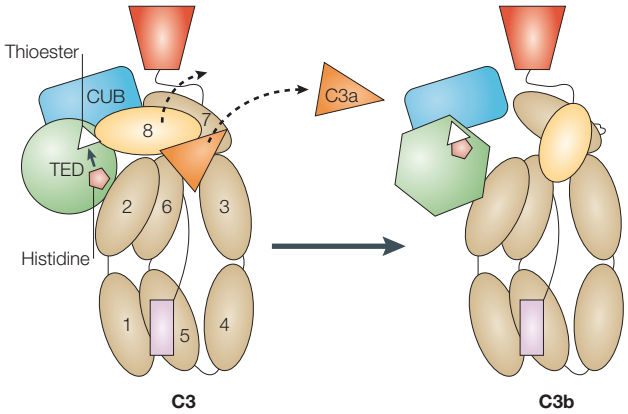
STRUCTURE

Long-awaited structures of an ancient system



Based on the structure by Janssen *et al*., this figure illustrates the conformational changes that occur on cleavage and activation of complement component C3 to yield the active fragments C3a and C3b. Release of C3a from C3 allows macroglobulin domain 8 to rotate away from the thioester-containing domain (TED). This exposes the reactive thioester and enables it to interact with a histidine residue to form a reactive intermediate. In this activated state, C3b can bind its target cells and other complement components, resulting in the formation of the membrane-attack complex. Modified, with permission, from *Nature* © (2005), Macmillan Magazines Ltd.

In a tour de force effort by Piet Gros and colleagues, the long-awaited structure of the central complement component C3 has now been resolved — possibly the largest multidomain protein structure that has been determined so far. With it, some of the mysteries that surround the origin of the complement system have been unlocked, indicating that its key defence mechanisms have changed little over hundreds of millions of years.

The crucial defensive functions of C3 are regulated by several proteolytic-cleavage steps that yield, first, the anaphylatoxin C3a and the active intermediate fragment C3b and, second, the inactive fragment C3c, following the release of C3f and C3dg. To understand how its activity is regulated, the authors resolved and compared the crystal structures of both C3 and C3c.

The core of C3 (and C3c) is composed of eight structurally homologous domains, which the authors term macroglobulin domains, six of which form a ring structure (see figure). This pattern of repeated domains indicates that these domains probably arose through duplication of a primordial gene, but in the intervening millennia, the sequences of the eight domains have diverged. Resting on top of the core ring structure are three domains with specialized functions: the anaphylatoxin domain, the CUB fold and the thioester-containing domain (TED). These domains seem to have been added to the core through gene-insertion events, possibly to provide necessary regulation of these active elements.

One of the key and most primitive elements of the complement defence mechanism is the presence of an activated thioester in the TED. In C3b, the thioester is exposed, allowing it to bind covalently to its target cells (such as bacteria and apoptotic cells). Importantly, in full-length C3, the thioester is buried between the TED and macroglobulin

IMMUNOTHERAPY

CD8$^+$CD25$^+$ regulatory T cells get the OK

Therapy with modified CD3-specific monoclonal antibodies has been shown to have both immediate and long-term benefits in some human autoimmune and allograft-transplantation settings. Now, a report in *The Journal of Clinical Investigation* indicates that one mechanism by which these antibodies might modulate the immune response is by inducing a population of CD8$^+$CD25$^+$ regulatory T cells.

The humanized CD3-specific monoclonal antibody hOKT3γ1(Ala-Ala) contains a modified Fc portion that reduces its ability to induce cytokine release (thereby reducing the adverse side-effects that are associated with its use). Previous studies have shown that those patients with new-onset type 1 diabetes who respond to treatment with hOKT3γ1(Ala-Ala) have a concomitant increase in the number of CD8$^+$ T cells. Bisikirska *et al*. found that hOKT3γ1(Ala-Ala) induced human peripheral-blood mononuclear cells (PBMCs) to proliferate *in vitro*. More specifically, whereas the CD8$^+$ T cells in the cultures divided multiple times, the CD4$^+$ T cells divided only once or twice. Importantly, 3 months after treatment with hOKT3γ1(Ala-Ala), the ratio of CD4$^+$ T cells to CD8$^+$ T cells in the peripheral blood of patients correlated with the ratio of CD4$^+$ T cells to CD8$^+$ T cells following culture of their PBMCs with hOKT3γ1(Ala-Ala). This indicates that it might be possible to predict whether a patient will respond to treatment with hOKT3γ1(Ala-Ala) by analysing the *in vitro* response of their PBMCs to stimulation with the antibody.

Further analysis showed that CD4$^+$ T cells proliferated in response to hOKT3γ1(Ala-Ala) if CD8$^+$ T cells were removed from the PBMCs and that the response of CD4$^+$ T cells to tetanus toxoid was inhibited in a dose-dependent manner by CD8$^+$ T cells that had been pre-activated by hOKT3γ1(Ala-Ala). Inhibition was mediated by cell–cell contact and not by soluble factors such as interleukin-10 and transforming growth factor-β. When the hOKT3γ1(Ala-Ala)-stimulated CD8$^+$ T-cell population was separated into CD25$^+$ and CD25$^-$ populations, it was found that only the CD8$^+$CD25$^+$ T cells could inhibit the response of CD4$^+$ T cells to superantigen. These CD8$^+$CD25$^+$ T cells were also shown to express increased levels of intracellular cytotoxic T-lymphocyte antigen 4 (CTLA4) and increased levels of mRNA encoding the transcription factor forkhead box P3 (FOXP3), which has been shown to be a marker of naturally occurring, CD4$^+$CD25$^+$ regulatory T cells in mice. A similar increase in *FOXP3* mRNA was observed in CD8$^+$CD25$^+$ T cells isolated from patients with type 1 diabetes who had been treated with hOKT3γ1(Ala-Ala).

These data indicate that a population of cells with a phenotype similar to the CD8$^+$CD25$^+$ regulatory T cells that are induced *in vitro* by stimulation with hOKT3γ1(Ala-Ala) is induced *in vivo* following treatment with the antibody, leading the authors to suggest that these regulatory cells might be central to the beneficial effects that therapy with hOKT3γ1(Ala-Ala) has for patients with type 1 diabetes.

Karen Honey

References and links

ORIGINAL RESEARCH PAPER Bisikirska, B., Colgan, J., Luban, J., Bluestone, J. A. & Herold, K. C. TCR stimulation with modified anti-CD3 mAb expands CD8$^+$ T cell population and induces CD8$^+$CD25$^+$ T_{Regs}. *J. Clin. Invest.* **115**, 2904–2913 (2005)

domain 8 and is therefore protected from hydrolysis (and thereby inactivation). In addition, the orientation of the TED of C3, which is in part maintained by the anaphylatoxin domain, prevents a histidine residue in the TED from interacting with the thioester and forming a reactive intermediate. Cleavage of the anaphylatoxin domain of C3 to produce C3b induces a conformational change that disrupts both of these protective mechanisms and exposes the reactive thioester.

These structures should provide further clues about the function and origin of the complement system and create new avenues for treating diseases that are associated with defects in complement.

Lucy Bird

References and links

ORIGINAL RESEARCH PAPER Janssen, B. J. C. *et al*. Structures of complement component C3 provide insights into the function and evolution of immunity. *Nature* **437**, 505–511 (2005)

FURTHER READING Liddington, R. & Bankston, L. Origins of chemical biodefence. *Nature* **437**, 484–485 (2005)

SIGNALLING

Achieving stimulus-specific gene expression

Nuclear factor-κB (NF-κB) is a transcription factor that is commonly active in various immune and inflammatory responses downstream of many cell-surface receptors. So, how can these different receptors induce different patterns of gene expression if they all have the same signalling end-point in terms of inhibitor-of-NF-κB kinase (IKK)-mediated NF-κB activity? Two studies reported in *Science* have used a combination of computer models and *in vitro* experiments to explain the stimulus specificity of the NF-κB pathway, using tumour-necrosis factor (TNF)-mediated signalling through TNF receptor 1 (TNFR1) and lipopolysaccharide (LPS)-mediated signalling through Toll-like receptor 4 (TLR4) as examples.

Both groups noted that TNF and LPS induce different profiles of IKK activity: TNF-dependent activation of IKK reached its peak between 5 and 15 minutes and then showed oscillatory behaviour if the stimulus continued, whereas LPS-mediated signalling resulted in a slower biphasic IKK response, which consisted of a small initial increase in IKK activity followed by a larger increase and a slowly attenuating later phase. Using a computational simulation of the IKK–NF-κB pathway, which was then confirmed by *in vitro* measurements, Hoffmann and colleagues showed that these differences in IKK profile correlate with distinct NF-κB-activity profiles. The oscillating response to TNF is thought to be the result of a negative-feedback mechanism by which NF-κB activation results in the synthesis of inhibitor of NF-κB (IκB) protein, so why does LPS not induce a similar pattern?

Baltimore and colleagues analysed the MyD88-dependent and MyD88-independent (TRIF-dependent) pathways of TLR4 signalling separately, using mouse embryonic fibroblasts that were deficient in MyD88 or TRIF. They showed that stimulation with LPS in the presence of either of the TLR4-signalling pathways alone resulted in oscillatory NF-κB activation. Using a computer model to simulate the two TLR4-signalling pathways, they predicted that the MyD88-independent pathway requires a time delay before it is activated, which might occur if protein synthesis is required for this pathway. Indeed, in LPS-stimulated, MyD88-deficient cells treated with the protein-synthesis inhibitor cycloheximide, NF-κB activation was prevented. Hoffmann and colleagues also reported that the second phase of IKK activity in response to LPS was inhibited by cycloheximide. Both groups showed that stimulation with LPS induced expression of TNF and that the second phase of IKK activity in response to LPS depends on TNF-mediated signalling. Hoffmann and colleagues showed that this second phase of TNF-induced IKK activity is essential for LPS-specific gene expression.

Therefore, the authors of both studies propose that the biphasic IKK response to LPS is the result of a positive-feedback mechanism in which early NF-κB activity through the MyD88-dependent pathway is accompanied by TNF production induced by the MyD88-independent pathway, which results in late NF-κB activity through TNF-mediated signalling. According to Baltimore and colleagues, each of these pathways individually has oscillatory behaviour, due to negative feedback, but their combination out of phase results in a more stable response. Therefore, the stimulus-specific expression of genes mediated by the same transcription-factor pathway can be controlled by positive- and negative-feedback mechanisms that determine the timing of transcription-factor activity.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPERS Werner, S. L., Barken, D. & Hoffmann, A. Stimulus specificity of gene expression programs determined by temporal control of IKK activity. *Science* **309**, 1857–1861 (2005) | Covert, M. W., Leung, T. H., Gaston, J. E. & Baltimore, D. Achieving stability of lipopolysaccharide-induced NF-κB activation. *Science* **309**, 1854–1857 (2005)