

## Sjogren's syndrome: apoptosis by anti-SSA and anti-SSB antibodies

### *Sindrome di Sjogren: apoptosi indotta da autoanticorpi anti-SSA e anti-SSB*

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Sirs,

The pathogenesis of the Sjogren's Syndrome (SjS) has not yet been completely defined. However, the cell-mediated immunity plays an important role and the apoptosis of the ductal and acinar epithelial cells is responsible of the glandular tissue damage, through the cytotoxic T-cells, particularly of the CD4<sup>+</sup> subpopulation, by the release of proteases (such as perforin and granzyme B) and by the interaction of the Fas Ligand (FasL; CD95L) of the T-lymphocytes, with the Fas (Apo-1; CD95) of the epithelial cells. The apoptotic death of the epithelial cells is the autocrine Fas/FasL interaction also. The anti-SSA and anti-SSB antibodies are the immunological markers of the Sjogren's syndrome, but it is not yet understood if they have pathogenetic implications.

We have studied 6 patients with SjS and 6 voluntary healthy donors, to investigate, on human salivary gland cell line HTB-41, the possible activation of apoptotic pathways by the whole serum or by IgGs from the serum of patients with SjS or voluntary donors. Of the 6 serum of the SjS patients, 1 was with anti-SSA abs, 2 with anti-SSA and anti-SSB abs, 1 with anti-SSA abs and systemic sclerosis, 2 with anti-SSA and anti-SSB abs and rheumatoid arthritis. The sera were diluted 2%; the respective IgG were isolated from each serum by ammonium sulphate precipitation and concentrated to final concentration of 20mg/ml; the whole serum or the IgG isolated were added to human salivary gland cell line HTB-41. The eventually induced apoptosis was

measured by fluorescence microscopy after addition of YOPRO-1 (DNA-intercalating dye) to measuring the apoptotic cells. The DNA fragmentation was assessed by isolation of genomic DNA from HTB-41 cells, submitted to various treatments and to final separation by 1,8% (w/v) agarose gel electrophoresis. A colorimetric assay and the immunoblotting analysis evaluated the intracellular activation of the effector caspase 3.

The whole serum and the extracted IgG of the 6 pts with SjS all induced the morphological signs of the apoptosis in 70% of the HTB-41 cells. On the contrary, in the same conditions, no control serum induced signs of apoptosis. Furthermore, the DNA fragmentation and the caspase 3 activation were clearly evident only with the 6 serums of the 6 pts with SjS (alone or associated with other diseases), independently from the whole autoantibody pattern. The immunopathogenesis of Sjogren's syndrome start from a primary abnormality or from predisposing factors (1) or from infections agents (2). The apoptotic pathway plays a central role in *T cells* toleranza to tissue-specific self antigen, and may drive the autoimmune phenomenon. The antiestrogenic actions might be a potent factor in the formation of autoimmune lesions (3). The caspase-inhibitors prevent the development of autoimmune lesions in the salivary and lacrimal glands (4). In patients with SjS increase the expression of negative regulator molecules PD-1 (programmed cell death-1), CTLA4 (cytotoxic T lymphocyte-associated antigen-4), the apoptotic signal molecules Fas and FasL (5) and of BAFF (B cell activating factor belonging to the TNF family) (6), that have central role in immunopathology SjS. The our various results clearly show the apoptosis induced in vitro by the autoantibodies of serum of pts with SjS on human salivary gland cells.

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