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COMPLEMENT – CURRENT STATUS

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Summary

Complement was initially discovered as a system essential for the expression of antibody-triggered cytotoxicity. It is now known that the complement system has more than one effect. Complement probably evolved as an integral part of host defense against infections in that it effects opsonization, facilitates phagocytosis, kills cells and is phlogogenic[1]. The following article reviews the current status of complement research and diagnostic application.

Bordet and/or Buchner[2] are usually credited with the discovery of the complement system towards the end of the last century. They discussed the effects of cell-free blood on erythrocytes and bacteria, differentiating between thermolabile factors whose action was independent of immunization "alexin".

The term 'complement' was introduced by Paul Ehrlich to indicate that many reactions were only brought to completion by the participation of certain labile factors. Heidelberger characterized serum complement as an intensifier of the immune reaction for exactly the same reason. The existence of labile serum components which supplement the antigen – antibody complex and which have the ability to carry out haemolysis and to destroy gram-negative bacteria has been known for many years. The chemical complexity of the phenomenon was not appreciated by early workers. The activity was thought to lie in one single component. We now know that complement is in fact a complex group of serum proteins present in relatively low concentrations in normal serum.

BIOCHEMISTRY

The complement system comprises, according to present knowledge, twenty molecules which function as zymogens, potentiators and regulators taking part in two major pathways of activation, *viz:* the classical and alternative pathways. These molecules or components are synthesized at different sites in humans, *eg* C2, C4 C5, Factor B, Factor D and Properdin (P) in the macrophages;[3 4 5 6] C3 in the macrophages as well as in the liver and Clq, Clr and Cls in the epithelium of colon[7] and ileum as well as in the fibroblasts[8] and macrophages.[6]

The components of the complement system are named Clq *Clr Cls* (which are joined by calcium ions to give C1) *C4, C2, C3,* C5, C6, C7, C8, C9,[9] *Factor B, Factor D* and P (properdin), the regulators include *C3bINA* (C3b inactivator) *SCPB (serum carboxypeptidase B)*, B_1H, ClINH (C_1 inhibitor) S-protein and ClqINH (Fig. 1 & 2). (italics = enzymes or zymogens)

The process of the complement reaction in the classical pathway consists of activation of nine complement factors, one after another in strict order after reaction with the activator (usually the immune complex), particularly when the binding has taken place on a cell membrane.[10]

The following is a description of the *in vitro* activation of complement which leads to immune haemolysis. This model results from artificial isolation and simplification of processes which *in vivo* may be more complex (Fig. 3).

The first component of complement, C1 is activated by gamma-globulin that has been changed either by its reaction with antigen or through aggregation. The altered gamma-globulin binds C1q with activates C1r which in turn converts the proesterase C1s to the esterase C1s. The Cl activating potentials of the different classes of immunoglobulins varies: IgM is clearly more effective on a molecular basis than IgG; IgA does not possess these properties at all; IgG_1 and IgG_3 react with C1q very strongly whereas IgG_2 reacts weakly and IgG_4 normally does not bind at all. According to Franklin, the complementophile grouping (*ie* Clq binding

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FIGURE 4. Biological activity of various intermediatory complexes and/or degradation products and inhibitors of the complement reaction. (Modified after Rother Hadding Till 1974).

Reaction Step	Intermediatory Complex	Degradation Product
Ag + Ab	Anaphylactic reaction (release of histamine) Recognition and ligand function	
C4	Virus neutralization, immune adherance, immune conglutination	serotonin-releasing activity
C2		kinin-like activity
C3	Immune adherance; immune opsonisation including immune phagocytosis and immune clearance; conglutination and immune conglutination; retraction and lysis of coagulated blood, release of vasoactive amine from rabbit RBC	anaphylatoxin-like activity C3a chemotaxis leucocyte mobilisation serotonin release from platelets (C3a)
C5	immune opsonisation	classical anaphylaxtoxin, C5a chemotaxis
C6	coagulation	
C7	chemotaxis (C567)	
C8	vulnerability of complement loaded erythrocytes to monocytes or lymphocytes	plasminogen activation
C9	Cytolysis, cytotoxicity, *eg* immune haemolysis, immune bacteriocidity, mast-cell lysis (rats), releasing histamine, lysis of rabbit thrombocytes releasing histamine and serotonin, K-cell cytotoxicity (?)	
B		chemotaxis for neutrophils (Ba) macrophage spreading (Bb)
D	Activation of B	
P	Potentiation of C3b BbD complex to form C5-convertase	
C 1q INH	Inhibits C1q	
C 1 INH	Inhibits C1r C1s	
C3b INA	Inactivates C3b to form C3c + C3d (C3bi)	
SCPB	eliminates activity of C3a and C5a	
S protein	binds C5b 6 7 inactivating cytolytic potential of complex	
B_1 H	Affinity for C3b preventing complexing to Bb Dislodges Bb from C3 convertase as Bi Potentiates C3b INA	

Other inhibitors may include C2 — decay accelerator, C4-INA, C6INA, C5INA.

FIGURE 1. Complement – WHO Nomenclature (Bull World Health Org. 1968).

E	=	erythrocyte (normally from sheep)
A	=	antibody (usually amboceptor from rabbit)
EA	=	antibody-sensitized erythrocyte
C	=	complement
Cn1	=	complement component where 'n' refers to number of component
$\overline{Cn}$	=	component with enzymatic or biological activity
Cna, b,c. etc.	=	complement degradation products
Cni	=	complement component that has lost a defined biological activity
EAC$\overline{1s4b2a3b5b}$	=	exact method of writing the intermediatory complex fromed by the first five complement components
EAC$\overline{14235}$	=	a shorter form
EAC$\overline{1\text{-}5}$	=	shortest from of writing
S	=	site of antibody binding e.g. SAC142

FIGURE 2. Provisional new nomenclature for the alternative or properdin pathway 1974.

P	=	properdin
$\overline{P}$	=	activated properdin
D	=	pro-GBGase, precursor of C3-proactivator-convertase
$\overline{D}$	=	GBGase, C3-proactivator-convertase
B	=	B_2-glycoprotein-11, GBG (glycine-rich betaglycoprotein, heat labile factor, C3-proactivator
$\overline{B}$	=	C3-activator, GGG (glycine-rich gammaglobulin)
C3b	=	factor A
Cobra venom factor binding protein	=	CVF
C3b-inactivator	=	KAF, conglutinogen activating factor, C3b-INH

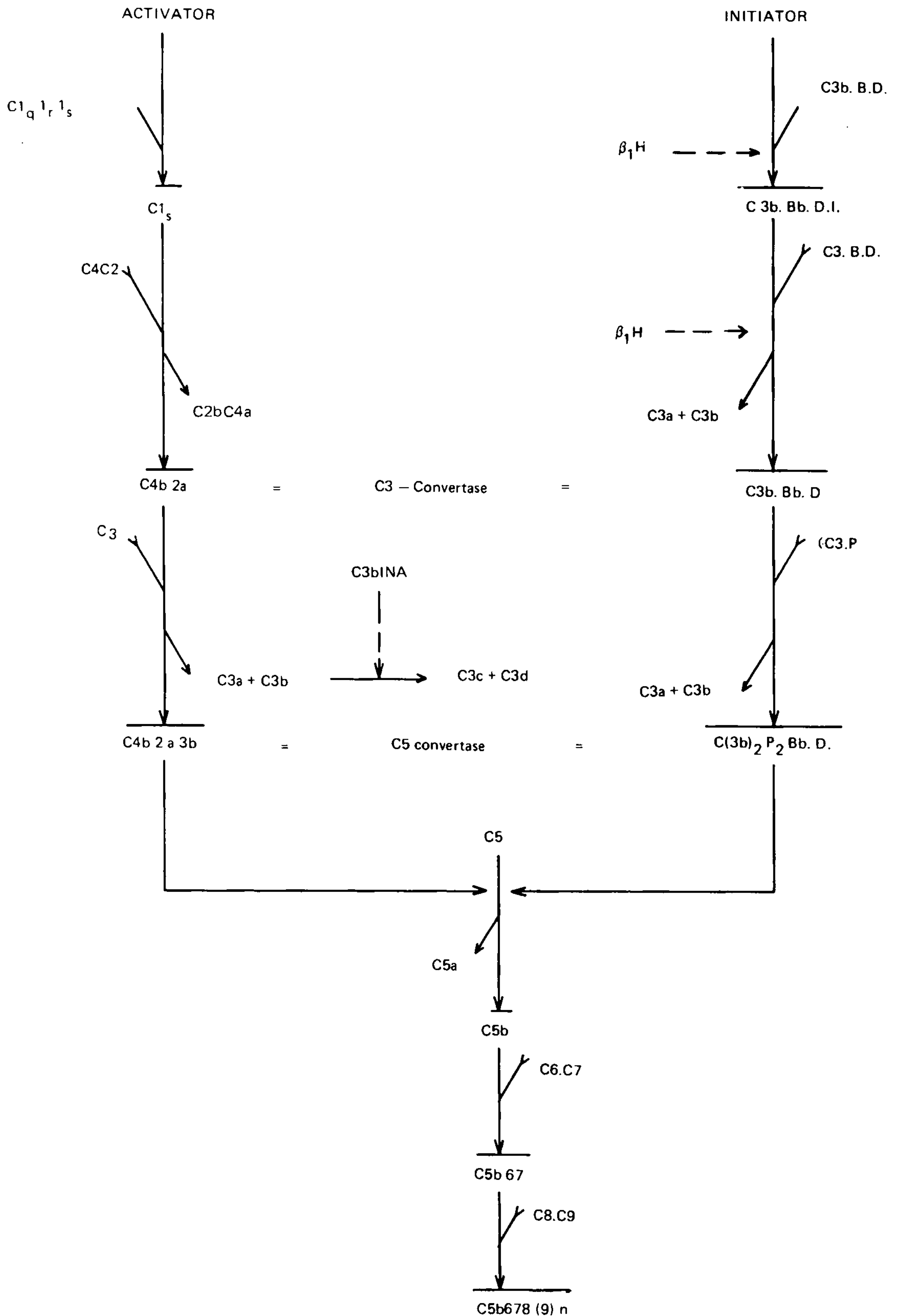

FIGURE 3. Complement activation.

site) is to be found on the Fc-fragment of the 7s – immunoglobulins in humans and rabbits. However, complement cannot be fixed until the 7s and/or the 3.5s – fragment is aggregated in a suitable way. The binding of antigens to antibody as well as alcohol, diazotisation and heat treatment leads to this aggregation.

C1s splits the fourth complement component in at least two parts, C4a and C4b. The reaction of the C1s with C4 uncovers the capability of C1 to react with its other natural substrate C2 to form with C4 the complex EAC $\overline{1s\ 4b\ 2a}$ or C3 – convertase. After formation of the C42 complex, C1 is no longer necessary for further reaction.[12]

The $\overline{C42}$ complex then splits C3 into C3a and C3b forming on the surface of the cell the complex EAC$\overline{1s\ 4b\ 2a\ 3b}$ (or EAC $\overline{423}$) and in the fluid phase the inactive C3bi which breaks down in the presence of H^+ and a serum enzyme, C3bINA to produce C3c and C3d.

The interaction of the EAC $\overline{1s\ 4b\ 2a\ 3b}$ complex with native C5 leads to the formation of two products C5a and C5b. C5b forms an intermediatory complex with $\overline{C6 \text{ and } C7}$ and finally with C8 and C9 giving $\overline{C5\ 6\ 7\ 8\ (9)n}$.[13,14]

C3 can also be activated without the presence of C1, C3 or C4 *via* the alternative or properdin pathway.

The exact activation mechanism of the alternative pathway is not yet perfectly clear; it has been proposed that distinct surface structures of activators of this pathway are recognized by the components of the initial enzyme, perhaps in a lectin-like fashion. Nevertheless, the generation of the initial enzyme is still under investigation; four models have been proposed.[15]

C3 is the dominant protein of the alternative pathway. Factor B is the key enzyme and Factor D its activating enzyme whereas properdin is the potentiator of the C3/C5 convertase (= SP C3b C3b BDP).[15]

Activators of the alternative pathway *in vitro* include endotoxins, agarose, zymosan, inulin, dextran and various polyanions; aggregated IgA can also function as an initiator. Pathologically important is the activation of the alternative pathway *via* the nephritic factor. Cobra venom factor can also activate C3 in combination with magnesium ions D and B.

PHYSIOLOGICAL FUNCTIONS

The biological activity of various intermediatory complexes and/or degradation products are shown in fig. 4.

Viruses can lose their infectivity through reaction with antibody and complement. It is believed that this is not always due to a specific activity of immune factors but to an enveloping of the virus protein with serum protein; C4 is involved in the first intermediatory step. C2 C3 play a role in vitro when the concentrations of C4 are relatively low.

Furthermore, C4 exhibits immune adherence properties and plays a role in immune conglutination. Conglutination is the agglutination of immune complexes through globulin bridges whereas in immune conglutination immunoglobulins are responsible for the bridging. A C4 reactive immune conglutinin has been reported. A serotonin releasing action has also been described for C4 degradation products.

C1s in the presence of C4 splits C2 into C2a and C2b and is accompanied by an increased capillary permeability which cannot be attributed to these two products. It is, therefore, suggested that, by the activation of C2, kinin-like activity is produced.

The activation of C3 is accompanied by many physiological phenomena. A single active $\overline{C142}$ site on the cell surface can activate hundreds of C3 molecules and bind C3 on the surrounding areas of the cell membrane. Anaphylatoxin-like activity (not identical with classical anaphylatoxin), chemotactic and leucocyte-mobilising activity have been associated with the small degradation products of C3, whereas the larger degradation products show immune adherance, immune opsonization, immune phagocytosis and immune conglutination stimulating or furthering properties. Opsonization is a process which increases the phagocytosibility of particles; C3b acts as a ligand in opsonization[15].

Activated C5 shows opsonizing, anaphylatoxic (very similar to classical anaphylatoxin) and chemotactic characteristics. C567 is also involved in chemotaxis.

C8 may be involved in the lysis of complement sensitized red blood cells by monocytes and leucocytes.

Attachment to the EAC $\overline{14235678}$ complex and activation of C9 in the presence of magnesium ions produces immune bacteriocidal pro-

perties. The complex can also lyse bacteria, red blood cells and viruses. In transplant rejection the complex may take part in the cytotoxicity due to 'Killer' – lymphocytes (K-cells).

The complement and coagulation systems are similar, both from their dependency on divalent ions and cascade modus of activation through two pathways pivoting on a central protein (Factor X and C5). A direct connection between the systems is to be found in C6 which can play a role in coagulation; the connection via the Kallikrein – kinin system remains to be explained. C8i has been reported to activate plasminogen. There is also a C3- dependent contraction and destruction of blood clots. Furthermore C3 can be split *in vitro* by trypsin and plasmin and C3a can cause serotonin release from platelets.[16]

In summary, the physiological importance of the complement system lies in its non-specific and specific roles in defence against bacterial and viral infection. Its biological role in other systems is yet to be elucidated.

DIAGNOSTIC APPLICATION

Complement levels vary in disease. Increased levels of C3, C4, Factor B, C9 and C1-INH occur in acute inflammatory processes. Decreased levels may be hereditary or acquired.

Patients with lymphopoenic hypogammaglobulinaemia often have very low serum levels of C1q whereas C1s-and C1r- serum concentrations are usually normal or even sometimes increased. The reason for these changes is not clear.

An inherited C1r deficiency has been observed in several families in combination with SLE – like symptoms and renal disease.

C2 deficiencies have been reported as a genetic defect in many families. Once again these patients show an increased incidence of lupus-like symptoms. It is possible that this deficiency results in an incomplete elimination of immune complexes.

A few patients with SLE-like symptoms were also found to have an inherited lack of C4; phagocytosis was very limited and other immune defects were present.

C3 deficiencies have been associated with repeated severe infection in a few cases.

Functionally defective but immunologically normal levels of C5 were found in several families; the patients suffered from repeated severe infections.

C6 deficiencies are accompanied by coagulation disturbances.

C1s - INH deficiencies are found in patients suffering from hereditary non-allergic angioneurotic oedema.

Diseases producing immune complexes, particularly IgG- and IgM – immune complexes, are accompanied by consumption of the components of the classical pathway (C1, C4, C2 and C3), *eg* in SLE, lupus nephritis, immune haemolytic anaemia, cryoglobulinaemia, hepatitis B infections, malaria, acute glomerulonephritis, and Goodpasture's syndrome.

Reduced complement levels are seen in the nephritides: acute post-streptococcal glomerulonephritis is characterized by reduced levels of C3C5 and P; C1g C4 and C2 levels are also sub-normal during the first phase of the disease. Patients with chronic membrano-proliferative glomerulo-nephritis have very low levels of C3 due to the presence of the nephritic factor. Incidentally, decreased C3 and presence of C3 NeF in the circulation have been observed in patients with partial lipodystrophy. Reduced levels of C1q, C4 and C3 are found in lupus nephritis and Good pasture's syndrome. The nephritis accompanying parasitic infections such as malaria is also associated with consumption of the components of the classical pathway due to the presence of immune complexes.

Immune complexes are also responsible for the haemorrhagic shock syndrome seen in dengue fever. Both pathways of activation are, however, involved. Active $\overline{C56}$ have been demonstrated in blood which can release platelet factor resulting in intravascular coagulation. C3a and C5a are probably involved in the shock state because of their anaphylactic activity.

The anaphylactic reactions seen after dextran infusions are associated with alternative pathway activation. The same is observed in patients with gram-negative infections. The abnormal erythrocytes found in paroxysmal nocturnal haemoglobinuria are responsible for the activation of the alternative pathway resulting in their own destruction.

Patients with rheumatoid arthritis often show normal or increased C3 and C4 in plasma whereas decreased levels in synovial fluid indicate localized immunological processes.

The determination of the CH_{50} in patients

with a history of severe repeated infection or suspected complement deficiency is a useful screen for suspected deficiencies. However, the diagnostic importance lies in the quantitation of the individual components in serum and/or other body fluids and in the demonstration of C3- or C1q- bound to immune complexes deposited in tissue using fluorescein or peroxidase techniques. Reduced C3 and C4 serum concentrations can indicate the presence of immune complexes in the circulation thus pointing to the presence of infection or autoimmune diseases.

Furthermore, quantitation of C3, C4, C1q and Factor B can assist in the control of the patient, *eg* in SLE, and can indicate whether therapy is successful.

The lack of international standards for complement components and the variation in normal ranges given by various manufacturers makes it mandatory that each laboratory establishes its own normal ranges.

FUTURE

The future will see more research on the mode of initiation of the alternative pathway and on the non-lytic cellular interactions of complement. Complement studies will certainly be established as a useful laboratory aid to diagnosis, prognosis and control of therapy.

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