Complement-Mediated Cellular Injury

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Summary: Complement activation and recruitment of inflammatory leukocytes is an important defense mechanism against bacterial infection. However, complement also can mediate cellular injury and contribute to the pathogenesis of various diseases. With the appreciation that the C5b-9 membrane attack complex can injure cells in the absence of leukocytes, a role for the terminal complement pathway in inducing cell injury and kidney disease was shown in several experimental models, including the rat passive Heymann nephritis model of human membranous nephropathy. In podocytes, sublytic C5b-9 activates a variety of downstream pathways including protein kinases, lipid metabolism, reactive oxygen species, growth factors/gene transcription, endoplasmic reticulum stress, and the ubiquitin-proteasome system, and it impacts the integrity of the cytoskeleton and slit diaphragm proteins. C5b-9 also injures other kidney cells, including mesangial, glomerular endothelial, and tubular epithelial cells, and it contributes to the pathogenesis of mesangial-proliferative glomerulonephritis, thrombotic microangiopathy, and acute kidney injury. Conversely, certain C5b-9 signals limit complement-induced injury, or promote recovery of cells. In addition to C5b-9, complement cleavage products, such as C5a and C1q, can injure kidney cells. Thus, the complement system contributes to various kidney pathologies by causing cellular damage in both an inflammation-dependent and inflammation-independent manner.
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The complement system is composed of three pathways, namely the classic (antibody-activated), alternative, and mannose-binding lectin pathways, which contain more than 30 proteins involved in activation and regulation.[1–3] The functions of the complement system are numerous, and although this review focuses on complement-mediated cellular injury, the complement system acts as a rapid and efficient immune surveillance system, eliminates cellular debris and infectious microbes, contributes substantially to homeostasis, and even may be involved in the repair and regeneration of damaged tissues.[2,3] Complement-mediated cellular injury has been implicated in various diseases including glomerulonephritis, sepsis, lupus, rheumatoid arthritis, myocardial infarction, multiple sclerosis, myasthenia gravis, organ transplant rejection, and, more recently, osteoarthritis and age-related macular degeneration.[2–5] In early studies of complement in experimental glomerulonephritis, the focus was on antibody-dependent complement activation as a mechanism for recruitment of inflammatory leukocytes. With the appreciation that the C5b-9 membrane attack complex can injure cells in the absence of inflammation, a role for the terminal complement pathway in inducing podocyte injury and proteinuria was shown in rat passive Heymann nephritis (PHN), an experimental model of human membranous nephropathy (see Table 1 for a list of animal models).[6–8] Consistent with induction of proteinuria, in PHN the C5b-9 complex has been localized in immune deposits, and in plasma membranes on the soles of the foot processes of visceral glomerular epithelial cells (GECs, commonly known as podocytes). C5b-9 also has been shown to induce podocyte injury in experimental anti–glomerular basement membrane (GBM) nephritis, and is the key mediator of mesangial injury in the anti-Thy1 model of human mesangial-proliferative glomerulonephritis.[4,9] Other human kidney diseases or experimental models in which complement is activated and there is C5b-9 assembly and injury include acute kidney injury (AKI), tubulointerstitial disease, and atypical hemolytic uremic syndrome (aHUS).[2–5,9] This article describes the mechanisms by which complement induces cellular injury and its role in the pathogenesis of renal disease. The main focus is on signaling pathways activated by the terminal pathway, that is, membrane attack complex, C5b-9, in kidney glomerular and tubular cells. Other components of the complement system, such as C5a and C1q, are discussed briefly in the context of renal cell injury. The effects of complement on non–kidney cell injury also are presented where appropriate to provide additional insights into relevant mechanisms.

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Table 1. Animal Models of Complement-Induced Injury and Corresponding Human Diseases

Animal Model	Corresponding Human Disease	Site of Injury	Complement Component
PHN	Membranous nephropathy	Visceral GEC (podocyte)	C5b-9
Chronic BSA overload	Proteinuric glomerulopathy	Glomerular and tubular cells	C5b-9, C3
Anti-Thy1 nephritis	Mesangial-proliferative glomerulonephritis	Mesangial cells	C5b-9, C5a, C1q
Anti-GBM nephritis	Anti-GBM nephritis	Visceral GEC and GBM	C5b-9, C5a, C1q
Thrombotic microangiopathy (anti–glomerular endothelial cell antibody nephritis)	HUS	Glomerular endothelial cells	C5a and/or C5b-9
Ischemia-reperfusion	Acute tubular nephrosis	Tubular epithelial cells	C5b-9, C5a
Cisplatin-induced AKI	Cisplatin nephrotoxicity	Tubular epithelial cells	C5a, C5a receptor

Abbreviation: BSA, bovine serum albumin.

C5b-9–INDUCED RESPONSES IN CELLS

The assembly of C5b-9 in the plasma membrane of a cell results in the formation of transmembrane channels or rearrangement of membrane lipids, with loss of membrane integrity. A single C5b-9 complex leads to lysis of an erythrocyte, whereas lysis of nucleated cells requires multiple C5b-9 lesions, and lower doses of C5b-9 induce sublethal (sublytic) injury in nucleated cells.[1,10–12] In vivo, there are relatively few examples of complement lysis,[2] and complement-mediated cellular injury generally is associated with sublytic amounts of complement. In addition to disrupting the plasma membrane, sublytic doses of C5b-9 lead to cell injury through the activation of specific signaling pathways (Table 2).[1,10–12] Such pathways have been characterized in numerous cell lines, as well as in in vivo disease models. Pathways activated by C5b-9 in GECs include protein kinases, phospholipases, reactive oxygen species (ROS), transcription factors, growth factors, proteinases, stress pathways, and others. Ultimately, these signals may impact on metabolic pathways, and the structure or function of lipids and the cytoskeleton, filtration slit diaphragm, or other cellular compartments.

Although various signals of C5b-9 injure nucleated cells, it should be noted that other signals are activated in parallel to limit/restrict the complement-induced injury, or to promote recovery. Indeed, nucleated cells are equipped with several mechanisms that support resistance to complement-dependent cytotoxicity.[1,12] Thus, multiple pathways may be activated simultaneously by sublytic C5b-9 in nucleated cells, and there is likely an equilibrium among pathways that lead to cellular injury with those that are cytoprotective.

ACTIVATION OF PROTEIN KINASES

The activation of a signaling pathway after assembly of sublytic C5b-9 in all probability starts at the plasma membrane, where C5b-9 is assembled. Given that C5b-9 forms a membrane channel or a leaky patch, a C5b-9–induced increase in cytosolic free Ca^{2+} concentration resulting from a calcium influx has been reported in various cell lines, including GECs.[1,6,7,12] Sublytic C5b-9 also can induce Ca^{2+} release from intracellular storage sites. Together, such changes in intracellular Ca^{2+} concentration can lead to the activation of protein kinases, among which is protein kinase C.[1,6,12] At the plasma membrane of lymphoblastoid B cells, C5b-9 assembly was shown to couple to heterotrimeric G proteins, which then can activate downstream effectors.[1] Sublytic C5b-9 induced transactivation of receptor tyrosine kinases at the plasma membrane, in cultured GECs and in PHN.[6,7] This resulted in activation of the Ras–extracellular signal-regulated kinase (ERK) pathway and phospholipase C-γ1. Transactivated receptor tyrosine kinases potentially serve as scaffolds for assembly and/or activation of proteins, which then lead to activation of downstream effector pathways, either independently or in conjunction with the increased cytosolic Ca^{2+} concentration. Complement-mediated activation of ERK was blocked by expression of a dominant-inhibitory mutant of Ras or a constitutively active RhoA mutant, or disassembly of F-actin, indicating that the mechanisms involve the Ras pathway and are facilitated by an intact actin cytoskeleton.[6,7] The role of ERK in complement-induced injury may be cytoprotective (see later).

Besides ERK, sublytic C5b-9 can activate the p38 kinase pathway in GECs.[6,7,13] The functional role of p38 appears rather complex. In GEC culture, the p38 pathway reduced complement-mediated cytotoxicity, and the cytoprotective effect involved heat shock protein-27. p38 activity was increased in glomeruli of rats with PHN, and treatment of these rats with a p38 inhibitor exacerbated proteinuria. In keeping with these experimental data, enhanced phosphorylation of p38 in podocytes was reported in biopsy specimens of human membranous nephropathy.[7,14]

Apoptosis signal-regulating kinase-1 (ASK1) is a mitogen-activated protein kinase kinase kinase, which is regulated by ROS, and can stimulate the p38 and c-Jun N-terminal kinase (JNK) pathways. ASK1 was activated in glomeruli of rats with PHN, and exposure of