

Therapeutic complement inhibition – from experimental to clinical medicine

The complement system plays a central part in the body's infection defence mechanism, but it is also involved in tissue homeostasis generally. Undesirable activation of the system occurs in connection with a number of diseases, so inhibition of the complement system has become an interesting therapeutic option for several different conditions.

The complement system forms part of our innate immune system. It protects us against infections by recognising microbial structures. However, research in recent years has revealed that the complement system also plays a key part in tissue homeostasis generally, in close cooperation with a number of biological systems, and that it is involved in the pathophysiology of a large number of disease processes.

Inhibition of complement activation has therefore become an interesting approach to the treatment of several diseases. This article provides a brief overview of the complement system, how complement inhibitors are used to day, and how they may conceivably be used in the future.

The complement system

The complement system (1–3) is one of the body's cascade systems, and consists of several dozen proteins (Fig. 1). One part of the system consists of a series of plasma proteins, which are activated in a particular sequence and form activation products with various functions. The other part consists of membrane proteins, which function as receptors for the activation products. A large number of the proteins are regulatory proteins, which keep the system under control. Factor H (FH) is crucial here.

There are three main activation pathways.

Classical activation. In this process, C1q binds antibodies or other molecules, for example C-reactive protein, that are bound to a surface. This causes activation of C1r and C1s, which in turn activate C4 and C2 with subsequent activation of C3, which then cleaves into C3a and C3b. C3a has both pro- and anti-inflammatory effects, while C3b functions as an important opsonin by binding covalently to a surface.

Lectin activation. This takes place when, for example, mannose-binding lectin (MBL) or ficolins react with microbes or damaged tissue, but also when naturally occurring IgM antibodies react with the body's own structures. This activates mannose-associated serine proteases (MASP1 and MASP2),

which are analogues of C1r and C1s in the classical pathway, and which in turn activate C4. The subsequent activation process is the same as in the classical pathway. C1 inhibitor (C1-INH) and C4b-binding protein (C4BP) are important regulators of both classical and lectin activation.

Alternative activation. Under physiological conditions, this takes place through spontaneous hydrolysis of the C3 molecule, which, however, is kept under control by factor I (FI) and factor H (FH). Under non-physiological conditions, for example contact of blood with a foreign surface, this equilibrium is upset, and alternative pathway activation is reinforced by the action of factor B (FB), factor D (FD) and properdin (FP). C5 cleaves into C5a and C5b. C5a is a highly potent peptide with a number of effects in the inflammatory process. C5b reacts with C6, C7, C8 and C9 and forms the terminal C5b-9 complement complex (TCC), which occurs in a membrane-bound form that can cause lysis, particularly of *Neisseria* bacteria and red blood cells. In soluble form (sC5b-9) it can be measured in plasma and is a marker for complement activation. sC5b-9 binds the regulators vitronectin (VN) and clusterin (CL), causing the resulting complex to remain in liquid form.

There are a number of complement receptors on the surface of the body cells. They fall into three different categories. Some emit signals to the cells and thus activate them. For example, C5a receptors are stimulated to an inflammatory response by the biologically active C5a peptide. Others are fagocyte receptors (e.g. CR3, CD11b/CD18), while the third group has the important function of protecting cells against attacks from their own complement.

The overarching function of the complement system is precisely to trigger an inflammatory reaction as part of the battle against microbes (infection-induced inflammation) and as part of tissue regeneration after non-microbial damage (sterile inflammation). The activation is very strictly con-

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MAIN POINTS

The complement system is involved in a number of disease processes.

Inhibition of the complement system is central to the treatment of paroxysmal nocturnal haemoglobinuria and atypical haemolytic uraemic syndrome.

Complement inhibition may also be applicable to the treatment of other conditions.

THE COMPLEMENT SYSTEM

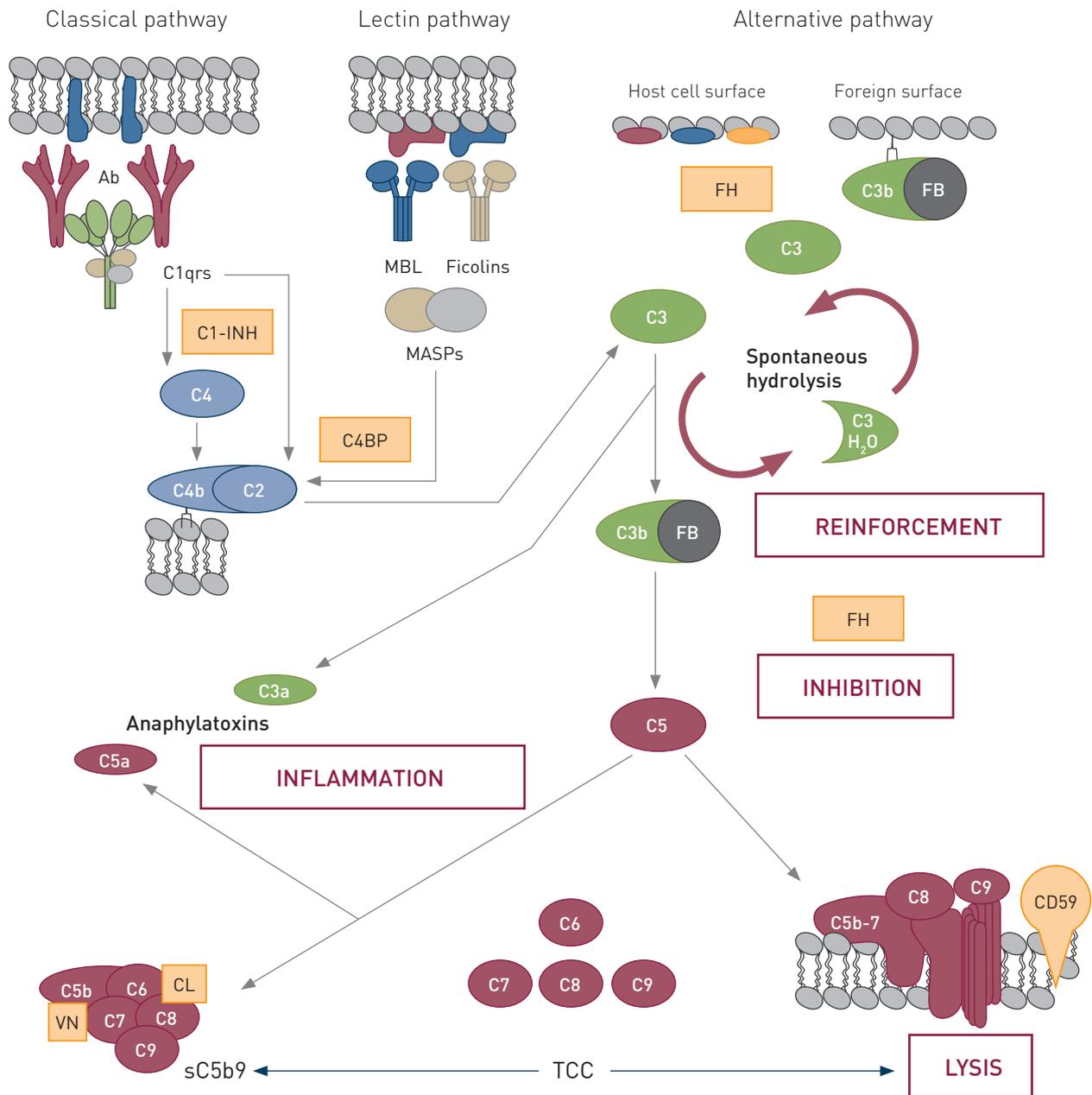


Figure 1 A simplified presentation of the three activation pathways of the complement system, and how they converge in C3 and then in C5, which subsequently cleaves into C5a and C5b. C5a is a potent anaphylatoxin, while C5b forms part of the terminal complement complex. Based on an original figure by Tom Eirik Mollnes

trolled by regulators in both the liquid phase and on the cell surface, to prevent the system being inappropriately activated and causing tissue damage and disease. The receptors CR1, MCP/CD46 and DAF/CD55 are important inhibitors at the C3 level, while CD59 inhibits incorporation of C5b-9 in the membrane. Patients with paroxysmal nocturnal haemoglobinuria (PNH) lack DAF and CD59 receptors on the cell surface, and the red blood cells are then lysed by complement (Fig. 2).

Complement activation and disease

The complement system works closely with the body's other cascade systems: coagulation, fibrinolysis and the kinin-kallikrein systems. For example, C1 inhibitor is involved in regulating all these systems. A genetically conditioned lack of C1 inhibitor causes angioedema, and C1 inhibitor has been used for decades as therapy during attacks of hereditary angioedema. However, the pathogenesis of the disease is not complement-mediated, but due to release of bradykinin

(4). Thus C1 inhibitor is not a specific complement inhibitor, and will therefore not be discussed at greater length here.

As with all the cascade systems, the complement primarily acts locally; systemic activation is undesirable and at worst can be fatal. Both local and systemic complement activation have been found in a large number of other pathological conditions, but it is often unclear whether the complement activation has pathogenic significance or whether it is merely a marker of the ongoing inflammation process.

Principles of complement inhibition

Three central questions form the point of departure for determining which strategy should be chosen to inhibit the complement system (1, 5).

Where in the cascade do we want to attack? If the pathogenesis has been determined and only one of the pathways is involved, it is possible to specifically block the start of one of the three activation pathways; for example C1 in the classical pathway, MBL in the lectin pathway, or factor D in the alternative pathway.

If more than one of the pathways is involved, it is possible to inhibit one of the central common molecules: C3, if a strong blockade is wanted early in the cascade (6) or C5 if only inhibition of C5a and C5b-9 formation is wanted (7). Alternatively, a specific activation product or a receptor can be blocked; for example C5a or C5a receptor 1 (C5aR1).

What type of inhibitor do we want to use? The most relevant are small-molecule peptides, which are simple and cheap to produce. They often have a short half-life, but can be linked to larger molecules, for example an immunoglobulin fragment, or equipped with a site that binds albumin after it has been injected, thereby substantially increasing the half-life.

Alternatively, large, recombinant proteins can be made that inhibit the complement system, or monoclonal antibodies can be used that block complement components or receptors (8).

Do we want to inhibit systemically or locally? Systemic inhibition will affect the complement system of the whole body, while local inhibition is organ-specific. Implementing systemic inhibition at an early stage, for example by blocking C3, will probably mean considerably increased risk of infection, since opsonisation is the complement system's most important infection control mechanism. Systemic C5 inhibition results in substantially less risk of infection.

Local therapy can be administered, for example eyedrops or injection into the eye in cases of age-related macular degeneration (AMD) (9). Alternatively, the inhibitor can be linked to a target-seeking molecule that binds to specific tissue, for example in joints, and accordingly seeks out and settles at sites where we want inhibition.

Complement inhibitors in use today

The monoclonal antibody eculizumab is the only specific complement-inhibiting drug that has been approved by the European Medicines Agency (EMA). At present there are only two indications: paroxysmal nocturnal haemoglobinuria, and atypical haemolytic uraemic syndrome (aHUS).

In paroxysmal nocturnal haemoglobi-

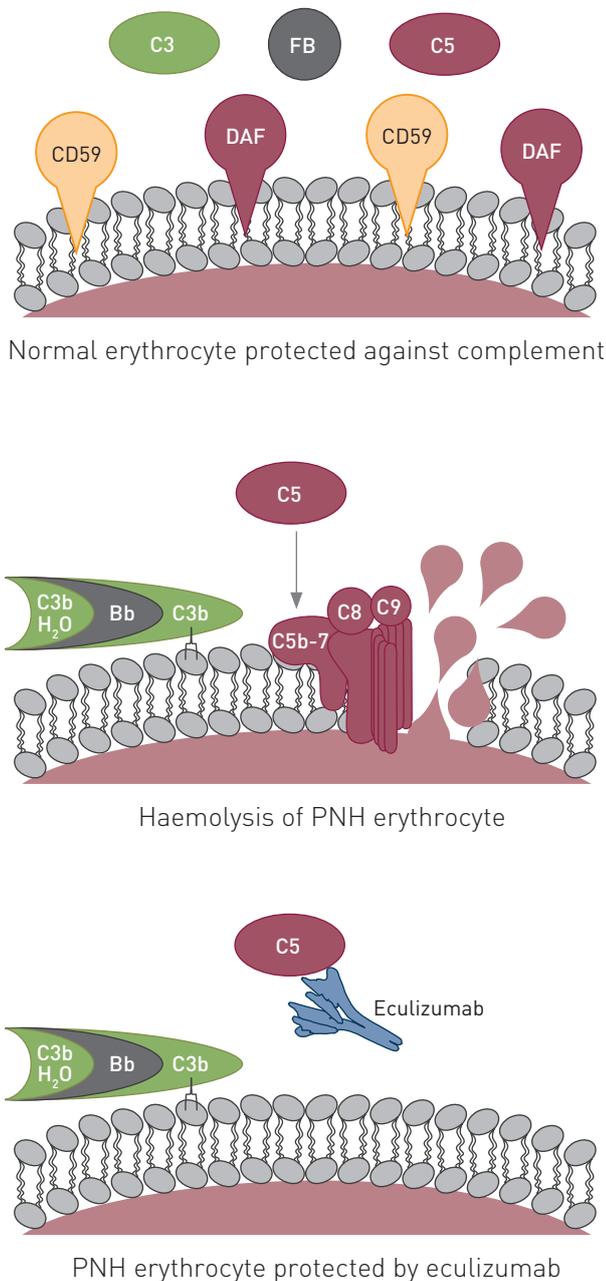


Figure 2 A schematic presentation of how eculizumab protects the erythrocyte against lysis in paroxysmal nocturnal haemoglobinuria. Based on an original figure by Tom Eirik Mollnes

nuria, DAF and CD59 are lacking at the cell surface, and the red blood cells are therefore susceptible to complement-mediated lysis (10). Eculizumab prevents complement-mediated lysis of the red blood cells in this condition (Fig. 2). In atypical haemolytic uraemic syndrome, it inhibits the inflammation reaction, in both cases by blocking the terminal cascade reaction at C5 level. A number of other complement inhibitors are undergoing preclinical and phase I trials (Table 1).

In Norway, eculizumab was first used to treat a patient with paroxysmal nocturnal

haemoglobinuria in 2008, and as at 15 August 2015, 20 patients (16 with paroxysmal nocturnal haemoglobinuria and four with atypical haemolytic uraemic syndrome) are undergoing treatment (G.E. Tjønnfjord, personal communication). Special national guidelines have been drawn up for the diagnosis and treatment of paroxysmal nocturnal haemoglobinuria (11). The therapy is reserved for patients with the most severe clinical phenotype.

Special national guidelines have not been drawn up for the treatment of atypical haemolytic uraemic syndrome, but there is an international consensus report (12). There are two

Table 1 Some selected examples of complement inhibitors in use and undergoing trial

| Name | Type | Point of attack | Indication/potential use |
|---------------|-----------------------------------|--|---|
| Eculizumab | Monoclonal antibody | C5 | Paroxysmal nocturnal haemoglobinuria, atypical haemolytic uraemic syndrome |
| Compstatin | Peptide | C3 | Paroxysmal nocturnal haemoglobinuria, atypical haemolytic uraemic syndrome, dense deposit disease, age-related macular degeneration |
| NM9401 | Monoclonal antibody | Properdin | Atypical haemolytic uraemic syndrome, dense deposit disease |
| TNX-234 | Monoclonal antibody | Factor D | Age-related macular degeneration |
| TA106 | Monoclonal antibody | Factor B | Age-related macular degeneration, asthma |
| TNT003 | Monoclonal antibody | C1s | Cold agglutinin syndrome |
| NN8209/NN8210 | Monoclonal antibody | C5a receptor | Rheumatoid arthritis |
| NOX-D19 | Aptamer (RNA that binds proteins) | C5a | Transplants |
| AMY_301 | Peptide | Binds factor H to biomaterial surfaces | Preclinical |
| Complin | Peptide | C2 and factor B | Preclinical |
| PIC1 | Peptide | C1 | Transplants, acute intravascular haemolytic transfusion reaction |
| siRNA | Small, inhibiting RNA pieces | C2 and several other potential attack points | Preclinical |

main scenarios where treatment with eculizumab is relevant because of a genetic defect in the complement system: to prevent or delay the development of dialysis-dependent renal failure, and to prevent recurrence after a kidney graft (13). Eculizumab can also be used with atypical haemolytic uraemic syndrome due to auto-antibodies against complement factor H. In most cases, however, the therapy for this condition will be aimed primarily at maintenance treatment, aimed at keeping the anti-factor H antibodies under control (14).

Controlled clinical trials – and the lack thereof

One placebo-controlled trial of eculizumab on paroxysmal nocturnal haemoglobinuria has been carried out, with the degree of haemolysis and need for transfusions as endpoints (7). The need for transfusion was reduced by the eculizumab therapy. Non-placebo-controlled studies of safety and efficacy have been conducted (15, 16). Studies have also been conducted using historical controls, with patient survival as endpoint. The use of eculizumab was associated with reduced mortality (17).

Randomised, placebo-controlled studies have not been conducted for atypical haemolytic uraemic syndrome, but there are several studies that document the effect on endpoints such as renal function and proteinuria (13, 18). A number of case studies and series with a few patients have shown very high

efficacy, and this, coupled with the rarity of the disease, has formed the basis for approval by the authorities (19).

Several randomised, placebo-controlled trials of the complement inhibitor pexelizumab, the predecessor of eculizumab, have been conducted in the field of ischaemic heart disease. Pexelizumab was compared with placebo in large trials in connection with myocardial infarction and various kinds of cardiac surgery. The studies revealed no difference between the treatment groups with regard to the primary endpoints. However, a retrospective analysis of these studies indicates that the dose and time of administration of the drug might not have been optimal (20). At this time, however, the manufacturer had stopped the trials of pexelizumab for ischaemic heart disease, and was concentrating on eculizumab with the indications paroxysmal nocturnal haemoglobinuria, atypical haemolytic uraemic syndrome and other rare diseases (Table 1).

Possible future indications

Complement inhibition has also shown some effect in connection with C3-glomerulopathy (C3GN) (13) (including dense deposit disease, DDD) (21), antibody-mediated acute rejection of kidney transplants (21), severe antiphospholipid syndrome (22), vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA) (23), myasthenia gravis (24) and neuromyelitis optica (25), but the

indications have not been verified. Studies are also in progress on such varied areas as sepsis, obstructive pulmonary disease, inflammatory bowel disease and various types of ischaemic reperfusion injury (5). It is also possible that complement inhibition plays a part in some kinds of autoimmune haemolytic anaemia and acute haemolytic transfusion reactions (26, 27).

Animal models have yielded promising results for complement inhibitors linked to antibodies with a special affinity for molecules exposed in an arthritic joint, as well as for injection of a DNA vector that codes for the production of a C5-inhibiting mini-antibody, so that local production is achieved in the affected joint (28). It remains to be seen whether it will be possible to use this type of targeted therapy clinically in the future to treat arthritis and other conditions with complement-induced tissue destruction.

Adverse effects

Patients with a congenital lack of C5 or some other terminal component are healthy on the whole, but at increased risk of contracting a *Neisseria* infection, because these bacteria are killed by C5b-9 in particular. Systemic C5 inhibition will entail a similar risk. Patients must therefore be vaccinated against *Neisseria meningitidis* (29). Other than the risk of *Neisseria* infection, eculizumab therapy does not appear to present any risk so far (17).

It is possible that adverse effects of complement inhibitors will manifest themselves with continuous systemic treatment (30). Systemic treatment at the C3 level must be assumed to present a major risk of infection, since C3 deficiencies are often associated with infections (31). Specific inhibition of C5a (using antibody or receptor blocker) preserves C5b-9 formation, so that the defence against *Neisseria* remains intact (32). Treatment of diseases with targeted organ-specific therapy, where systemic complement is not inhibited, is unlikely to cause adverse effects. At any rate, none have been observed in animal experiments (28).

Summary

Internationally, the use of the C5-inhibiting monoclonal antibody eculizumab has in the course of just a few years become the first choice of treatment of atypical haemolytic uraemic syndrome and the most severe phenotypes of paroxysmal nocturnal haemoglobinuria. At present eculizumab is the only complement inhibitor in ordinary clinical use.

This despite the fact that there only exists one randomised, placebo-controlled trial of eculizumab for paroxysmal nocturnal haemoglobinuria and none for atypical haemolytic uraemic syndrome, and that the therapy is very costly. There is reason to believe that complement inhibition as therapy will increase in the future, and that other drugs will also prove to be effective.

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