Apopotosis—new clues to the pathogenesis of Sjögren’s syndrome?

Sjögren’s syndrome (SS), characterized clinically by xerostomia and xerophthalmia, is associated with a destruction of glandular tissue and a resultant impaired secretory capacity of mainly salivary and lacrimal glands. In addition, salivary gland samples from patients with SS show characteristic mononuclear cell infiltrates centred around the feeding vessels of glandular lobules, often in myoepithelial islands and germinal centre-like structures. The mononuclear infiltrates consist predominantly of T cells (CD4 > CD8), with some B cells and plasma cells. Glandular atrophy is largely restricted to the acinar and ductal epithelial cells and there is progressively less destruction in the periphery of lobules than around the central blood vessels [1]. Interestingly, this pattern of centri-lobular destruction is also associated with the highest number of lymphocytic infiltrates, suggesting either an attraction of lymphocytes to the area by adhesion molecules and/or that the lymphocytes mediate destruction (by cytotoxic CD8 and/or CD4 cells) of glandular epithelial cells.

A major mechanism of cellular destruction is apoptosis, a highly organized form of cell death that is critical for maintaining homeostatic function in a variety of tissues [2]. Apoptosis is characterized by morphological changes such as cellular shrinkage, nuclear condensation, DNA fragmentation, membrane blebbing, and the generation of membrane-bound cellular material (apoptotic vesicles) which is available for rapid removal by the phagocytic system so as to avoid major inflammatory tissue reactions as occurs with necrosis. The induction and regulation of apoptosis is a complex process. A number of cell surface molecules [tumour necrosis factor (TNF)-receptor, Fas], intracellular proteins (Bcl-2 family, P53, c-myc), and enzyme pathways (caspases, protein kinases, sphingomyelinase, phosphatases, etc.) have been implicated in apoptosis in particular situations. In keeping with the trend of research into the role of apoptosis in disease pathogenesis, a variety of studies has now been published in relation to SS [3].

Is there any evidence that apoptosis occurs in salivary or lacrimal tissue of patients with SS? The answer is a resounding yes. In fact, there is evidence of apoptosis in normal salivary gland tissue probably as part of homeostatic regulation of cell number and differentiation. Using in situ cell staining techniques for apoptosis (for example, TUNEL: terminal deoxyribonucleotidyl transferase-mediated d-UTP nick-end labelling), it has been shown that apoptosis is enhanced in glandular tissue from SS patients as compared with both normal controls and patients with non-specific sialadenitis [4, 5]. This is supported by a variety of reports of increased apoptosis in salivary tissue from experimental mouse models of SS (including NZB, NOD, C57BL/6, NFS/sld).

A closer analysis reveals a greater amount of apoptosis in acinar and ductal epithelial cells, with only limited apoptosis in the infiltrating lymphocytes. There is greater cellular expression of pro-apoptotic molecules in acinar and ductal epithelial cells (CD95/Fas, Bax) than the anti-apoptotic proteins Bcl-2 and Bcl-X [4, 5]. Bcl-X comprises either Bcl-XL or Bcl-XS, the former protecting from apoptosis whilst the latter has the opposite effect. There is no direct evidence from published data that the increased Bcl-X expression in SS refers to Bcl-XL. Infiltrating lymphocytes also express Fas (reflecting their activated state) but have considerably more Bcl-2, which probably provides a measure of protection from Fas-ligand, which in turn is expressed by glandular epithelial cells as well as by activated lymphocytes. The role of other inhibitors of apoptosis, like FLIP [FLICE(Fas-associated death domain-like interleukin 1 beta converting enzyme) inhibitory protein] and caspase inhibitors, have not been defined in SS, but could perhaps be studied easily in salivary cell lines such as HSG [6]. Cytokines like interferon-γ and
interleukin-10 (IL-10) have also been shown to modulate Fas-mediated apoptosis in SS [6, 7]. All of these data are consistent with the idea that infiltrating lymphocytes may be responsible for the acinar/ductal epithelial cell destruction.

Cell-mediated death could be on the basis of Fas-mediated cytotoxic cell damage of acinar/ductal cells by Fas-ligand. Fas-ligand on activated T cells or soluble Fas-ligand released by activated T cells are both known to mediate apoptosis [8], but the role of Fas-ligand expressed by epithelial duct cells themselves remains speculative (discussed later). Another mechanism whereby cytotoxic T lymphocytes may cause apoptotic destruction of glandular epithelial cells is by the perforin–granzyme pathway [9].

Apoptotic destruction of glandular tissue is not only restricted to exocrine salivary or lacrimal glands in SS. Attias et al. [10] have reported an interesting case of SS with recurrent breast lumps which contained lymphocytic infiltrates similar to those found in salivary glands with predominantly CD4+ cells. The tissue expressed elevated levels of both Bcl-2 and Fas. Despite elevated Fas expression these cells do not undergo apoptosis because of the protection offered by Bcl-2. The exact role of the mononuclear cell infiltrates in the pathogenesis of the breast lesions remains speculative, but nevertheless interesting. SS has been associated with malignancies, especially lymphoma. Could these infiltrating lymphocytes, relatively protected from apoptosis by their expression of proteins like Bcl-2 and Bcl-XL, be responsible for the high prevalence of lymphoma and pseudo-lymphoma in SS? Why is it that not all patients with SS develop lymphoma or pseudo-lymphoma? Is there a need for a second trigger, such as that provided by a virus leading to the release of oncogenes in those patients that do develop malignancy?

Commitment to apoptotic cell death is not a simple matter of the balance of pro- and anti-apoptotic proteins. A complex series of enzyme pathways is activated and in turn regulated to determine the ultimate fate of the cell following surface engagement by apoptotic ligands such as Fas-ligand or TNF [11]. One major enzyme cascade that appears to be the final common pathway for cellular commitment to death comprises the caspase family of proteases (cysteine proteases that cleave after an aspartate residue). Robinson et al. have shown increased caspase activity in submandibular glands of NOD mice with SS which corresponds closely with acinar cell loss [12]. Interestingly, even greater caspase activation and acinar cell loss is evident in NOD-scid mice, suggesting that the induction of apoptosis of acinar cells may precede lymphocytic infiltration. Caspases cleave a variety of protein substrates within the cell, including fodrin, poly-ADP ribose polymerase, topoisomerase I and others. Alpha-fodrin constitutes a major component of fodrin, part of the cortical cytoskeleton of most eukaryotic cells. Alpha-fodrin is cleaved during apoptosis, and the resultant 120 kDa subunit has been implicated as an autoantigen in primary SS [13, 14]. Other autoantigens in SS such as Ro and La proteins have been demonstrated in apoptotic vesicles following Fas-mediated apoptosis [15]. It is conceivable, therefore, that epithelial cell apoptosis may provide cellular proteins as autoantigens which then perpetuate the autoimmune response in SS. Lymphocyte infiltrates may then represent secondary phenomena rather than being the primary inducers of the disease. These lymphocytes would then be responsible for the autoantibody production (anti-Ro, anti-fodrin) and further damage to epithelial cells by the cytotoxic mechanisms outlined above.

How then does the initial apoptotic event occur in salivary gland epithelial cells prior to the infiltration of lymphocytes? The role of viral proteins needs to be elucidated further in this regard. A number of viruses have been implicated in the pathogenesis of SS, but thus far no definitive evidence exists for enhanced apoptosis in patients with SS who have for example coexistent HTLV-1 infection [16]. Other factors that play a role in the regulation of apoptosis may be faulty, as in systemic lupus erythematosus (SLE) for example, where macrophage phagocytosis of apoptotic particles is defective. In an analogous situation in SS, the initial apoptotic destruction of epithelial cells may be a normal response to the viral infection (whether that be HTLV-1, Epstein–Barr virus or other retroviruses), but the inability to regulate the apoptotic process may then perpetuate epithelial cell damage and the resultant cellular and humoral features that characterize SS. The lymphocytic infiltrate in this situation may be responsible for further cellular destruction through cytotoxicity and maintenance of the autoantibody responses by CD4+ helper T cell functions. Further research is required to demonstrate conclusively the hypothesis, but the experimental work in the NOD-scid mouse goes a long way in supporting the idea [17]. The management of SS currently consists primarily of supportive and replacement therapy. However, a greater understanding of the cellular mechanisms that lead to glandular destruction may allow us to direct therapy more specifically in future in an attempt to limit the loss of secretory capacity of the exocrine glands affected in SS.

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