CHAPTER II

REVIEW OF LITERATURE

Structure of C4

The fourth component of complement (C4), a serum glycoprotein, of which 7% are carbohydrate, is primarily synthesized in the liver by extrahepatic monocytes and macrophages, as a single chain promolecules called pro - C4 (23). The synthesis of pro-C4 is directed by a polyadenylated mRNA of approximately 5.6 kilobases coding for 1722 amino acids (24). The promolecule of C4, an approximate 200 kilodaltons (Kd) glycoprotein (25), was then processed by two intracellular cleavage to yield the secreted three-subunit molecule ($C4^S$) (26,27). This three-chain disulphide-linked structure consists of an alpha chain (95,000 M_r), a beta chain (75,000 M_r), and a gamma chain (35,000 M_r) (25) (Figure 1). The beta chain is equivalent to the amino-terminal subunit of pro-C4, the alpha chain, the central, and the gamma chain, the carboxy-terminal. These three chains are separated in the precursor protein by arginine - rich intersubunit linking peptides similar to the linking peptide between the beta and alpha subunits of pro-C3 and pro-C5 precursors of the third and fifth complement components (27). Post synthetic processing of pro - C4 involves excision of the linking peptide , sulfate modification of residues within the region of the thiolester site of alpha chain and glycosylation of alpha and beta chains. Finally, extracellular modification of secreted form of C4 ($C4^S$) by removing a 5 Kd fragment from the carboxy-terminal of alpha chain yields the plasma form, $C4^P$ (28,29).

In the classical pathway of complement activation (30), C4 is cleaved by C1S, a subcomponent of activated $C\bar{1}$, into two fragments, C4a and C4b. The larger fragment, C4b, or activated C4, is composed of beta and gamma chains, and the carboxy-terminal portion of alpha chain. It has transient ability to bind to the Fc part of antibody and must therefore be very close to both antibody and antigen. The C4 molecule forms a covalent bond with antibody or cell surface through a reactive acyl group released on activation by C1, from an intra-chain thioester bond.

When $C\bar{4}$ is bound, C2, also activated by proteolysis by $C\bar{1}$, interacts non-covalently with C4 to give the active protease $C\bar{4}\bar{2}$, or C3 convertase, which activates C3. Activated $\bar{C3}$, binds to the $\bar{C4}\bar{2}$ complex to give $C\bar{4}\bar{2}\bar{3}$, or C5 convertase, which activates C5 and initiates the formation of the lytic complex of complement system (Figure 2). Complement activation is limited in several ways of which one is the half life of C3 convertase. The other is the degradation of C4 by an enzyme factor I and its protein cofactor, C4 binding protein (C4bp), yielding C4c and C4d fragments (Figure 3).

C4 has a central role in the activation of complement by the classical pathway. Dissolution of immune aggregates by complement and uptake by phagocytic cell depends on the binding of C3 and C4 to the aggregates and on the presence of receptors for bound C3 and C4 and their breakdown products on macrophages and other cells. The quantity of bound C3 will depend on the efficiency of the C3 convertase and hence indirectly on the C4 surface. Thus reactions of C4 with

antibody, cell surface molecules, C1, C2 and C3, both as substrate and as component of C5 convertase, factor I, C4 bp and also C4 receptor will depend on the pattern of amino acid residues on the surface of the C4 molecule and if all the polymorphic forms recognized so far do have different amino acid sequences the strength of binding or rate of proteolysis is likely to vary between the different forms (8,9).

The Structural Polymorphism of C4 Protein.

Polymorphism in human C4 was first detected by Rosenfeld and coworker in 1969 (31), who used two-dimensional immunoelectrophoresis or prolonged agarose gel electrophoresis of EDTA-plasma followed by antigen-antibody crossed immunoelectrophoresis to define C4 patterns. They noted differences in individuals but were unable to construct a simple genetic model to explain these differences in populations or in families, suggesting genetic polymorphism.

Teisberg et al, 1976, (32) using high voltage agarose gel electrophoresis followed by immunofixation with specific C4 antibody, postulated the structure locus determining an electrophoretic variation in C4 as two common alleles at a single locus, $C4^F$ and $C4^S$. This has been shown to be HLA linked loci. Nevertheless, various attempts to resolve these patterns consistent with Mendelian inheritance of alleles at a single locus has failed (33,34). O' Neill et al 1980 (35) also used agarose gel electrophoresis with a discontinuous buffer, intrepreted the electrophoretic pattern in a completely different way. They stated that only three kinds of patterns could be reproducibly observed : C4F, C4S and C4FS, and they

concluded that C4F and C4S could not be produced by C4 alleles at a single locus. They then postulated that the genetic loci controlling the fourth component of human complement was two distinct, closely linked loci, C4F and C4S, controlling an electrophoretically fast and slow form respectively, and that deficiency state for one or the other of these loci were common but rarely occur together. Furthermore, O'Neill and co-worker (36) also made the very important observation that two blood groups, Chido (Ch) and Rodgers (Rg) antigenic determinants, previously shown to be closely linked to HLA were carried on the C4 molecule. In this model, the C4F individuals exhibit Rodgers serological activity or Rg (a+) Ch (a-), the C4S individuals, Chido activity or Ch (a+) Rg (a-) and the C4FS individuals, Rg(a+) Ch(a+). In addition, Rodgers and Chido antigenic determinants expressing C4F and C4S respectively reside in the proteolytic degradation fragment of C4 molecule, C4d (37,38), which is in accordance with Mevag's finding that the part of the C4 molecule determining the electrophoretic polymorphism lies in the C4d fragment (39).

Awdeh and Alper improved the technique in determining the extent of structural polymorphism of the two C4 loci by pretreatment of plasma or serum with neuraminidase (40). This enzyme was used to reduce the heterogeneity of the C4 molecule by removing sialic acid and increased resolution of C4F and C4S that partially overlap in the electrophoretic pattern. In addition, they proposed the C4 nomenclature that the protein of which most variants have more acid

(anodal) or fast migration (C4F) be called C4A, the more basic (cathodal) or slow migration (C4S), C4B (41).

On the basis of charge separation by eletrophoresis, more than 35 alleles (13 alleles of C4A and 22 alleles of C4B) have been identified , including non expressed or "null alleles" at each locus (10). These null alleles (or silent genes) corresponding to the absent (deletion) gene are designated by the abbrivation "QO" (Quantitative Zero).

<u>The Two Isotypes of the Fourth Complement Component.</u>

The molecular basis for the two isotypes of C4, C4A and C4B have shown marked differences in their structure and function.

Lundwall et al 1981 (38) have separated and distinguished large homologous tryptic peptides from different forms of C4, approximately equivalent to the C4d fragment, by size and by ability to inhibit anti Rodgers or anti Chido antiserum. They were able to demonstrate that a tryptic C4d fragment of 30,000 M_r carried the Rodgers (C4A) and that of 28,000 M_r carried the Chido antigen (C4B). This finding agreed with Roos et al 1982 (42) who demonstrated that the alpha chain of C4 carrying the Chido determinant (96,000 M_r) differ in molecular weight from that carrying the Rodgers determinant (94,000 M_r). The apparent 2000 daltons molecular weight difference of the two alpha chains resides in the C4d fragment. Moreover, amino acid sequence analysis of the fragment of C4A and C4B from pooled serum showed the difference between C4A and C4B (43). The specific characteristics of

the two classes of C4 found from the derived amino acid sequence have been called the class-specific difference or isotypic difference (Table 1).

The functions of the products of the C4A and C4B loci differ considerably, for instance, both forms exhibit a difference in thiolester reactivity.(11,12,13) C4A binds preferentially to amino groups of peptide antigens or nucleophiles and C4B more efficiently binds to hydroxy groups of carbohydrate antigen. Thus, hydralazine (an amino group nucleophiles) binds more efficienly to C4A than C4B (44). The intrinsic difference in covalent binding affinity also results in 3-to 4-fold higher binding of C4B to antibody-coated red blood cell which explains its well-known higher activity in the classical haemolytic assay (41,45,46).

The molecular basis for the difference in immune haemolysis is that once C4 has been cleaved by activated C1, the covalent binding of nascent C4b to cell surface or insoluble protein involves a specific internal thioester bond located in the alpha chain (47). This thioester may transacylate onto hydroxyl or amino groups present on the acceptor surface or protein to form ester and amide bond respectively. Since C4B forms preferentially ester bond, its more efficient haemolytic activity implied that the red cell surface have abundant hydroxyl acceptor sites, whereas the lower C4A binding is explained by less numerous amino group on erythrocyte membrane (13).

The two forms of C4, C4A and C4B, have been shown to differ not only in their capacity to mediate hemolysis but also in their ability to inhibit immune precipitation (11,12,14). While C4B was more efficient than C4A in the haemolytic assay, the reverse was true in inhibition of immune precipitation. Thus, C4A is likely to be preferentially involved in immune complex clearance reaction (12). For instance, two C4 allelic forms, C4A3 and C4B1 have been compared for the solubilization and inhibition of immune complex formation. Both appeared to be equal in their ablity to solubilize immune complex but differ with respect to the inhibition of immune complex formation. C4A3 was 1.5 to 2-fold more efficient in inhibiting immune precipitation than C4B1 (11). Nevertheless, there was no difference between the two isotypes in reaction with other complement component such as activation by C1 esterase, interaction with C2 to give C3 convertase, nor inactivation by C4 bp and factor I (11,13).

C4 Gene.

The region on the short arm of human chromosome 6 encoding class I and class II antigens of the major histocompatibility complex (MHC)involved in immune recognition also encodes a group of molecules termed class III which includes C2, factor B (BF), C4A and C4B. The four genes encoding the complement proteins occupy about 120 Kb of genomic DNA between HLA-B and HLA-DR. C2 and factor B are less than 1Kb apart, and are about 30 Kb from the two C4 genes which are separated by about 10 Kb (48,49,50). Interestingly, a gene coding for

steroid 21-hydroxylas (21-OH) was later identified at less than 2 Kb 3' to each C4 gene (50,51,52)(Figure 4).

Of the human C4 A genes analyzed to date, all are about 22 Kb, as are some C4B (C4B long); however, about 45% of C4B gene are only 16 Kb in size (C4B short) (53,54). The difference in size can be explained by a single large intron (6-7Kb) located about 2-2.5 Kb from the 5' end of the gene (53,54,55).

The Genetic Polymorphism of C4.

The primary structure of C4 was determined using recombinant DNA techniques to clone and sequence cDNA prepared from C4 mRNA (24,56,57). Complete comparison of nucleotide sequence of C4A and C4B demonstrated that there were only 14 nucleotide sequence differences out of more than 4600 compared between C4A and C4B (24,57). Twelve of the differences were clustered in the C4d region, which contains the internal thiolester, and the other two were in the beta and gamma chain respectively. Of the 12 nucleotide differences in C4d, nine resulted from codon changes leading to amino acid substitutions at positions 1101, 1102, 1105, 1106, 1188 and 1191 (24,57)(Table 1).

The structural basis for the polymorphism of human C4 was further determined in order to correlate the structure and function of the two C4 isotypes particularly with respect to the structural basis of the differential thiolester reactivity and the antigenicity of Rh/Ch blood group (55,58). It had been previously shown that there are

two Rodgers (Rg:1,2) and 6 chido (Ch:1,2,3,4,5,6) antigenic determinants (59). For example, C4 A3 was deduced to be Rg:1,2 / Ch:-1,-2,-3,-4,-5,-6 and C4B1 Rg;-1,-2/Ch;1,2,3,4,5,6. On the other hand C4A1 and C4B5 are exceptional variants which possess the class-specific chemical reactivities, but express essentially the reversed antigenicities. Thus, C4A1 was typed Rg :-1,-2 / Ch :1,-2,3-4,5,6 whereas C4B5 as Ch :-1,-2,-3,4,-5,6 and Rg positive. By comparing the structural data of C4A3 and C4B1 with C4A1 and C4B5, C4A and C4B can be defined by only four isotypic amino acid differences at position 1101-1106 of the pro-C4 molecules. Over this region C4A has the sequence of PCPVLD (Proline-Cysteine-Proline-Valine-Leucine-Aspartic) while C4B the sequence of LSPVIH (leucine-Serine-Proline-Valine-Isoleucine-Histidine) (55). The four amino acid differences at position 1101-1106 are solely responsible for the difference in thiolester reactivity between the C4A and C4B isotypes (55,58,60). However, the Rodgers/Chido antigenicity are not related to the four amino acid differences at position 1101-1106. Instead, the expression of Rg/Ch antigenicity is partly determined by amino acid sequence of VDLL (Valine-Aspartic-Leucine-Leucine for Rg(+) and ADLR (Alanine-Aspartic-Leucine-Serine) for Ch(+) at position 1188-1191 (55,58).

The C4d isotypic region (1101-1106) is solely responsible for modulating the thiolester reactivity although the putative thiolester and the isotypic residues are separated by 106 amino acid. It is likely that the proposed conformational change during activation by C1 would bring the isotypic and thiolester residue close together for contain interaction. The negatively charged Aspartic acid (1106) of

C4A might increase the nucleophilicity of the nearby amino/amine groups towards the thiolester carboxyl. This may provide an explanation for the higher covalent binding affinity of C4 A to peptide antigen. By the same analogy, the positively charged histidine (1106) of C4 B might participate in hydrogen bonding charge shift in hydroxyl groups of carbohydrate antigen. Beside the Aspartic acid/Histidine isotype (1106), the other three isotypic residues and the sequence nearby might be important in modulating the thiolester reactivity as well. Subsititution of Cysteine for Serine at residues 1102 reduced the hemolytic activity to 2- fold but did not result in an increased binding to immune aggregate (60). Interestingly, a Proline/Leucine interchange at position 1101 resulted in a shift of 2,000 M_r of the alpha chain on SDS-polyacrylamide gel electrophoresis, yet it did not appear to affect hemolytic activity (60). In addition, the Aspartic acid/Histidine change at position 1106 may contribute to the charge difference between the respective C4 A and C4 B that resulted in the electrophoretic mobility difference.

Furthermore, comparison of the nucleotide sequence of the C4d region of three C4 A and three C4 B showed that allotypic difference may be due to single amino acid substitution (60). Based on known haplotypes from the constructed libraries, tentative assignments of allotypes were made (Table 1). One possible allelic difference was observed between the two C4A sequences at position 1182. C4A3 showed Serine while C4A4, Threonine. The Serine - Threonine difference propabably represents an allelic variation between the C4A4 and C4A3. In addition, position 1054 in C4B was identified in which

Aspartic acid in C4B2 was substituted for Glycine in C4B1, explaining the faster mobility of C4B2.

Molecular Basis for C4 Null Allele

Genetic studies in human have demonstrated that deficiency in the serum of either C4A or C4B is relatively common. O'Neill et al has defined the absence of C4A or C4B protein in the serum as the result of a null allele, C4A*Q0 or C4B*Q0 (35). The molecular basis for the deficiency was not known, but Raum et al (1984) proposed that the null allele was due to deleted gene resulted from unequal crossover (61)(Figure 5). Thus, some chromosomes would have only a single C4 locus and other would have three loci encoding C4. Alternatively, C4 was not duplicated on all chromosome. In order to understand the molecular basis for the null alleles at the DNA level, haplotypes with either C4A*Q0 or C4B*Q0 were analyzed by the technique of Southern Blot, using the C4 and steroid 21 - hydroxylase (21-OH) probe. It was able to show that about half of the null alleles are the result of deletion of a 28 Kb of DNA (53,54). Although the precise limits of deletion have not been determined, they appeared to include either the 21 - OHA or 21 - OHB gene. The three common deletions mapped include the C4A and 21 - OHA,C4B and 21 - OHA,C4B and 21 - OHB gene. Deletion of C4A and 21 - OHB together has not been observed (54).

Unequal crossing-over mechanism has been suggested to account for the generation of deletion and duplication. An alternative hypothesis first suggested by Palsdottir et al is that the non-deleted null allele are transcriptionally active but have been converted to code for a product similar to the adjacent locus. Thus, both loci encode proteins of the same isotype (50).

C4 and Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of unknown origin that may affect many different organs (62). It is an autoimmune disease charaterized by disorder throughout all compartments of the immune response (63,64,65). Serologically, autoantibodies against components of the cell nucleus including double-stranded DNA, single-stranded DNA, RNA, nucleoproteins and histones can be detected. Other serological abnormalities include raised levels of circulating immune complex, hypocomplementaemia and hypergammaglobulinemia. At the cellular level, several independent defects are evident including B cell hyperactivity, a loss of T suppression and a decrease in the numbers of post-thymic precursor cells. In addition, both the natural killer cell (NK) and the reticuloendothelial system have been shown to have impaired functions. Some of these defects may be due to a loss of responsiveness or production (or both) of the immunomodulating factor, interferon and interleukin 2.

The etiologies of SLE and other lupus like disease are unknown. However, evidence from both human and animal studies suggest that multiple factors including genetic, endocrine and environmental elements are involved (63,65).

Genetic factors have long been suspected to be involved in the development of systemic lupus erythematosus (SLE). Indirect evidence has been derived from : (1) family studies showing multiple members affected with SLE, other autoimmune disorders, and autoantibodies, (66,67). (2) Twins studies demonstrating a greater concordance for SLE in monozygotic twins (68). (3) Population studies reporting that approximately 7-12% of patients with SLE have another first-or second-degree relative with SLE (67). Moreover, other evidence is derived from the association between SLE and genetically determined antigens of the HLA system (15,19,20,69) (Table 2).

Studies from Hauptman et al, Ballow et al, Urowitz at al, Kjellman et al and Marcart-Lemone et al, suggested that SLE is associated with hereditary deficiencies of the fourth component of complement (70,71,72,73,74). According to Hauptman, thirteen of the 17 subjects with homozygous C4 deficiencies have had SLE or lupus like disease (74). Although total C4 deficiency is rare (75),partial genetic deficiency of C4 occurs in 30-40% of the Caucasian population and has also been associated with susceptibility to SLE (15,19,76,77). Several studies have shown an increased frequency of C4A null allele (C4A*QO) in Caucasian and Black patients with SLE and that appeared to be one risk factor for SLE (15,19,76,78). According to Bruun et al,

and Awdeh et al, C4A*QO linked to certain extended HLA haplotypes, particulary HLA-A1, B8, DR3 (79,80). In addition, HLA-B8, DR3 have been reported to be in linkage disequilibrium with the C4A*QO in Caucasian SLE (19,78,81). In contrast, there is evidence of an increase in the frequency of C4A*QO in Chinese SLE although DR3 was of low frequency indicating that no DR3 association was present (69). Unlike the HLA-DR and DQ association in SLE, C4A null allele was significant in Caucasian, Black, Chinese and Japanase patient with SLE (19,22,68,82). Furthermore, when the two risk factors DR2 and C4A*QO, occured simultaneously, they appeared to act synergistically, increasing the relative risk (19). Similarly, it has been found that if two C4 null alleles occur in combination (C4A*QO, QO), it markedly increases the relative risk supporting the significance of the C4A null allele in SLE (19).

The molecular genetics of C4 null allele and C4 deficiency of patients with SLE further analyzed by Southern blotting technique revealed that the common cause of the C4A null phenotype is the deletion of the C4A gene and 21-hydroxylase A gene (81,83,84).

C4 and Rheumatoid Arthritis (RA)

Rheumatoid arthritis (RA) is a chronic relapsing inflammatory disease usually affecting multiple diarthrodial joints with varying degree of systemic involvement (64,65). Females are more affected than males by a three to one margin and the prevalence increases with age, peaking at 35 to 45 years of age.

Rheumatoid arthritis is probably the best known of the autoimmune rheumatic disease. Immunological abnormalities are evident, the most remarkable being the presence of autoantibodies, rheumatoid factor (RF), antinuclear antibodies (ANA), immune complex in the joints and characteristic complement level.

Immunologic reaction appears to play a major role in the perpetuation of rheumatoid inflammation. The interaction of IgG and rheumatoid factor set in the rheumatoid synovial menbrane and joint fluid. The sustained formation and presence of immune complexes in the joint can lead to a series of pathological event giving rise to joint damage and eventual destruction. The complex fixes complement by either the classical or alternative pathways. The subsequent release of biologically active fragments recruits polymorphonuclear cells into the intraarticular space in an attempt to phagocytose and eliminate the immune complexes. Inflammation is a product of this reaction. In addition, the complexes may induce the release of hydrolytic and proteolytic enzymes from lysosomes. These enzymes are believed to play a major role in the proliferative and destructive change characteristic of rheumatoid joint disease.

The etiology of rheumatoid arthritis remains unknown. However, genetic factor may contribute to the pathogenesis of the disease. As suggested by Grennan et al, part of this genetic susceptibility may be accounted for by genes within the HLA region, and a class II MHC antigen, HLA-DR4, which increases the relative risk for rheumatoid arthritis by up to fourfold (85). The association of

HLA-DR4 in RA patients is found in many ethnic populaitons including Caucasian, Oriental and Black North Americans. However, a second, weaker association with HLA-DR1 has been seen in different ethnic groups, for instance, in the Asian Indian populaiton (86). Furthermore, various subtypes of DR4 exist, only some of which was found associated with RA. DNA sequence analysis of the DR-beta chain genes encoding the DR4 subtypes and DR1 revealed that similar third hypervariable regions sequences are found on RA - associated DR - beta chain alleles (87). This led Gregerson and cowerkers to hypothesized that genetic susceptibility for RA is due to a group of similar third hypervariable region sequences that share conformational and functional attributes important in antigen presentation and immune regulation.

It has been observed that there is a high incidence of a rare C4B variant closely resembling the C4B3 variant in RA (16,17). This C4B variant was further differentiated from C4B3 by a slightly cathodal mobility and was termed C4B 29 (Perth). In contrast, Thomson et al reported no such increase (88). However, they did find an association between the C4B null allele (C4B*QO) and Felty's syndrome, an uncommon complication of rheumatoid disease characterized by a combination of chronic arthritis, leukopenia and splenomegaly. Moreover, the association between C4B*QO and HLA-DR4 in Felty's syndrome have been found; 19 of 20 Felty's patients were DR4-positive and 11 of 19 DR4-positive arthritis subjects typed for C4B*QO (89). Both rheumatoid arthritis and Felty's syndrome has been described previously that they are associated with the presence of HLA-DR4(90).

As a result, Thomson suggested that C4B*QO may identify individuals within the rheumatoid population at risk of developing Felty's syndrome. They also found that in all cases where haplotypes could be assigned, DR4 and C4B*QO were in "cis" haplotypic combination.

In 1986 Tokunaga (22) and Takeuchi (91) described a significant increase in both C4A*QO (32% versus 12%) and C4B*5 (36% versus 17%) associated with HLA-DR4 in 157 japanese rheumatoid arthritis patients but no significant difference concerning C4B*QO between RA patients and controls was observed. Whether C4 gene is really involved in susceptibility to rheumatoid arthrithis remains to be elucidated.

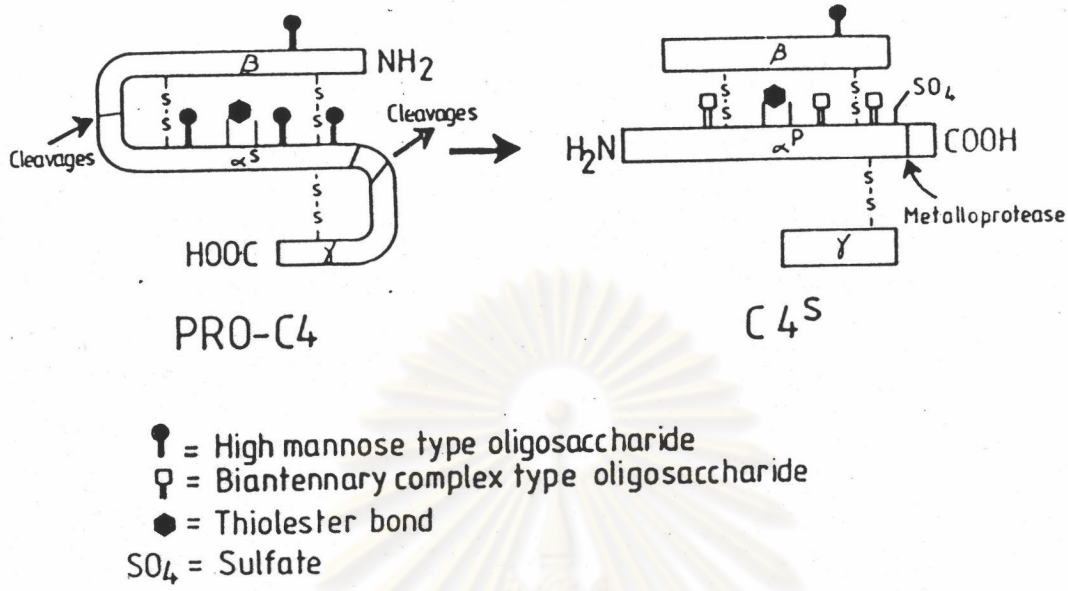

Figure 1 Structural features of human C4. Pro C4 is synthesized as a single peptide chain of 1722 amino acids and is processed by proteolytic removal of a leader peptide, short basic peptides between the beta and alpha, alpha and gamma chains to give the three chain structure and subsequently further hydrolysis of a peptide from the C-terminal end of the alpha chain. It is glycosylated by two types of polysaccharide on asparagine residues as shown, a thiolester bond is formed and a sulfate residue is added.

From : Chan, A., J. Immunol., 134, 1790 - 1798, 1985.

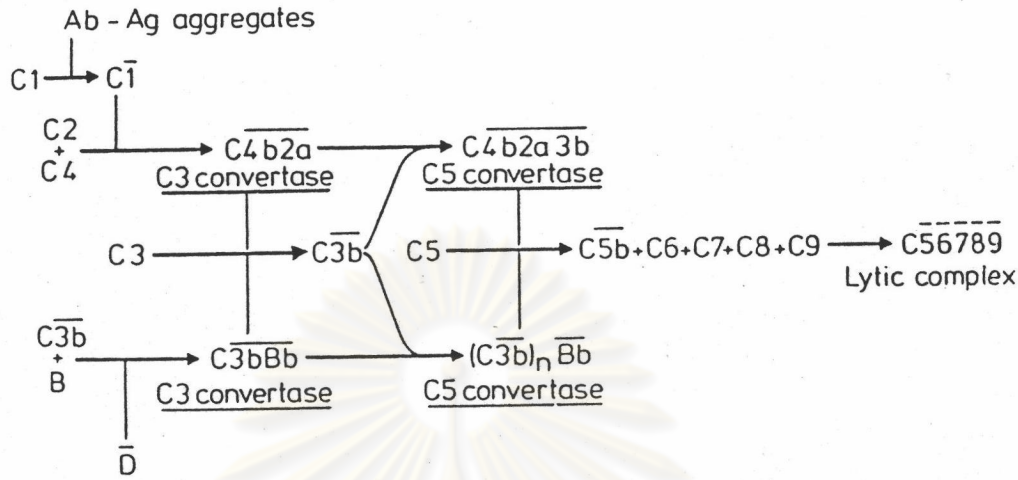

Figure 2 Activation pathway of Complement. The upper section shows the classical pathway activated primarily by antibody - antigen aggregates, and the lower section shows the alternative pathway activated by antibody - antigen aggregates or polysaccharides.

From : Reid, K.B., Ann. Rev. Biochem., 50, 433-464, 1981.

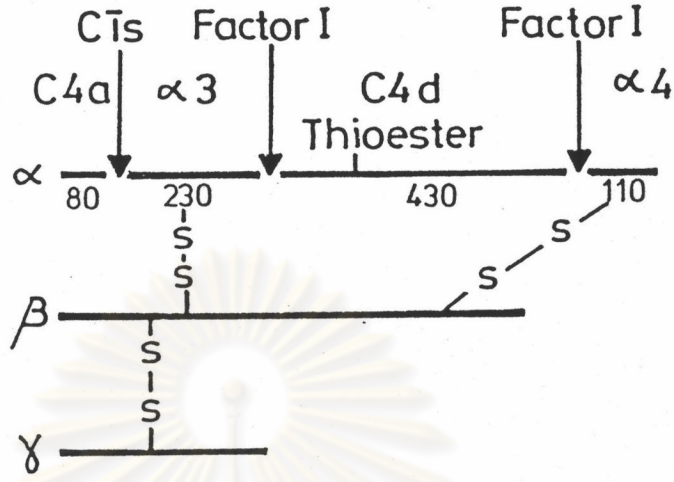

Figure 3 The three - peptide chain structure of C4 showing the chain activated by C1S. the latter is inactivated by hydrolysis at two position by factor I and the protein cofactor C4bp.

From : Porter, R.R., CRC. Critial. Rev. Biochem., 16(1), 1-19, 1983.

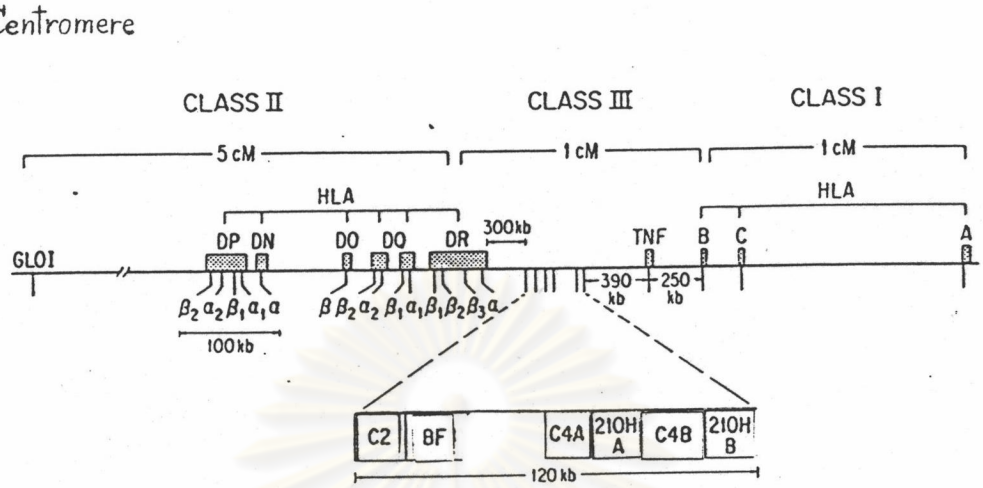

Figure 4 The gene map of human MHC on the short arm of chromosome 6. The class III region is shown the alignment of the complement genes C2, factor B, C4A, C4B and the steroid 21-hydroxylase genes (21-OHA and 21-OHB).

From : Alper, C.A., Complement. Inflamm., 6, 8-18, 1989.

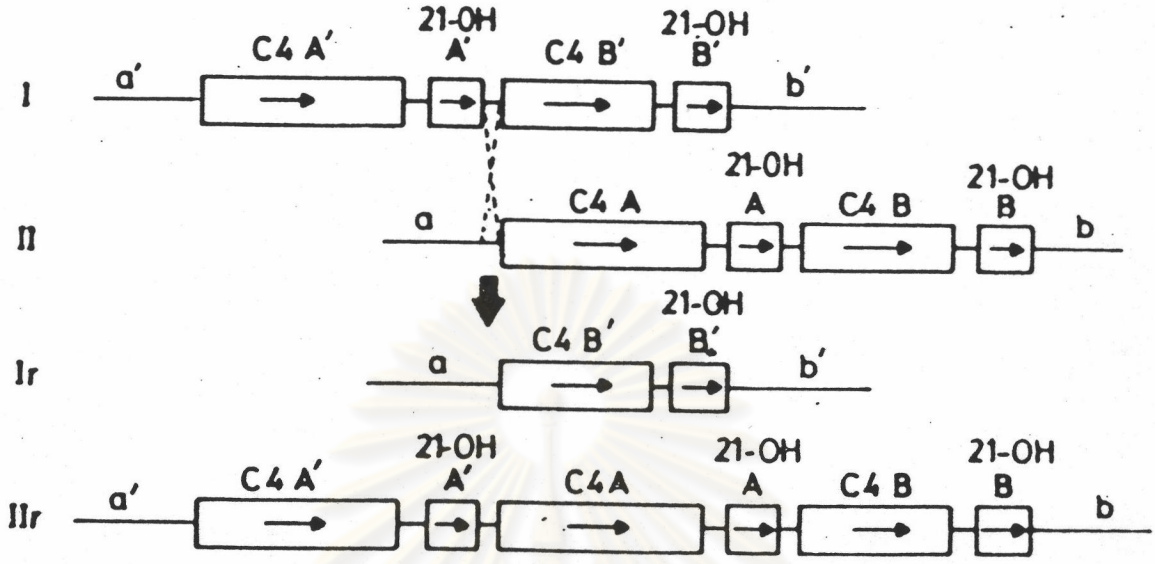

Figure 5 Model proposed to explain deletion of C4 and 21-OH genes on some haplotypes and duplication on others. Gene deletion and duplication would result from unequal crossover events between sister chromatids during meiosis.

From : Carroll, M.C., EMBO.J., 4, 2547-2552, 1985.

Table 1 Proposed C4 isotypic and allotypic differences.

Position	C4 allotype				
	A3	A4	B1a	B1b	B2
1054	Asp	Asp	Gly	Gly	Asp
1001[1]	Pro	Pro	Leu	Leu	Leu
1002[1]	Cys	Cys	Ser	Ser	Ser
1005[1]	Leu	Leu	Lle	Lle	Lle
1106[1]	Asp	Asp	His	His	His
1157	Asn	Asn	Asn	Ser	Ser
1182	Thr	Ser	Thr	Thr	Thr
1188[1]	Val	Val	Ala	Ala	Ala
1191[1]	Leu	Leu	Arg	Arg	Ala
1267	Ser	Ser	N.D.	N.D	Ala

ND = Not determined

[1] Proposed isotypic positions.

From : Carroll, M.C., Fed. Proceed., 46(7), 2457-2462, 1987.

Table 2 The Association of MHC Antigen in Systemic Lypus Erythematosus.

Ethnic groups	Associated MHC antigen	Reference
1. Caucasian (United Kingdom)	HLA-A, 1B 5, B7, B8 HLA DR3	Fielder et al 1983 (15)
2. Caucasian (USA)	HLA-DR2	Howard et al 1986 (19)
3. Japanese	HLA-DR2 low with HLA-DR3	Hashimoto et al 1985 (20)
4. Sounthern Chinese	HLA-DR2	Hawkins et al 1987 (69)