Cell Death in the Colonic Epithelium During Inflammatory Bowel Diseases: CD95/Fas and Beyond

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Abstract: CD95 is a member of the death receptor family. It is a prototypical inducer of apoptosis that, upon binding of its cognate ligand (CD95L), forms a death-inducing signaling complex composed of adaptor molecules and initiator caspases that transmit the apoptosis signal. The CD95/CD95L system was implicated in the etiology of inflammatory bowel disease (IBD) based, primarily, on the finding that CD95 is highly expressed in the intestinal epithelial cells and that epithelial apoptosis is increased in IBD. In recent years it has been recognized that CD95, while playing an important role as an apoptosis-inducing receptor in the immune system, also has multiple nonapoptotic functions on nonimmune cells. This review critically discusses the data on the possible function of CD95 as an apoptosis-inducing receptor in IBD and discusses alternative mechanisms for epithelial cell loss in IBD.

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Key Words: cell death, colitis, CD95

Crohn's disease (CD) and ulcerative colitis (UC), collectively termed inflammatory bowel disease (IBD), are chronic diseases that result from inappropriate mucosal immune activation. CD and UC are among the most common and broadly studied diseases in the US. Although the etiology of IBD is still awaiting elucidation, genetic, immunological, microbial, and environmental aspects are believed to contribute to IBD.[1] Both apoptosis and proliferation of epithelial cells could be found in the process of colitis,[2] suggesting that the balance between these 2 processes must be tightly regulated. Once deregulated, chronic colitis is believed to be one cause of colon carcinogenesis.[3] In this review we focus on the role of the apoptosis inducing receptor CD95 (Fas/APO-1) in IBDs.

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CELL DEATH PATHWAYS

Maintaining the balance between cell proliferation and cell death is of pivotal importance to homeostasis of tissues and organs. In general terms, imbalances that favor cell proliferation lead to neoplasms, while excess cell death triggers inflammatory responses. Programmed cell death responses are typically classified as apoptosis (type I), autophagy (type II), or necrosis (type III).[4]

Apoptosis is a physiological process that eliminates unwanted, damaged, or virus-infected cells in a way that does not evoke an inflammatory response. Upon binding by their cognate ligands, cell surface death receptors (DRs), CD95 (Fas/APO-1), TNFR1, DR3, DR4, and DR5[5] form homotrimers that recruit death domain (DD) containing molecules, such as FADD (Fas-associated death domain)[6] or TRADD (tumor necrosis factor [TNF]-receptor associated death domain),[7] through the interaction with their intracellular DDs. This initiates the extrinsic pathway of apoptosis, so-termed because it is activated by extracellular signals. Following the recruitment of FADD to DRs, its death effector domain binds to caspase-8, an enzyme that serves as the initiator of apoptosis through dimerization and self-cleavage followed by activation of downstream effector caspases, such as caspase-3.[8] In contrast, the intrinsic pathway of apoptosis is triggered by an intracellular death signal involving mitochondrial dysfunction and resulting in formation of the apoptosome, which contains Apaf-1, cytochrome c, procaspase-9, and ATP/dATP.[9] Conversion of procaspase-9 to the active form allows caspase-9 to cleave, and thereby activate, procaspase-3, the most important executioner caspase. Knockout mouse studies have shown that both caspase-3 and the related caspase-7 are critical to the intrinsic apoptosis pathway.[10]

Autophagy occurs during conditions of nutrient deprivation and is also associated with tumor initiation and progression.[11] During the process of autophagy, intracellular proteins, organelles, or viruses together with a portion of cytosol are wrapped into a double-membrane structure and later digested by lysosome hydrolases.[12] Although there are contradicting views on whether autophagy is a cell survival or death pathway, recent findings suggest it is more likely a cell survival pathway.[13–15] Interestingly, apoptosis and autophagy share a number of positive molecular regulators such as p53,[16] Bcl-2, Bcl-x_L, Beclin-1,[17,18] and FADD.[19] Conversely, the PI3 kinase/Akt pathway inhibits both apoptosis and autophagy.[20]

Necrosis was long viewed as an unphysiological form of cell death that is not "programmed." However, recent data on the identification of RIP1 as a central mediator of necrosis have resulted in a different view, resulting in a new term, necroptosis.[4,21] Necrosis/necroptosis is a caspase-independent process that involves molecules like endonuclease G, HMGB1, DNAse II, RIP1, and RIP3.[21–25] However, there is also crosstalk between autophagy and necrosis through the PI3 kinase-Akt-mTOR pathway,[26] indicating that the three types of programmed cell death are related to and may be modulated by one another. For the remainder of this review, we focus on apoptosis unless otherwise stated.

CD95 RECEPTOR

Like all DRs, CD95 carries the conserved 80 amino acid long DD, within its cytoplasmic tail. The DD is essential for initiation of apoptosis. CD95L binding causes CD95 to assemble the death-inducing signaling complex (DISC) at its DD. Recruited proteins essential for DISC function include the adaptor molecule FADD, procaspase-8, procaspase-10, and the caspase-8/10 regulator c-FLIP.[27–31] Oligomerization is thought to allow caspase-8 to be cleaved and released from the DISC as an active heterotetramer containing two p18 and 2 p10 subunits.[32] Active caspase-8 then initiates the apoptotic program. We introduced a model of two CD95 signaling pathways to better describe the differences between cells that undergo apoptosis independently of mitochondrial signal amplification and those cells that rely on mitochondria for cell death. We termed them Type I and Type II cells, respectively.[33,34] Type I cells release large quantities of active caspase-8 from the DISC through efficient recruitment of FADD to the DD of CD95. This caspase-8 amount is sufficient to directly cleave and activate caspase-3. In contrast, Type II cells produce very little active caspase-8 at the DISC, and this is sufficient to cleave the proapoptotic BH3 domain only containing the Bcl-2 family member Bid,[35,36] causing its translocation to the mitochondria, where it induces the release of mitochondrial factors that ultimately enhance the apoptotic signal. Hepatocytes are Type II cells, while thymocytes and peripheral T cells are Type I (reviewed Ref. 34). While Type II cells are sensitive to apoptosis induced by both soluble and membrane-bound forms of CD95L, Type I cells are almost completely resistant to the killing effects of physiological CD95L.[37] Type I cells, therefore, may receive signals that activate processes other than apoptosis via CD95.

Recently the apoptosis inhibitor XIAP was found to be a critical regulating switch between the two apoptosis pathways.[38] XIAP deficiency converts Type II cells, such as hepatocytes, into Type I cells. However, this activity is likely limited to the apoptosis-inducing activity of CD95 and the differences in CD95 signaling between cell types. Consistent with this, Type I and Type II cells do not only differ in the way CD95 signals apoptosis, but also differ in their expression of two major microRNA families, let-7 and miR-200.[39,40] Expression of these microRNAs impacts cellular differentiation status and functions including drug sensitivity[37] and sensitivity to CD95 mediated-apoptosis (unpubl. data).

CD95 IN UC: YESTERDAY AND TODAY

UC was one of the first diseases of the gut in which the CD95/CD95L system was suggested to be involved. In contrast to CD, which seems to be characterized by resistance of activated T cells to apoptotic signals,[41] CD95L expression was significantly increased in lamina propria T cells of UC lesions and was associated with increased numbers of apoptotic cells.[42–46]

Localization and expression level of CD95/CD95L or related molecules in colonic epithelial cells, lamina propria cells, and mucosa lymphoid cells may exert distinct functions and have a different influence on the outcome of colon inflammation. Increased expression of CD95L in lamina propria cells together with decreased expression of CD95 on T cells and macrophages were noted in IBD patients, implying a reduced response of CD95L-induced apoptosis of lymphoid cells.[47] In addition, TIA-1, an intracellular antigen of T cells, which alters apoptosis sensitivity through alternative splicing the premessenger RNA, was found to be elevated in patients with active UC and CD, suggesting reduced apoptosis sensitivity of cytotoxic T lymphocytes (CTLs).[48]

Normal, freshly isolated colon crypt epithelia rapidly undergo apoptosis when incubated in vitro with a CD95-activating antibody.[43] This apoptosis generated small epithelial defects.[43] Thus, CD95-induced mucosal damage was thought to contribute to decreased epithelial barrier function in UC.[49,50] Consistent with this, soluble CD95L was increased in the serum of UC patients.[43,51] CD95 expression was also increased in these patients, but not in healthy controls or in non-IBD colitis patients. Data from some mouse models also suggested a role of the CD95/CD95L system in UC. For example, increased in vitro CD95-dependent cytotoxic activity was detected in lamina propria CD4+ T cells isolated from mice with colitis induced by adoptive transfer.[52] Moreover, treatment of diseased mice with CD95 antagonist antibodies significantly reduced lamina propria T-cell apoptosis, suggesting that CD95-dependent cytotoxicity is present in vivo.[53] Consistent with this is a report that demonstrated that anti-CD95 ligand monoclonal antibody was able to ameliorate the clinical manifestations of $CD4^{+}CD45Rb^{high}$ T-cell adoptive transfer colitis, including weight loss and diarrhea. Anti-CD95 monoclonal

antibody also suppressed production of TNFα and interleukin (IL)-1β by lamina propria mononuclear cells.[54]

CD95-dependent cytotoxic activity in vitro was shown using lamina propria cells from mice in which colitis was induced by transfer of wildtype (WT) bone marrow (BM) into T-cell and natural killer cell-deficient mice.[55] In this model, however, colitis also developed when BM cells from *gld* (expressing nonfunctional CD95L) instead of WT mice were transferred, whereas colitis was less severe after transferring cells with defects in another cytotoxic molecule, perforin. These results suggest that expression of CD95L is not the only way by which cytotoxic T-lymphocytes kill target cells. This was further highlighted by studies showing that acute anti-CD3 antibody-induced enteritis was reduced by perforin knockout mice, but was not altered by TNF receptor or CD95 deficiency.[56] However, mice with perforin and CD95L defects were protected to a greater extent than those lacking only perforin, suggesting that several cytotoxic molecules may synergize to cause mucosal injury in this model. In contrast, MRL/*lpr* mice, which bear a CD95 mutation, have increased sensitivity to trinitrobenzene sulfonic acid (TNBS) colitis,[57] perhaps due to decreased CD95-dependent apoptosis of mucosal lymphocytes. Nevertheless, despite these intriguing data, most people studying IBD lost interest in CD95 as a causative agent for several reasons.

- Most reports implicating CD95 in colitis were correlative. It was therefore cautioned that the mere expression of CD95 within colonic epithelium together with increased CD95L expression does not necessarily indicate CD95-induced apoptosis.[58]
- More recent studies did not find a correlation between CD95L expression and apoptosis or inflammation during active colitis. In contrast, perforin expression did correlate with apoptosis.[47,48]
- Although neutralizing anti-CD95L antibodies ameliorated disease in murine models of colitis, histological analysis failed to demonstrate reduced local inflammation, suggesting that CD95L was not directly involved in tissue damage.[54]
- Finally, a great deal of confusion was caused by a number of reports that used antibodies of questionable CD95L specificity.[59–61] Although a recent editorial suggested that certain anti-CD95L antibodies should be removed from the market,[59] such antibodies have been some of the most widely used reagents for immunohistochemical detection of CD95L.

CD95 AND COLON CANCER

Colonic epithelial cell homeostasis is tightly regulated; minor perturbations can lead to colitis or neoplasia.[62] These may also occur in response to a single stimulus, such as azoxymethane (AOM), which can induce epithelial apoptosis as well as neoplastic transformation, alone or in combination with dextran sodium sulfate (DSS).[2,3,63] The role of CD95 in these processes is only beginning to be understood, as recent data indicate that CD95 is not only a dedicated apoptosis inducing receptor but also as a regulator of cell proliferation and migration.[64,65] We have summarized these novel activities in a recent review.[66] A dominant nonapoptotic function of CD95 in most tissues, as opposed to major proapoptotic roles in immune cells[67,68] and certain disease situations such as hepatitis,[69] is beginning to emerge. This new perspective has been advanced by the recent development of mouse models with CD95 deficiency limited to nonhematopoietic tissues. We have shown that genetically modified mice lacking CD95 expression in intestinal epithelial cells (IECs) have slightly, but consistently, elevated, disease indices following DSS or AOM/DSS treatment relative to WT littermates.[70] This indicates that CD95 is cytoprotective and prevents, rather than enhances, cell loss during colitis. It is, therefore, interesting to note that while colonocytes from healthy subjects or patients in UC remission undergo dose-dependent apoptosis in response to CD95L stimulation, apoptosis is reduced in colonocytes from patients with active UC.[71]

Remarkably, the incidence of neoplasia was not increased in IEC-specific CD95 knockout mice despite the more severe colitis that developed.[70] We recently demonstrated that CD95 is a general tumor promoter for epithelial ovarian cancer, endometrioid ovarian cancer and hepatocellular carcinoma.[72] In addition knocking down either CD95 or CD95 ligand in various cancer cell lines of different tissue origin including the colon significantly reduced their growth.[72] Because an increase in inflammation has been shown to promote colon carcinogenesis[73] we suggest that in the mice lacking expression of CD95 in the IECs the tumor promoting activity of DSS-induced inflammation may have been cancelled out by the reduction of the expression of tumor promoting CD95. In summary, CD95 has now been demonstrated to act as a tumor promoter in vivo for glioblastoma, lung, ovarian and liver cancer and it may also be tumorigenic for colon cancer.[72,74,75] The observation that increased CD95L expression was beneficial to in vivo tumor growth and progression of colon cancer has so far been interpreted in the context of the "CD95 counterattack" model which proposes that CD95L expression on tumor cells induces apoptosis of tumor infiltrating lymphocytes and neutrophils.[76–78] Recent data now suggest that tumor cells produce small amounts of CD95L acting as an autocrine and paracrine growth factor.

OTHER MECHANISMS OF CELL LOSS DURING COLITIS

Cellular changes during colitis (i.e., in response to DSS treatment) are highly complex and involve many genes.[79] Although CD95 can be excluded as a causative agent for the

apoptosis observed in the IECs in IBD, the mechanism of this cell loss remains to be determined. The Th1 cytokines interferon gamma (IFNγ) and TNFα have been linked to the epithelial damage observed during CD,[80] whereas the Th2 cytokine IL-13 is a key effector promoting apoptosis in UC.[81]

The TNF$^{\Delta ARE}$ mouse model, in which the 3′AU-rich element (ARE) of TNF mRNA is deleted, overproduce TNF and develop enterocolitis that is similar to CD.[82] The development of enterocolitis in TNF$^{\Delta ARE}$ mice depends on IL-12 and IFNγ and involves CD8$^+$ T cells. Protein kinases such as Tpl2 and JNK2 also promoted disease, while MK2 limits inflammation. Consistently, reduction of TNF expression by using a pharmaceutical JNK inhibitor reduced the severity of colitis.[83] However, depending on the context, TNF through binding to its receptor TNFR1 was also shown to enhance colon epithelial cell survival, through an RAF and NF-κB activation-dependent mechanism.[84] Genetic disparities for TNF expression can also influence the sensitivity and outcome of bowel inflammation and oncogenesis. A recent report implied that an intrinsic resistance mechanism to apoptosis could contribute to the high susceptibility of A/J mouse strain to dimethylhydrazine-induced colon tumorigenesis when compared to AKR/J mice, which are very sensitive to dimethylhydrazine-induced colitis through the persistent expression of TNF and activation of caspase-3, causing a low incidence of colon cancer formation.[85]

The involvement of TNF in the pathology of IBD has resulted in a successful treatment of IBD using anti-TNF therapeutic reagents. These include partially or completely humanized anti-TNF antibodies (infliximab, CDP571, adalimumab), soluble TNF receptors (etanercept, onercept), or small molecules with anti-TNF activity (thalidomide, CNI-1493).[86] While anti-TNF therapy is being used effectively for the treatment of active CD and UC and for maintaining remission[87] the mechanism of its action is not completely clear. While TNFα is known to contribute to cell death under certain conditions and has been suggested to be involved in loss of epithelial barrier function,[88] anti-TNF therapy most likely acts by neutralizing the function of TNF as a cytokine fueling inflammation. Recently, large-scale genome-wide association studies using single nucleotide polymorphism (SNP) analyses have identified a linkage between the autophagy-regulating genes ATG16L1[89,90] and IRGM.[91] However, it is not known yet what function of autophagy, cell survival, or cell death, is dysregulated and results in cell loss. In summary, the mechanisms that underlie the loss of IECs remain elusive.

CONCLUSIONS

The concept of CD95 mediating apoptosis and contributing to the loss of cells in many diseases needs to be reconsidered. Accumulating evidence points primarily at a nonapoptotic function of CD95 in various nonhematopoietic tissues. Multiple other mechanisms have been identified to contribute to the loss of IEC in IBD and one needs to consider whether to enhance the activity of CD95 during colitis rather than blocking it for colitis treatment.

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