same region 0.191. The differences between the rheumatoid and non-rheumatoid patients and between the rheumatoid patients and blood donors were statistically significant ($\chi^2 = 9.49$, 1 d.f., $P<0.005$, respectively $\chi^2 = 5.89$, 1 d.f., $P<0.025$). Independently Farhud et al. (63) have reported an increase of the phenotype F among rheumatoid patients, thus confirming the results of the present study.

The C3F gene seems to be associated with clinically diagnosed rheumatoid arthritis but not with the occurrence of rheumatoid serum factors since seropositive non-rheumatoid patients had a low frequency of the gene C3F (0.077).

Many investigators favour the hypothesis of an infectious aetiology of rheumatoid arthritis, e.g. Svartz (64) and others. Consequently a somewhat impaired immune defence mechanism might be a predisposing factor for the development of rheumatoid arthritis. Since C3 is an important part of the immune defence system, a difference in the biological function or the efficiency of the C3 variants might cause an increased susceptibility to some infections for some individuals and therefore also to rheumatoid arthritis. As a working hypothesis it is proposed that the C3 variant F might be inferior to S concerning the immune defence.

Several different observations of impaired immune mechanisms in patients with rheumatoid arthritis have also been reported, though none with obvious connection to the complement system. Thus there have been reports of association between agammaglobulinemia and rheumatoid arthritis (65), impairment of the chemotaxis of the leukocytes (66), impaired response to a chemical sensitizing agent and impaired antibody response in patients with seropositive rheumatoid arthritis (67), impaired mixed leukocyte reaction with cells from rheumatoid patients (68) and impaired response to stimulation of lymphocytes with phytohemagglutinin (69).
Since complement is involved in the immunological reactions resulting in local tissue injury in rheumatoid arthritis (70), a different efficiency of the C3 variants in these reactions could provide alternative explanations to the present results.

If the working hypothesis that the variant F is inferior to S is correct then an increased frequency of F would be expected in some other groups of patients with infections or impaired immune mechanisms. The observation by Farhud et al. (63) of an increased frequency of the phenotype F in a series of hepatitis patients is of interest in this connection.

5. Relationship between C3 phenotypes and antibody titres (VII).

The relationship between C3 phenotypes and antibodies against blood group antigens A and B was studied in sera from newly delivered mothers and in blood donor sera. The frequency of the gene C3^F in the group of 64 mothers with high titres of anti-A or anti-B (≥1/128) was 0.164 and in the group of 87 mothers with low titres (≤1/16) 0.264. This difference was statistically significant ($\chi^2 = 4.30$, 1 d.f., $P<0.05$). The difference was most pronounced among mothers with incompatible infants. When only the incompatible antibodies were taken into consideration the difference between the groups with high and low titres was larger ($\chi^2 = 10.16$, 1 d.f., $P<0.005$). No association between C3 phenotypes and titres of isoagglutinins in the blood donor sera was found.

It was assumed that the observed association between C3 phenotypes and blood group antibodies in the maternal material concerns the "immune" antibodies against A and B antigens and not the isoagglutinins. This assumption was supported by the fact that the material was selected, many of the sera had hemolysins and a large proportion of the mothers belonged to blood group O.
The importance of C3 for the antibody production is not known. The observation by Ellman et al. (71) that C4-deficient guinea pigs had an impaired antibody synthesis against 2 of 3 tested antigens might, however, suggest a role for the complement system in the antibody response.

The observation in the present study that the gene C3^ is associated with higher antibody titres supports the working hypothesis that the gene C3^ is superior to C3^ in the immune defence mechanism.

SUMMARY AND CONCLUSIONS

In different Swedish subpopulations the frequency of the gene C3^ varied between 0.18 and 0.23. No significant differences between materials from different areas were found. The Swedish frequency figures were similar to those found in other Caucasian populations. Four different rare variants were found, as in Norway, the most frequent of these rare alleles was C3^.

Swedish Lapps had a very low frequency of the gene C3^, around 0.03. These results have been confirmed by studies of Norwegian and Finnish Lapps.

The C3 level measured by electrophoresis in agarose gel containing antibodies showed a range of 52-147 mg/100 ml with a mean and standard deviation of 87.8 ± 16.1 mg/100 ml. No significant difference was found between the C3 levels of the sera of the three different phenotypes.

The stability of C3 in different sera was estimated by means of antigen-antibody crossed electrophoresis after storage at 4°C. No relationship between conversion rate in vitro and C3 phenotype was found.

The distribution of C3 phenotypes was studied in sera from patients with clinically diagnosed rheumatoid arthritis. In
comparison with non-rheumatoid patients and blood donors
the rheumatoid patients had a significantly higher frequen-
cy of the gene $C3^F$. The presence of rheumatoid factors was
not associated with the $C3$ types.

The relationship between $C3$ phenotypes and antibodies
against blood group antigens A and B was studied in sera
from newly delivered mothers and in blood donor sera. Among
the mothers an association was found between high antibody
titres and the $C3^S$ gene. This relationship was most pronoun-
ced in cases of ABO incompatibility between mother and
infant thus suggesting an association between $C3$ types and
immune antibodies. Among the blood donors no relationship
was found between $C3$ type and isoagglutinin titre.

A working hypothesis is presented suggesting that the
variant $F$ might be inferior to $S$ in some immune defence
mechanism. The presence of $C3^F$ might thus cause an increa-
sed susceptibility to some infections and, theoretically,
to rheumatoid arthritis. The observation of an association
between high antibody titre and $C3^S$ might also fit into
this hypothesis.

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REFERENCES


