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In Sjögren's syndrome, B lymphocytes induce epithelial cells of salivary glands into apoptosis through protein kinase C delta activation

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ABSTRACT

Sjögren's syndrome (SS) is a chronic autoimmune epithelitis associated with diffuse lymphocytic infiltration that varies in composition and differs according to lesion severity. T lymphocytes have been viewed as competent in their own right in the destruction of epithelial cells, whereas B lymphocytes that predominate in severe lesions have never been implicated in direct tissue damage. Using co-culture experiments with human salivary gland (HSG) cell line cells and tonsilar B lymphocytes, we observed that direct HSG cell-B lymphocyte contacts were able to induce apoptosis in epithelial cells. This B lymphocyte-mediated cell death could not be ascribed to Fas–Fas ligand interactions but required translocation of protein kinase C delta (PKC δ) into the nucleus of epithelial cells. Ultimately, activation of PKCδ resulted in histone H2B phosphorylation on serine 14 and poly (ADP-ribose) polymerase cleavage. Our results suggest that B lymphocytes infiltrating the SGs of patients with SS could evoke epithelial cell apoptosis.

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1. Introduction

Sjögren's syndrome (SS) is a common [1] autoimmune disorder characterized by the infiltration of lacrimal and salivary glands (SGs) by mononuclear cells with the ensuing destruction of the

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parenchymal tissue. This disorder may develop alone, as primary SS, or against a background of connective tissue disease, as secondary SS. Damage of the exocrine cells accompany lymphocyte infiltrates of the ductal epithelial cells (ECs), and results in oral and ocular dryness [2]. However, these aggregates evolve from wild to focal, and from focal to diffuse, and vary in composition according to the severity of the disease.

In the SGs, activation of ECs [3] promotes lymphocyte settlement and infiltration. Here, T lymphocytes, of which the majority are $CD4^+$, predominate over B lymphocytes. On the other hand, antigen-presenting cells (APCs), such as macrophages and dendritic cells consist of a minority of infiltrating cells. Of note is, however, that they are detectable even in wild lesions. Furthermore, the number of T lymphocytes correlates inversely with the infiltration grade and focus score, whereas those of B lymphocytes and macrophages increase in proportion [4]. Consequently, total T-$CD4^+$ lymphocytes predominate in wild lesions, whereas B lymphocytes substitute for them along with the severity. This view confines their role to the production of antibody (Ab) and, following proliferation of one clone [5], restricted to the secretion of a monoclonal Ab (mAb).

Most of the studies have hitherto been concentrated on the role of $CD4^+$ T lymphocytes in tissue destruction [6], neglecting potential damage accounted for by the B lymphocytes [7]. Thus, T lymphocytes induce ECs into apoptotic death through the release of proteases, or via the insertion of the Fas ligand (Fas L) of activated $CD4^+$ T lymphocytes into the Fas receptor harbored by the target ECs [8]. Accordingly, agonistic anti-Fas Ab promotes apoptosis of cultured ECs. The unstimulated human SG (HSG) duct cell line cells that we used in the present study express low levels of Fas, this is upregulated by interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Apoptosis induced by the CH11 anti-Fas mAb is subsequently exaggerated [9].

The delta isoform of protein kinase C (PKCδ) has also been implicated in regulation of apoptotic cell death in ECs [10]. Its activation is triggered by tyrosine phosphorylation, and followed by translocation from the cytoplasm to the nucleus [11]. Pre-treatment with rottlerin prevents nuclear accumulation of PKCδ, suggesting that, for its nuclear translocation to occur [12], some degree of kinase activity is required. In the nucleus, PKCδ encourages apoptosis by phosphorylating multiple proteins, such as lamin B which is degraded [13] or histone H2B which is phosphorylated at serine 14. These events condense the chromatin [14] and fragment the nucleosomal DNA [15].

Thus, T lymphocytes have long been viewed as competent in their own right by destroying ECs by cell-mediated mechanisms. A wealth of evidence support now that B lymphocytes solicit their help from T lymphocytes, release a number of cytokines [7], and serve as APCs. However, there are no studies on the direct effect of B lymphocytes on EC apoptosis within the SGS of patients with SS.

2. Material and methods

2.1. Cell preparations

HSG cell line cells were incubated in Dulbecco's modified Eagle's medium (Lonza, Verviers, Belgium) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine (Invitrogen, Carlbad, CA), 100 μ/ml penicillin, 100 μg/ml streptomycin (Panpharma, Fougères, France) and 1% non-essential amino acids (Sigma-Aldrich, Saint-Louis, MO).

Tonsils were collected from five children undergoing routine tonsillectomy, and their mononuclear cells isolated by a 30-minute 1800-rpm centrifugation on Ficoll Hypaque (PAA, Pasching, Austria). These were then incubated with neuraminidase-treated sheep red blood cells for 90 min at 4 °C, layered onto Ficoll-Hypaque and centrifuged for another 30 min at 1800 rpm. B and T lymphocytes were isolated from sheep red blood cells using ACK solution, and T lymphocytes further-purified using the Easy-Sep human T lymphocyte enrichment kit (StemCell Technologies, Grenoble, France).

Biopsy specimens of SGs were obtained from patients who fulfilled the American–European Consensus Group criteria for primary SS [16] and displayed a focus score ≥ 3 [17]. All patients and donors' parents gave informed consent, and the study was approved by our Ethics Committee.

2.2. Co-culture of B- and T-lymphocytes with HSG cells

HSG cells were seeded into 96-well flat-bottomed culture plates. Once they were grown to confluence, 1.5×10^5 B or T lymphocytes were added in 1 ml RPMI-1640 medium (Lonza) supplemented with 10% FCS, 2 mM L-glutamine, and antibiotics. To avoid allogenic response and long-term culture artifacts, cells were collected 24 h later. Viability of HSG cells was assessed by using fluorescein isothiocyanate (FITC)-labeled annexin V and propidium iodide (PI). To construct the dose-effects curves, HSG cells were distributed into two series of three aliquots. In the first three aliquots, there were one, five and 10 B lymphocytes, respectively, for one HSG cell, and in the three aliquots one, five and 10 T lymphocytes, respectively, for one HSG cell. Transwells (Corning, Corning, NY) enabled to verify whether cell–cell contacts were discernable or not. To address this issue, confluent HSG cells were placed in the lower chamber and B- or T-lymphocytes in the upper.

2.3. Stimulation of HSG cells

In order to determine the implication of Fas in the signaling pathway, HSG cells were incubated with 0 to 10 μg/ml of the ZB4 anti-Fas mAb (Beckman Coulter, Villepinte, France) for 1 h at 37 °C. The entry of PKCδ into the nucleus was blocked by treatment with 50 μM rottlerin (Sigma-Aldrich) for 30 min at 37 °C before being added to the lymphocyte in culture.

2.4. Immunofluorescence staining for PKCδ location

Co-cultured HSG cells and B lymphocytes were stained with an anti-PKCδ mAb from BD Biosciences (Franklin Lakes, NJ) and a rabbit anti-CD20 polyclonal Ab (pAb) from Thermo Scientific (Waltham, MA). The former Ab was revealed by an FITC-conjugated donkey anti-mouse pAb, and the latter by a rhodamine Red-X-conjugated donkey anti-rabbit pAb (both from Jackson ImmunoResearch, West Grove, PA).

2.5. Subcellular proteome extraction

HSG cells in culture were separated from B cells using the Biomag goat anti-mouse IgG kit, according to the Qiagen's instructions (Hilden, Germany). Briefly, co-cultured HSG cells and B lymphocytes were incubated with mouse anti-CD19 mAb (Beckman-Coulter) for 30 min at 4 °C. Then, magnetic beads coated with goat anti-mouse IgG pAb were added, and HSG cells eluted, whereas the B lymphocytes were retained in the magnetic separator.

After isolation, non-denaturated cytoplasmic, membrane, and nuclear extracts were prepared from HSG cells, cultivated alone or with B lymphocytes using the ProteoExtract Subcellular Proteome Extraction kit according to the Calbiochem's instructions (Darmstadt, Germany). The cell fractions were harvested separately. The protein concentrations were measured with the Quanti-Assays kit (Invitrogen). The fractions were then resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to a polyvinylidene difluoride membrane (GE Healthcare, Buckingham, England). After blocking of unbound sites with 5% non-fat milk in 10 mM Tris 0.1% Tween-20, the membrane was probed with one