The immunoglobulin variable gene repertoire generates enormous diversity through both combinatorial and somatic mutation mechanisms. Consequently, it has the capacity to produce high-affinity, exquisitely specific antibodies to a vast number of potential antigenic targets. Also, the constant domains of antibody molecules can harness effector mechanisms of the humoral and cellular immune systems in vivo. Thus, monoclonal antibodies hold great promise for application to a wide range of diagnostic and therapeutic clinical settings, as evidenced by the current clinical use of monoclonal antibody-derived products in transplantation, myocardial revascularization and tumour imaging.

Antibody engineering
Recent advances in the molecular engineering of immunoglobulin genes have further broadened the potential utility of monoclonal antibody-based therapeutics. For example, Fab fragments, as well as covalently linked single-chain fragments consisting of the heavy and light chain variable regions (scFv's), have been derived that retain the specificity and affinity of the original intact antibody. These smaller molecules display distinct properties, such as reduced serum half-life and enhanced tissue penetration, which may be particularly useful for certain clinical situations such as tumour imaging and therapy, or for the treatment of acute inflammation. In addition, they are amenable to modifications, such as covalent linkage to genes encoding toxins, enzymes or cytokines, or to the incorporation of dual specificity or catalytic function, which can impart novel functional properties to these monoclonal antibody-derived molecules.

Complement-specific antibodies: Designing novel anti-inflammatory agents

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Chronic application of monoclonal antibody therapy requires 'humanization' to prevent a host immune response from developing. Current methods to accomplish this include complementarity-determining region (CDR) grafting, or replacement of the hypervariable loops of a human antibody with those of the murine monoclonal antibody of desired specificity, selection by phage display, or potentially using HumAb mice, in which endogenous Ig loci have been inactivated by mutation and then replaced by large segments of human immunoglobulin genomic loci.

**Complement and inflammation**

Complement proteins represent an attractive target for the development of monoclonal antibody-based anti-inflammatory therapeutics. The complement system is composed of more than 20 serum proteins that interact in a precise series of enzymatic cleavage and membrane binding events leading to the generation of products with immunoprotective, immunoregulatory, and pro-inflammatory properties.

Complement can be activated through either of two distinct enzymatic cascades, referred to as the classical and alternative pathways (Fig. 1). The classical pathway is generally initiated by the interaction of C1q with antibody/antigen complexes, whereas the alternative pathway is initiated by deposition of C3b on a variety of substrates including bacterial lipopolysaccharide and cell membranes. The formation of C3b is necessary for the amplification and progression of the complement cascade through both pathways. It is the primary opsonin for many pathogenic microorganisms and, in addition, promotes the clearance as well as solubilization of immune complexes.

Both the classical and alternative pathways converge at C5, which is cleaved to form products with multiple proinflammatory effects (see Fig. 1). C5a is the most potent anaphylatoxin, inducing alterations in smooth muscle and vascular tone, as well as vascular permeability. It is also a powerful 'chemotaxin' and activator of both neutrophils and monocytes. C5a-mediated cellular activation can significantly amplify inflammatory responses by inducing the release of multiple additional inflammatory mediators, including hydrolytic enzymes, cytokines, arachidonic acid metabolites and reactive oxygen species. C5 cleavage also leads to the formation of C5b-9, or the membrane attack complex (MAC). There is now strong evidence that the MAC may play an important role in inflammation in addition to its role as a lytic pore-forming complex, as it also stimulates the release of many of the same proinflammatory molecules as C5a and promotes thrombosis following deposition on platelets and endothelium.

**Complement in disease**

With improved methodology for the detection of activated components in inflamed tissue and biological fluids, the complement system has been increasingly implicated as contributing to the pathogenesis of numerous disease states (see table). These include immunological diseases characterized by antibody-mediated classical pathway activation, as well as vascular inflammatory conditions in which the alternative pathway is induced after reperfusion of ischaemic tissue. Distinct mechanisms of complement activation are associated with some diseases, such as Alzheimer's disease, since the β-amyloid protein has been shown to bind to C1q directly and thereby activate the classical pathway in vitro. In many conditions there is evidence for simultaneous activation of both classical and alternative pathways.

**Modelling anti-C5 therapy**

Because activation of C5 represents a critical step in the inflammatory cascade and as there are no known naturally existing molecules that uniquely block C5 activation, the C5 molecule represents an attractive target for development of a monoclonal antibody-based complement inhibitor. Therapeutic inhibition of the complement cascade at C5 would block the formation of the potent inflammatory mediators C5a and C5b-9 via both the classical and alternative pathways, while preserving the patient's

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**Fig. 1** Components of the complement cascade.
ability to generate the critical immunoprotective and immunoregulatory functions of C3b-mediated opsonization and immune clearance. Therefore, we have developed recombinant C5-specific monoclonal antibodies and their engineered derivatives as soluble anti-inflammatory biopharmaceuticals. The principle of anti-C5 monoclonal antibody therapy of inflammatory disease has been examined in several preclinical models. Using a monoclonal antibody specific for mouse C5, we have shown that systemic anti-C5 monoclonal antibody administration efficiently inhibited complement in vivo (Inhibiting serum haemolytic activity for as long as six to seven days after a single intravenous injection), and that treatment with anti-C5 monoclonal antibody was therapeutically effective in two distinct models of immune complex nephritis and autoimmune disease (Y. Wang et al., manuscript in preparation). In these models, continuous treatment with anti-C5 monoclonal antibody for up to six months was not associated with any negative side effects. In murine collagen-induced arthritis, anti-C5 monoclonal antibody therapy not only prevented the onset of disease, but, most importantly, was also highly effective in ameliorating the course of established arthritis in an ex vivo model of cardiopulmonary bypass (CPB)-induced inflammation, administration of a prototype anti-human C5 monoclonal antibody completely blocked C5a and C5b-9 generation in whole human blood, as well as both the platelet and leucocyte activation that normally occur during CPB. Thus, anti-C5 monoclonal antibody therapy effectively modulated inflammatory responses in both in vivo and ex vivo models.

Recombinant anti-C5 antibodies

To generate a human C5-specific monoclonal antibody for clinical development, mice were immunized with purified human C5 and candidate monoclonals were screened in high throughput in vitro assays for their ability to block both C5a and C5b-9 generation via the classical and alternative pathways. From this effort a highly potent anti-C5 monoclonal antibody was derived with very high affinity (Kd < 100 pM), capable of blocking complement activation at monoclonal antibody/C5 molar ratios as low as 0.5:1. The genes encoding the variable regions of this monoclonal antibody have been cloned and several recombinant forms have been engineered. Both recombinant Fab and scFv variants have been derived and shown to bind C5 with similar affinity and to block C5 activation at the same molar ratio as the intact antibody. In addition, humanized recombinant anti-C5 monoclonal antibody and scFv that retain the binding affinity and complement inhibitory activity of their murine counterparts have been produced by CDR grafting (M. Evans, manuscript in preparation).

The ability of an scFv derivative of a C5-specific monoclonal antibody to inhibit complement in vivo was tested by generating and administering intravenously a C5-specific scFv cross-reactive with primate complement. This scFv-inhibited serum complement haemolytic activity for up to 2 hours following administration of a single intravenous bolus, consistent with the more rapid clearance of scFvs relative to intact monoclonal antibodies. C5 inhibition by an intravenously administered scFv also demonstrated that the functional domains of the immunoglobulin constant region were not required for complement inhibition in vivo. The efficacy and pharmacokinetic profile of the anti-C5 scFv suggest that it may be a useful anti-inflammatory agent in acute settings such as CPB or ischaemia/reperfusion injury associated with myocardial infarction.

In summary, we have shown that inhibition of C5 activation with high-affinity monoclonal antibodies represents a novel and potentially safe and effective approach to ameliorate inflammation in a variety of clinical settings. The potent anti-C5 activity of the scFv molecule further suggests that it may be possible to define minimal peptide sequences that retain C5 inhibitory activity, and thereby ultimately design orally available small molecule peptidomimetics. With recent advances in antibody engineering, monoclonal antibody-based therapeutic approaches appear to be well poised to achieve their anticipated clinical potential.

Diseases associated with complement activation

| Renal LUPUS nephritis | Rheumatological Lupus arthritis | Neurological Cerebral lupus |
|-----------------------|-------------------------------|---------------------------|
| Membranoproliferative GN | Rheumatoid arthritis | Guillain-Barré syndrome |
| Membranous nephritis | SJögren's syndrome | Alzheimer's disease |
| IgA nephropathy | Behçet's syndrome | Multiple sclerosis |
| Vascular/pulmonary Biocompatibility | Systemic sclerosis | Myasthenia gravis |
| Atherosclerosis | Post-CPB inflammation | Dermatological Vasculitis |
| Myocardial infarction | Haemodialysis | Pemphigus |
| Stroke | Catheter reactions | Bullous pemphigoid |
| ARDS | Platelet storage | Phototoxic reactions |
| Reperfusion injury | Transplant rejection | Thermal burns |
| Allergy | Infection | Other |
| Anaphylaxis | Septic shock | Inflammatory bowel disease |
| Asthma | Viral infection | Thyroiditis |
| Skin reactions | Bacterial infection | Infertility |
| | | PNH |
| | | Haemolytic anaemia |

1. Disease association with complement activation has been demonstrated by the presence of activated complement components in biological fluids and/or deposition of activated complement components in diseased tissue. GN, glomerulonephritis; ARDS, adult respiratory distress syndrome; CPB, cardiopulmonary bypass; PNH, paroxysmal nocturnal haemoglobinuria.

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