the cells even in the absence of ischemia. TECs from Crry−/−fB−/− mice and wild-type mice were grown in primary culture. The expression of complement regulatory proteins on TECs from wild-type mice was examined by flow cytometry. We found that the TECs express Crry, but CD55 and CD59 were not detected. Next, TECs were incubated with 10% normal mouse serum, and C3 deposition on the surface was examined by FACS analysis. Spontaneous C3 deposition was greater on the Crry−/− cells than on Crry+/+ cells. Because Crry expression is polarized to the basolateral surface of TECs, cells were also grown on transwell filters and exposed to 10% serum in the bottom chamber. More C3a was generated by contact of serum with the Crry−/− cells than with Crry+/+ cells. Next, when mice were reconstituted with 50 μg of factor B, deposition of C3 was observed along 63 ± 9% of the proximal tubules of Crry−/−fB−/− mice but only 3 ± 3% of the tubules in Crry+/+fB−/− controls ($P < 0.003$). Tubular epithelial cells in the reconstituted Crry−/−fB−/− mice demonstrated epithelial cell injury and detachment as well as the presence of neutrophils in the glomeruli and around the tubules. These observations were not present in the factor B reconstituted Crry+/+fB−/− control mice. These studies demonstrate that TECs that do not express Crry are susceptible to spontaneous complement mediated injury in vivo even in the absence of ischemia or other cellular stressors. More generally, these findings help explain why inherited deficiency of complement regulatory proteins causes tissue-specific inflammation by the alternative pathway.

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Sensitive and specific assays for C3 nephritic factors permit dissection of mechanisms underlying complement dysregulation

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C3 nephritic factors (NeFs) are autoantibodies which prolong half life of the alternative pathway C3 convertase and/or prevent its decay by regulators resulting in uncontrolled complement activation. NeF are strongly associated with renal disease but their role in pathogenesis remains unclear. We have designed sensitive and specific ELISAs and haemolysis assays to interrogate the different properties of NeF, such as: (1) ability to stabilise C3 and/or C5 convertase; (2) dependence on properdin; (3) capacity to induce resistance to regulation by decay accelerators soluble complement receptor 1 (sCR1), decay accelerating factor (DAF), and factor H (fH). Using these assays we have characterised a panel of 31 different NeFs and investigated the mechanism(s) of dysregulation. We divided the NeF into two groups according to their ability to stabilise C3 convertase in the absence (type I) or presence (type II) of properdin. We found a prevalence of NeF type I (19 out of 31), all prevented decay by fH and most also prevented decay by DAF and sCR1. In contrast, C3 convertases stabilised by type II NeF were, with a single exception, decayed by fH although degree of resistance to other regulators varied. Both groups of NeF bound only the complexed enzyme and not the individual components (C3b, factor B, Bb, properdin), suggesting either that they bound conformational neoepitopes or that the binding site spanned more than one protein moiety. Our data show that type I and II NeFs are functionally distinct, differing not only in their ability to stabilise C3 convertase in the absence or presence of properdin, but also in their ability to prevent accelerated decay by regulators, suggesting that they bind to different regions of the assembled convertase. Dissection of the mechanisms underlying these differences may provide insight into the association of specific disease phenotypes with different NeFs and guide strategies for treatment of patients harbouring different classes of NeF.

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Innate immune proteins C1q and MBL modulate clearance and metabolism of modified lipoproteins by human monocytes and macrophages

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Atherosclerosis is a chronic inflammatory disorder that in early stages is characterized by the accumulation of modified lipoproteins in the arterial intima. Mechanisms governing the removal of modified lipoproteins and processing of cholesterol are critical to the progression of atherosclerosis. C1q and MBL are not only recognition components of the complement cascade, but we and others have shown that they are pattern recognition receptors able to modulate phagocytosis and cytokine responses in phagocytic cells. Therefore we have studied the role of C1q and MBL in the clearance of native and modified lipoproteins by human monocytes and macrophages. Both C1q and MBL bind and enhance the monocyte/macrophage clearance of modified forms of low density lipoprotein (LDL) including oxidized LDL (OxLDL) and acetylated LDL (AcLDL), but not native LDL. Modified forms of LDL also activated the classical complement pathway in a C3b deposition ELISA, but no lectin pathway activation was detected. C1q also suppressed levels of free cholesterol accumulation in monocytes that had ingested OxLDL, and enhanced HDL-specific cholesterol efflux from monocytes and HMDM. These results suggest that beyond their role in activation of complement, C1q and MBL may also play an important role in the atherosclerotic region by enhancing cholesterol clearance and metabolism.

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Acquired and hereditary complement abnormalities in atypical hemolytic and uremic syndrome and membranoproliferative glomerulonephritis

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Membranoproliferative glomerulonephritis (MPGN) is an uncommon cause of chronic nephritis that occurs primarily in children and young adults. In contrast atypical hemolytic uremic syndrome (aHUS) is an acute disease of microangiopathic hemolytic anemia, thrombocytopenia, and renal impairment