Complement inhibition by anti-C5 antibodies – from bench to bedside and back again

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Summary

The complement system is an important part of the innate immune system with pro-inflammatory and regulatory functions. Although many experimental studies have demonstrated that complement inhibition might be advantageous in a number of different human diseases, complement inhibition is still not part of the clinical treatment routine. With blocking antibodies against complement C5 a new generation of therapeutic complement inhibitors has now been investigated in some human diseases. This review gives an overview on the new complement inhibitors and the results obtained in clinical studies thus far.

Key words: complement system; anti-C5 antibodies; C5 blockade

Introduction

The complement system is a group of plasma and cell membrane proteins that play a key role in the immune system [1, 2]. The complex cascade is activated by at least three major pathways, the classical pathway which is typically activated by immune-complexes, the alternative pathway that can be activated by unprotected cell surfaces, and the mannose binding lectin (MBL) pathway (figure 1). All three pathways lead to the cleavage of C3 and the formation of the cytolytic membrane attack complex C5b-9. The best known function of the complement system is to cause lysis of bacteria, cells and enveloped viruses. Complement has also a role in bridging the innate and adaptive immunity and mediates the process of opsonisation, in which foreign cells, bacteria, viruses, fungi, immune-complexes and dying cells generated by apoptosis or necrosis are prepared for phagocytosis. Furthermore, activation of complement leads to the generation of peptide fragments that regulate features of the inflammatory and immune responses. These fragments play a role in vasodilatation at the site of inflammation, in adherence of phagocytes to blood vessel endothelium, in egress of the phagocytes from the vessel, in directed migration of phagocytic cells into areas of inflammation, and, ultimately, in clearance of inflammatory agents (such as bacteria) from the body.

Figure 1

The complement cascade and therapeutic complement inhibitors. The anti-C5 antibodies eculizumab and pexelizumab (red circle) inhibit the cleavage of C5 into its fragments C5a and C5b. Compared to other complement inhibitors, both antibodies interfere in a relatively late stage of the complement cascade.

Physiologic regulation of complement

In order to prevent complement-mediated destruction of the individual's own tissues, the mostly pro-inflammatory functions of the complement system require strict regulatory control. As a consequence, many control proteins have evolved to defend against such an attack. Clinically, the most prominent complement regulator is C1 inhibitor but the cascade is controlled by many other plasma or membrane bound regulatory proteins such as factor I, factor H, C4 binding protein, complement receptor 1 (CR1, CD35), decay accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46), CD59, and CRIT [3, 4].

However, despite the presence of a large number of complement inhibitors they might not be able to sufficiently control inflammation in the context of disease. When complement is involved in causing disease, it usually is functioning normally but is misdirected, that is, damaging host tissues. Such inflammatory disorders include not only immune-complex and autoimmune diseases but also severe trauma, sepsis, ischaemia-reperfusion injury or systemic inflammatory response syndrome (SIRS). Thus, treatment approaches to control excessive complement activation might have substantial therapeutic potential in many clinical situations.

Pharmaceutical complement regulation

Complement activation is most effectively controlled by the system's physiological regulators. The specificity and the absence of toxic side effects of endogenous complement inhibitors provide an excellent prerequisite for their therapeutic application. In this context, recombinant soluble complement receptor 1 (sCR1) and C1 inhibitor are the two best investigated complement regulators (reviewed in 5, 6). Up to now the use of sCR1 was limited by the relatively difficult synthesis of the protein and its rapid clearance from blood. However, in a number of animal models for different diseases sCR1 has been shown to be effective. In addition, in a more recent study on patients undergoing coronary-bypass grafting, sCR1 significantly decreased the incidence of mortality and myocardial infarction in male patients without increase in the incidence of adverse effects [7].

In contrast to sCR1, C1 inhibitor is available for the clinical use since many years. C1 inhibitor has been widely used in patients with angioedema and has been shown to prevent deleterious complement activation in many animal models. Further indications for the treatment with C1 inhibitor are still lacking, potentially due to its regulatory functions outside the complement cascade, such as the coagulation and kinin systems, that bear the risk of unwanted side effects. However, recent studies suggested an advantageous effect in the prevention of cardiac cell injury during acute myocardial infarction [8].

Next to these two physiological complement inhibitors, several other complement regulators have been developed, in part based on physiological mechanisms. Despite promising results achieved in animal experiments, most of these potential therapeutics are not yet available for clinical use. However, recent studies suggest that an antibody inhibiting the cleavage of C5 might become available in the near future.

Complement inhibition by an antibody preventing the cleavage of C5

Cleavage of C5 resulting in the fragments C5a and C5b is a critical event during the activation of the complement cascade. C5b is the basis for the formation of the membrane attack complex (C5b-9) that seems to have multiple functions beyond cytolysis. In nucleated cells that are resistant to cytolysis by C5b-9, sublytic quantities of C5b-9 can cause cellular activation which results in cell proliferation, generation of pro-inflammatory mediators and production of extracellular matrix [3]. The other C5 fragment, C5a, is a very potent pro-inflammatory mediator that induces chemotactic migration, enhances cell adhesion, stimulates the oxidative burst, and induces the release of various inflammatory mediators such as histamine or cytokines [9]. Thus, the prevention of C5 cleavage would have substantial anti-inflammatory consequences in a relatively late stage of the complement activation cascade without affecting the activation and function of the early components. The specific blockade of C5 cleavage can be achieved either by a humanised monoclonal antibody (eculizumab, Alexion Pharmaceuticals) or its 25kD single chain version (scFv) lacking the whole constant region including the Fc part of the antibody (pexelizumab, Alexion Pharmaceuticals). Whereas eculizumab is under investigation as a treatment of chronic inflammatory diseases, the short acting pexelizumab was designed for the use in acute cardiovascular complications [10–12]. The impor-

tance of a therapeutic inhibition of complement at the level of C5 has recently been supported by the finding that thrombin is able to cause direct activation of the late complement cascade in the absence of C3 [13].

C5 blockade in paroxysmal nocturnal haemoglobinuria (PNH)

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare acquired disease presenting as chronic haemolytic anaemia with acute exacerbations and increased risk of thromboembolism. Haemolysis is mediated by complement due to a deficient cell surface expression of DAF and, more importantly, CD59 which protects blood cells from complement attack [14]. In a recently published double-blind, randomised, placebo-controlled, multicentre phase III trial, eculizumab was investigated in 87 patients with PNH [15]. The drug was administered intravenously, initially weekly, then in 2-week intervals for up to 26 weeks. Interestingly, stabilisation of haemoglobin levels as the primary endpoint, defined as a haemoglobin value that remained above the earlier specified set point in the absence of transfusions during the 26-week treatment period, could be achieved in about half of the patients receiving eculizumab but in none of the patients receiving placebo. In parallel, treatment with eculizumab led to a significant reduction of haemolysis, absolute numbers of units of packed red cells transfused and an improved quality of life as judged by a chronic fatigue score. Furthermore, treatment with eculizumab was not associated with an increase in serious adverse events. Of the 13 patients with serious but non-fatal adverse events that were reported, 9 were in the placebo group. The most common specific adverse events reported for the eculizumab group were headache and back pain. These data support the central role of intravascular haemolysis in the pathogenesis of PNH and indicate that complement C5 blockade is an effective treatment in patients with PNH.

C5 blockade in patients with cardiovascular diseases

Results of several trials that investigated the role of blockade of complement C5 cleavage in cardiovascular diseases and/or complications are now available. The clinical settings in which pexelizumab has been investigated were coronary bypass surgery and patients with myocardial infarction undergoing revascularisation either by fibrinolysis or primary percutaneous coronary intervention (PCI). The rationale for the use of a complement inhibitor in cardiac surgery is the extensive complement activation by exposure of blood to bio-incompatible surfaces of the extracorporeal circuit and reperfusion of ischaemic organs. The so-called ischaemia-reperfusion injury is produced by inflammatory reactions including complement activation that can exacerbate the initial tissue damage caused by the ischaemic event [16]. Blockade of complement activation during or shortly before reperfusion of ischaemic organs has been shown to strongly reduce secondary organ damage in many experimental settings (reviewed in 17). Thus, complement inhibition using pexelizumab was also considered in patients with myocardial infarction undergoing revascularisation.

In patients undergoing coronary bypass surgery with or without additional valve surgery, phase I and II trials could demonstrate a significant reduction in myocardial damage by the administration of pexelizumab as judged by post-operative peak creatine kinase MB fraction (CK-MB) levels. In addition, in a subgroup analysis of patients with isolated coronary bypass grafting without valve surgery, the administration of pexelizumab led to a significant reduction of the composite endpoint of death and/or myocardial infarction [18, 19]. In a larger consecutive study, investigating the effect of pexelizumab in 2746 patients undergoing isolated coronary bypass grafting, no significant reduction of the composite endpoint of death and/or myocardial infarction through day 30 could be observed any more. However, there was a non-significant (p = 0.07) relative risk reduction of 18% [20]. Furthermore, post hoc analyses of the cohort could identify a beneficial effect of pexelizumab in high-risk patients [21–23].

Revascularisation after acute myocardial infarction might be the clinical situation that resembles best ischaemia-reperfusion injury as it is usually studied in animal models. In contrast to coronary bypass grafting, there is no overlapping complement activation due to an extracorporeal circuit. In a phase II study on patients with acute myocardial infarction receiving systemic fibrinolysis, there was no benefit for the addition of pexelizumab [24]. However, in another phase II trial on patients with acute myocardial infarction that underwent primary percutaneous coronary intervention [25], a significant reduction of the ninety-day mortality rate in the group receiving pexelizumab as a bolus plus infusion could be observed. The relative risk reduction of the composite end point of death, new or worsening heart

failure, shock, and disabling stroke was 70% compared to the placebo group. This mortality reduction appeared early, became larger over time and persisted at 6 months. Using this regimen (initial bolus followed by infusion for 20 h), pexelizumab was well tolerated and adverse events were not increased compared to placebo. The different outcome of the two studies mentioned before was unexpected and might be explained by the different ways of reperfusion: thrombolytics themselves are quick and powerful activators of complement and therefore, local complement inhibition at the site of reperfusion might not have been sufficient. In addition, using primary percutaneous coronary intervention (PCI) instead of thrombolysis might lead to critical differences in the reperfusion process with more abrupt restoration after PCI.

Based on the promising initial findings, a large-scale study of pexelizumab bolus plus infusion for patients with ST-elevation myocardial infarction undergoing PCI was designed (APEX AMI) in order to establish the effectiveness of pexelizumab in this setting [26]. However, in this prospective, multicentre, double-blind, placebo-controlled trial enrolling 5745 patients no effect of pexelizumab on mortality or the composite end point of death, shock, or heart failure could be found.

Back to the bench?

Although some studies have clearly shown an effect for the C5 blocking agents, the effect in cardiovascular diseases was not as pronounced as was expected based on *in vitro* and animal experiments. In particular, the impressive effect of pexelizumab on the outcome of patients with acute ST-elevation myocardial infarction undergoing PCI observed in smaller phase II trials could not be confirmed in a large phase III trial. The discrepancies between these studies and in particular between the expected effects based on observations made in experimental settings and results obtained from clinical trials remain unclear. Once again these results highlight the importance of large scale studies and the difficulty of extrapolating data obtained in animal models to a clinical setting. In addition, better knowledge of disease mechanisms might be required to predict the effectiveness of new treatment strategies. For example, one potential confounding factor that has not yet been sufficiently considered in human studies on ischaemia-reperfusion injuries is the impact of functional deficiencies of complement mannose-binding lectin (MBL). MBL is a pattern recognition molecule of the complement cascade and has originally been shown to play an important role in the immunological defence against bacterial infections. However, more recent experimental studies suggested an additional role in tissue ischaemia-reperfusion injury. Experimental data on cardiac ischaemia-reperfusion injury [27, 28] as well as other experimental settings of ischaemia-reperfusion injury [29–31] point towards a major complement activating and therefore pro-inflammatory role in this setting. As a consequence, MBL deficiency in patients undergoing coronary catheter-revascularisation might be beneficial. If confirmed in patients, such an effect could be of importance for the evaluation of complement inhibitors such as pexelizumab since functional MBL deficiency can be found in about 25% of the population.

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References

1 Walport MJ. Complement. First of two parts. New Engl J Med. 2001;344:1058–66.

2 Walport MJ. Complement. Second of two parts. New Engl J Med. 2001;344:1140–4.

3 Turnberg D, Botto M. The regulation of the complement system: insights from genetically-engineered mice. Mol Immunol. 2003;40:145–53.

4 Inal JM, Hui KM, Miot S, Lange S, Ramirez MI, Schneider B, Krueger G, Schifferli JA. Complement C2 receptor inhibitor trispanning: a novel human complement inhibitory receptor. J Immunol. 2005;174:356–66.

5 Kirschfink M. Trageting complement in therapy. Immunol Rev. 2001;180:177–89.

6 Cicardi M, Zingale L, Zanichelli A, Pappalardo E, Cicardi B. C1 inhibitor: molecular and clinical aspects. Springer Sem Immunopathol. 2005;27:286–98.

7 Lazar HL, Bokesch PM, van Lenta F, Fitzgerald C, Emmett C, Marsh HC Jr, Ryan U; OBE and the TP10 Cardiac Surgery Study Group. Soluble human complement receptor 1 limits ischemic damage in cardiac surgery patients at high risk requiring cardiopulmonary bypass. Circulation. 2004;110:II274–279.

8 De Zwaan C, van Dieijen-Visser MP, Hermens WT. Prevention of cardiac cell injury during acute myocardial infarction: possible role for complement inhibition. Am J Cardiovasc Drugs. 2003;3:245–51.

9 Gerard NP, Gerard C. The chemotactic receptor for human C5a anaphylatoxin. Nature. 1991;349:614–7.

10 Thomas TC, Rollins SA, Rother RP, Giannoni MA, Hartman SL, Elliott EA, et al. Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. Mol Immunol. 1996;33:1389–401.

11 Kaplan M. Eculizumab (Alexion). Curr Opin Investig Drugs. 2002;3:1017–23.

12 Whiss PA. Pexelizumab Alexion. Curr Opin Investig Drugs. 2002;3:870–7.

13 Huber-Lang M, Sarma JV, Zetoune FS, Rittirsch D, Neff TA, McGuire SR, et al. Generation of C5a in the absence of C3: a new complement activation pathway. Nat Med. 2006;12:682–7.

14 Holt DS, Botto M, Bygrave AE, Hanna SM, Walport MJ, Morgan BP. Targeted deletion of the CD59 gene causes spontaneous intravascular hemolysis and hemoglobinuria. Blood. 2001;98:442–9.

15 Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med. 2006;355:1233–43.

16 Riedemann NC, Ward PA. Complement in ischemia reperfusion injury. Am J Pathol. 2003;162:363–7.

17 Hart ML, Walsh MC, Stahl GL. Initiation of complement activation following oxidative stress. In vitro and in vivo observations. Mol Immunol. 2004;41:165–71.

18 Fitch JC, Rollins S, Matis L, Alford B, Aranki S, Collard CD, et al. Pharmacology and biological efficacy of a recombinant, humanised, single-chain antibody C5 component inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. Circulation. 1999;10:2499–506.

19 Shernan SK, Fitch JC, Nussmeier NA, Chen JC, Rollins SA, Mojcik CF, et al.; Pexelizumab Study Investigators. Impact of pexelizumab, an anti-C5 complement antibody, on total mortality and adverse cardiovascular outcomes in cardiac surgical patients undergoing cardiopulmonary bypass. Ann Thorac Surg. 2004;77:942–9.

20 Verrier ED, Shernan SK, Taylor KM, Van de Werf F, Newman MF, Chen JC, et al.; PRIMO-CABG Investigators. Terminal complement blockade with pexelizumab during coronary artery bypass graft surgery requiring cardiopulmonary bypass: a randomized trial. JAMA. 2004;291:2319–27.

21 Haverich A, Shernan SK, Levy JH, Chen JC, Carrier M, Taylor KM, et al. Pexelizumab reduces death and myocardial infarction in higher risk cardiac surgical patients. Ann Thorac Surg. 2006;82:486–92.

22 Carrier M, Menasche P, Levy JH, Newman MF, Taylor KM, Haverich A, et al. Inhibition of complement activation by pexelizumab reduces death in patients undergoing combined aortic valve replacement and coronary artery bypass surgery. J Thorac Cardiovasc Surg. 2006;131:352–6.

23 Smith PK, Carrier M, Chen JC, Haverich A, Levy JH, Menasche P, et al. Effect of pexelizumab in coronary artery bypass graft surgery with extended aortic cross-clamp time. Ann Thorac Surg. 2006;82:781–8.

24 Mahaffey KW, Granger CB, Nicolau JC, Ruzyllo W, Weaver WD, Theroux P, et al.; COMPLY Investigators. Effect of pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to fibrinolysis in acute myocardial infarction: the COMPlement inhibition in myocardial infarction treated with thromboLYtics (COMPLY) trial. Circulation. 2003;108:1176–83.

25 Granger CB, Mahaffey KW, Weaver WD, Theroux P, Hochman JS, Filloon TG, et al.; COMMA Investigators. Pexelizumab, an anti-C5 complement antibody, as adjunctive therapy to primary percutaneous coronary intervention in acute myocardial infarction: the COMplement inhibition in Myocardial infarction treated with Angioplasty (COMMA) trial. Circulation. 2003;108:1184–90.

26 Armstrong PW, Granger CB, Adams PX, et al. Pexelizumab fro acute ST-elevation myocardial infarction in patients undergoing primary percutaneous coronary intervention. JAMA. 2007; 297:43–51.

27 Jordan, et al. Inhibition of mannose binding lectin reduces postischemic myocardial reperfusion injury. Circulation. 2001; 104:1413–8.

28 Walsh MC, et al. Mannose-binding lectin is a regulator of inflammation that accompanies myocardial ischemia and reperfusion injury. J Immunol. 2005;175:541–6.

29 Moller-Kristensen, et al. Mannose-binding lectin recognises structures on ischemic reperfused mouse kidneys and is implicated in tissue injury. Scand J Immunol. 205;61:426–34.

30 De Vries, et al. The mannose-binding lectin–pathway is involved in complement activation in the course of renal ischemia-reperfusion injury. Am J Pathol. 2004;165:1677–88.

31 Hart ML, Ceonzo KA, Shaffer LA, et al. Gastrointestinal ischemia-reperfusion injury in lectin complement pathway dependent without involving C1q. J Immunol. 2005;174:6373–80.

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