

The Salivary Gland Epithelial Cell in Sjögren's Syndrome: What Are the Steps Involved in Wounding or Killing Their Secretory Function?



In their article in this issue of *The Journal*, Ping, *et al* used *in vitro* cultures of epithelial cells from salivary gland tissues of patients with Sjögren's syndrome (SS), and from control patients lacking inflammatory infiltrates¹.

The basic process of salivary gland cell death (apoptosis) was similar both in SS salivary epithelial cells and in normal salivary epithelial cells. Thus, the key difference in the salivary gland dysfunction of the patient with SS *in vivo* appears to be the presence of focal T cell infiltrate in the SS gland that serves as a source of CD40 ligand (CD40L).

Although their article is a nice exercise in molecular biology, with the expected molecular gymnastics (electrophoretic mobility shifts, Western blots, and TUNEL assays), the ultimate questions that arise are: What do these results mean for the patient with SS with dry mouth? How do these results influence the future for therapeutic selection of options?

The current study by Ping, *et al* is an extension of their report from 2005 on the role of CD40 and Fas in SS salivary gland cells². Subtle points in the present study include the observation that inhibition of p38 and nuclear factor- κ B (NF- κ B) activation does not fully prevent CD95-mediated apoptosis (see their Figure 4). So there must be other pathways that CD40 is activating.

Particularly in the regulation of p38 and NF- κ B, many different factors influence their canonical and noncanonical activation. Thus, additional factors *in situ* determine the epithelial cell's decision toward apoptosis or not.

Overall, the situation is a good bit more complicated *in vivo* than measured during *in vitro* cultures. Nevertheless, these studies provide a framework for preclinical evaluation of novel therapeutic agents to improve epithelial cell function. In particular, the regulation of p38 and NF- κ B may subsequently influence many different pathways (including both their canonical and noncanonical activation) to allow a return of normal functional status of salivary gland epithelial cells even in a proinflammatory microenvironment.

There has been extensive literature on the differences between salivary gland epithelial cells of healthy controls and of patients with SS. These include the upregulation of HLA-DR due to interferon- γ ^{3,4}, Fas and Fas ligand (FasL) induction by CD4 T cells^{5,6,7,8}, as well as changes in other intracellular markers such as aquaporin^{9,10,11} and costimulatory cell-surface markers¹². The paradox of these studies is that the initial steps of apoptosis (Fas and FasL) are expressed in SS biopsies, but the actual progression to apoptosis (i.e., DNA fragmentation) *in situ* is a rare event in the SS biopsy¹³.

In order to understand the state of the SS salivary gland epithelial cell in purgatory (i.e., the gland becomes dysfunctional but not actually destroyed with fragmented DNA), we need to learn more about the enzymatic activation cascade that leads to molecular "demise" or "dysfunction" of the salivary gland.

Since the initial flurry of activity about Fas and FasL expression in SS glands a decade ago, studies in this area have largely dropped from research interest in SS. However, research into pharmacologic inhibitors of p38 mitogen-activated protein kinases (p38MAPK) and NF- κ B has made extensive progress during this interval as agents for treatment of rheumatoid arthritis.

In short, the good news is that the SS gland is not destroyed (only about 50% loss of the acini or ducts in SS patients with severe dryness)¹⁴. We might be able to mitigate the dysfunction of the epithelial cells if we better understood the underlying mechanisms. This is in contrast to type I diabetes, endstage liver disease, or renal failure, where significant symptoms appear only after irreversible destruction of over 90% of the parenchymal cells.

It is time to see if we can use the knowledge of familiar molecules such as p38MAPK and NF- κ B (and the abundant supply of their inhibitors) to resuscitate the salivary gland epithelial cells of patients with SS. Thus, it is worth a new look at these familiar (and incredibly multifaceted) mole-

See p38MAPK, NF- κ B, and salivary epithelial cell death, page 1256

cles in SS salivary glands, as they have received most of their attention from lymphocyte pathways of rheumatoid arthritis:

- CD40 is a costimulatory protein found on salivary gland epithelial cells (dendritic cells) and is required for their activation
- CD154 (CD40L) is present on T helper cells to CD40
- Binding the CD40 on epithelial by the CD40L on the T cells leads to expression of more CD40 and tumor necrosis factor receptors on the epithelial cell surface
- FasL is a homotrimeric type II transmembrane protein. It signals through trimerization of FasR, which spans the membrane of the “target” cell. This trimerization usually leads to apoptosis, or cell death. Fas form the death-inducing signaling complex upon ligand binding
- P38MAPK are a class of MAPK responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in cell differentiation and apoptosis
- p38MAPK inhibitors¹⁵ represent one of the most intensively studied classes of treatment of inflammation. Although they have not achieved their final goals in rheumatoid arthritis¹⁶, the area of application in other autoimmune diseases such as SS may be promising
- NF- κ B light-chain-enhancer of activated B cells is a protein complex that controls the transcription of DNA. NF- κ B is found in almost all animal cell types and is involved in immune activation. NF- κ B plays a key role in regulating the immune response and mediating the beneficial effects of corticosteroids. It has also been implicated in processes of synaptic plasticity and neural memory, processes that may play a role in the functional “denervation” seen in the SS salivary gland.

To the practicing rheumatologist, the complex cascade of enzyme processes leading to salivary epithelial cell death looks like a cartoon created by a tortured graphic artist, but these pathways are important for at least 3 key reasons:

1. Each of the molecules in the pathway has been very well defined, and all have been the subject of intense screening for pharmacologic inhibitors
2. The pathway might not always end in death, but may lead to dysfunction of gland without overt apoptotic death. This scenario is more consistent with past immunohistologic studies, where the number of Fas- and FasL-positive cells greatly exceeds those with frank DNA fragmentation characteristic of apoptotic death. At such an intermediate stage, a knowledge of the relevant factors may lead to “resuscitation of the ailing” salivary gland epithelial cells
3. The factors involved (NFK and p38) are usually associated with activation processes rather than death processes. This makes us think in an entirely different manner about homeostasis in the gland — namely, the importance of apoptosis-induced cell activation, the process that reshapes the repertoire of both lymphocytes and epithelial cells.

In summary, Ping, *et al* note that CD40L upregulates the expression of p38MAPK and Fas, resulting in the induction of apoptosis. This process occurs equally in salivary gland cells derived from both healthy controls and patients with SS. Normal salivary epithelial cells could be “tricked” into expressing proapoptotic factors when incubated with interferon- γ and anti-CD40 monoclonal antibody to mimic the conditions of the SS epithelial cell.

The prior state of “inflammation” in the SS gland does not appear to be a necessary factor for “early death” of epithelial cells. Now that well defined factors such as p38MAPK and NF- κ B have been implicated, a new array of therapeutic options might be considered.

ROBERT I. FOX, MD, PhD,
Rheumatology Clinic,
Scripps Memorial-XiMED,
910-9850 Genesee Avenue,
La Jolla, California 92037, USA

Address correspondence to Dr. Fox. E-mail: robertfoxmd@mac.com

REFERENCES

1. Ping L, Ogawa N, Zhang Y, Sugai S, Masaki Y, Weiguo X. p38 mitogen-activated protein kinase and nuclear factor- κ B facilitate CD40-mediated salivary epithelial cell death. *J Rheumatol* 2012;39:1256-66.
2. Ping L, Ogawa N, Sugai S. Novel role of CD40 in Fas-dependent apoptosis of cultured salivary epithelial cells from patients with Sjögren's syndrome. *Arthritis Rheum* 2005;52:573-81.
3. Brookes SM, Price EJ, Venables PJ, Maini RN. Interferon-gamma and epithelial cell activation in Sjögren's syndrome. *Br J Rheumatol* 1995;34:226-31.
4. Fox RI, Bumol T, Fantozzi R, Bone R, Schreiber R. Expression of histocompatibility antigen HLA-DR by salivary gland epithelial cells in Sjögren's syndrome. *Arthritis Rheum* 1986;29:1105-11.
5. Attias MR, Kong L, Tortolero M, Maccarone BG, Nakabayashi T, Talal N, et al. CD4 mononuclear cell infiltrates and Fas/Fas ligand positive mammary gland cells in breast tissue from a patient with Sjögren's syndrome. *J Rheumatol* 1998;25:1226-31.
6. Gannot G, Bermudez D, Lillibridge D, Fox PC. Fas and Fas-mediated effects on a human salivary cell line in vitro: A model for immune-mediated exocrine damage in Sjögren's syndrome. *Cell death and differentiation. Cell Death Differ* 1998;5:743-50.
7. Ichikawa Y, Yoshida A, Horiki Y, Morita H, Uchiyama M, Shimiau H, et al. Abnormal expression of apoptosis related antigens, Fas and bcl-2 on circulating T-lymphocyte subsets in primary Sjögren's syndrome. *Clin Exp Rheumatol* 1995;13:307-13.
8. Kong L, Ogawa N, Nakabayashi T, Liu GT, D'Souza E, McGuff HS, et al. Fas and Fas ligand expression in the salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum* 1997;40:87-97.
9. Beroukas D, Hiscock J, Gannon BJ, Jonsson R, Gordon TP, Waterman SA. Selective down-regulation of aquaporin-1 in salivary glands in primary Sjögren's syndrome. *Lab Invest* 2002;82:1547-52.
10. Beroukas D, Hiscock J, Jonsson R, Waterman SA, Gordon TP. Subcellular distribution of aquaporin 5 in salivary glands in primary Sjögren's syndrome. *Lancet* 2001;358:1875-6.
11. Steinfeld S, Cogan E, King LS, Agre P, Kiss R, Delporte C. Abnormal distribution of aquaporin-5 water channel protein in salivary glands from Sjögren's syndrome patients. *Lab Invest* 2001;81:143-8.

12. Manoussakis MN, Dimitriou ID, Kapsogeorgou EK, Xanthou G, Paikos S, Polihronis M, et al. Expression of B7 costimulatory molecules by salivary gland epithelial cells in patients with Sjogren's syndrome. *Arthritis Rheum* 1999;42:229-39.
13. Ohlsson M, Skarstein K, Bolstad AI, Johannessen AC, Jonsson R. Fas-induced apoptosis is a rare event in Sjogren's syndrome. *Lab Invest* 2001;81:95-105.
14. De Vita S. Ductal Fas antigen and the pathobiology of Sjogren's syndrome. *Clin Exp Rheumatol* 1996;14:231-4.
15. Damjanov N, Kauffman RS, Spencer-Green GT. Efficacy, pharmacodynamics, and safety of VX-702, a novel p38 MAPK inhibitor, in rheumatoid arthritis: Results of two randomized, double-blind, placebo-controlled clinical studies. *Arthritis Rheum* 2009;60:1232-41.
16. Genovese MC. Inhibition of p38: Has the fat lady sung? *Arthritis Rheum* 2009;60:317-20.

J Rheumatol 2012;39:1117-19; doi:10.3899/jrheum.120278