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Title: Properdin-dependent activation and control of immune-homeostasis and autoimmunity
Issue Date: 2014-08-27
Summary

In a normal situation your body is protected from insults (viruses, bacteria and parasites) by the immune system. The immune system consists of a powerful set of tools which function to protect the host and maintain the normal physiological state. In autoimmunity, the immune system attacks the body itself inducing damage, generally due to a combination of genetic and environmental factors. The damage induced by autoimmunity can result in various illnesses known as autoimmune diseases. Systemic lupus erythematosus (SLE), anti-glomerular basement membrane (GBM) and anti-neutrophil cytoplasmic antibody (ANCA) vasculitides are examples of autoimmune diseases all of which can target the kidney.

The immune system is an intricate collection of cells and proteins which interact to form a potent tool for the body. The cells of the immune system include white blood cells such as dendritic cells and neutrophils, while the proteins vary from antibodies and clotting factors to the complement system. If the body is damaged (cut) and becomes infected (by bacteria), the site of injury becomes the focal point of cells and proteins of the immune system to help resolve and heal the damaged site. Resolution of the injury by the immune system is typically achieved when the key signs of inflammation e.g swelling, pain, redness and heat are removed from the site of injury.

The complement system is a key member of the immune system. The system is a collection of proteins found in circulation which have a role in removing infections or injured/dying cells. The complement system is divided into three pathways the classical pathway (CP), lectin pathway (LP) and alternative pathway (AP). The CP is activated by the initiator protein C1q while the LP generally requires the protein mannan binding lectin (MBL) to act as a recognition molecule for LP activation. The alternative pathway is the oldest of the pathways and can become auto-activated should it be left uncontrolled. It consists of various proteins including complement C3, factor B, factor D and properdin. The AP can become activated in seconds allowing it to act as a potent pathway in the complement system. Due to its swift capability in becoming activated, the AP requires tight regulation. Properdin promotes AP activation by stabilising the pathway, while factor H inhibits AP activation, allowing both proteins to regulate the level of complement activation.

In chapter 2, we investigated the role the complement system plays in lupus nephritis, the renal manifestations in SLE. Earlier studies from our department have shown that anti-C1q antibodies are strongly associated with renal disease in patients with SLE. During lupus nephritis, the endothelial cells are the principle target. We investigated how autoantigens targeted during SLE, e.g C1q, interact
with the cells and whether it supports complement activation. The autoantibodies bound to the endothelial cells and supported CP complement activation, suggesting a mechanism of damage in lupus nephritis. The findings demonstrate the close relationship between classical pathway components, such as C1q and anti-C1q autoantibodies, which may play a role in the development of lupus nephritis.

Properdin is a key member of the AP, with its traditional function stabilising the AP by binding activated C3. In recent years a novel role for properdin has been proposed whereby it acts as a pattern recognition molecule, initiating the AP thereby expanding its possible roles in disease states. The AP has been implicated in various renal autoimmune diseases including ANCA vasculitides and anti-GBM disease. Both diseases have an autoantibody component which would suggest CP activation. However the AP is also implicated in both diseases but the exact mechanism is unknown. In chapter 3, we investigated the role of the AP, in particular properdin, in an in-vivo model of anti-GBM disease. We found that deficiency of properdin or C3 in-vivo did not protect from renal damage as compared to the normal control. We demonstrated that properdin levels increased in circulation during the disease time course, while properdin was also deposited at the site of injury in the kidney. Interestingly, properdin was deposited in the kidney independent of C3, suggesting that also here properdin can act as a pattern recognition molecule. This is to our knowledge the first proof of properdin binding independent of C3 in-vivo, supporting the earlier proposed ability of properdin to act as a pattern recognition molecule.

The AP is intricately implicated in neutrophil mediated diseases including ANCA vasculitides, but its exact role in the disease is still unclear. In chapter 4 we isolated human properdin which in chapter 5 we used to investigate its interaction with neutrophil components and the capacity to direct AP activation. The neutrophil enzymes myeloperoxidase (MPO) and proteinase 3 (PR3) are the main targets in ANCA vasculitides. Properdin bound to lysozyme, cathepsin G, elastase, PR3 and in particular MPO. MPO exposed to serum is capable of inducing C3 deposition, which was significantly reduced with properdin-deficient serum. More in-depth analysis revealed that binding of properdin to MPO supported AP-mediated C3 deposition and increased generation of C5b-9, indicating AP activation. The results indicated a new role for MPO in activating the complement system and illustrate its ability to support properdin-directed complement activation. Interestingly, various neutrophil enzymes bound to properdin without the ability to activate complement (as shown for MPO), suggesting additional mechanisms of regulating properdin in the context of neutrophil degranulation.

As mentioned earlier, properdin may act as a stabiliser or initiator of the AP. In recent years, properdin has been shown to bind proximal tubular epithelial cells (PTEC) in the kidney following injury to the organ. Furthermore, in-vitro data
demonstrated that properdin can bind to the cells initially and act as an initiator for AP activation on its cell surface. Recent studies from various groups have indicated that an inhibitor of properdin may exist in-vivo, however the exact identity of the molecule remains unknown. In chapter 6, we investigated whether properdin binding could be inhibited by normal human serum, possibly confirming the existence of an inhibitor. Our data showed that human serum inhibited the ability of properdin to bind to PTEC. Our studies focused on C-reactive protein (CRP), both the pentameric CRP and the dissociated form, monomeric CRP. We demonstrated that specifically monomeric CRP could inhibit properdin binding to viable PTEC, whereas pentameric CRP did not exhibit such activity. Interestingly, monomeric CRP could in solution form a complex with properdin, while its larger pentameric form was unable to do so. The ability of monomeric CRP to inhibit properdin binding to PTEC, impeded properdin-directed C3 deposition and C5b-9 generation on these cells. More importantly, this inhibitory feature of monomeric CRP was not unique for the binding of properdin to PTEC, since it also prohibited properdin binding to necrotic Jurkat cells. These findings indicate that properdin is capable of binding to a dissociated form of pentameric CRP in solution, further expanding its capability to bind various other ligands independent of C3. Moreover, the binding of monomeric CRP to properdin and inhibition of properdin-directed AP activation reveals a novel role for CRP in regulating complement activation.

Properdin can exist in various forms including dimers, trimers, tetramers as well as pentamers and is found in the bloodstream at a concentration of approximately 25µg/ml. Typically most complement components are produced by the liver, with certain components such as C1q or properdin produced by white blood cells. The white blood cells can act as potent reservoirs of complement components at the site of damage or insult. The ability of the AP to become autoactivated makes it increasingly susceptible to the AP regulators, properdin and factor H. Activation of the AP by properdin is counter-acted by factor H, the most prominent negative regulator of the AP. Our aim in chapter 7 was to investigate whether human monocyte derived dendritic cells (DC) or tolerogenic (tolDC) can act as a source of properdin and factor H, and whether this production can be modulated. Our studies demonstrated the constitutive capability of DC to produce properdin and factor H, with higher levels of both AP components produced by tolDCs. Upon activation with IFNγ both cell types showed an increased FH production, while simultaneously decreasing the production of properdin. This was unique for IFNγ as LPS and IFNα or IFNβ did not demonstrate this dual regulation. IL-27, a member of the IL-12 family which elicits features of IFNγ, did increase production of FH, but FP remained unaffected. This prompted us to investigate if either DC-derived regulators of the AP had a role in the DC T cell synapse. Therefore
we silenced production of properdin or factor H in DCs, and investigated the
impact on allogenic T cell activation. Interestingly, silencing of factor H in DC
resulted in an increased T cell proliferation. In contrast, silencing of properdin
in DC diminished their allostimulatory capability. Cumulatively, these results
indicate the importance of both AP regulators, guiding the AP towards activation
or regulation.

Our studies mainly encompassed the alternative pathway focussing on regulation
by factor H and properdin. We demonstrated that properdin has a wider repertoire
of functions than only stabilising AP activation. Our *in-vivo* results supported
the ability of properdin to act as a pattern recognition molecule independent
of activated C3. Our *in-vitro* data illustrated that properdin can bind various
new ligands supporting the properdin-directed model of AP activation, and the
existence of a novel inhibitor of properdin to control complement activation.
DC exhibited the ability to act as a potent source of both AP regulators and
the unique effect of IFNγ stimulation on modulation of their production. The
unexplored importance of DC derived factor H and properdin was illustrated by
their opposing effects on T cell immunity.