The significance of the target cells in the development of autoimmune lesions:

Lessons from Sjögren's syndrome

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Sjögren's Syndrome

- Female disease
  - ♀/♂ : 9/1
- Common
  - 0.5-1% of adult females
- 4⁰⁻⁵⁰ decade of life
- Slowly progressive
Sjögren's Syndrome

- Center of autoimmune disorders
  - alone (primary)
  - with other (secondary)

- Wide clinical spectrum
  - organ-specific
  - systemic
  - neoplasia

- Prototype autoimmune disease
  - humoral *(anti-Ro, anti-La)*
  - cellular *(focal lymphocytic infiltrates - exocrine glands)*
Sjögren's Syndrome
Major Clinical Manifestations

<table>
<thead>
<tr>
<th>Clinical Manifestations</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry eyes</td>
<td>95</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>90</td>
</tr>
<tr>
<td>Parotid gland enlargement</td>
<td>49</td>
</tr>
<tr>
<td>Pulmonary involvement</td>
<td></td>
</tr>
<tr>
<td><em>small airway disease</em></td>
<td>23</td>
</tr>
<tr>
<td>Renal involvement</td>
<td></td>
</tr>
<tr>
<td><em>interstitial</em></td>
<td>9</td>
</tr>
<tr>
<td>Liver involvement</td>
<td></td>
</tr>
<tr>
<td><em>biliary cirrhosis</em></td>
<td>4</td>
</tr>
<tr>
<td>Lymphoproliferative disorders</td>
<td>4</td>
</tr>
</tbody>
</table>

Skopouli et al., Semin Arthritis Rheum 2000
Sjögren's Syndrome
Lesions: Major Histopathologic Findings

- Lymphocytic infiltrates

- Labial Minor Salivary Gland
- Kidney
- Lung
- Liver
Sjögren's Syndrome

Exocrine glands

Kidney
Liver
Lung

Autoimmune Epithelitis
Sjögren's Syndrome – Autoimmune Epithelitis

The Role of Epithelium

Labial Minor Salivary Gland

Salivary Gland Epithelial cells
(non-neoplastic, long-term cultured)
Sjögren's Syndrome – Autoimmune Epithelitis
Autoimmune Phenomena: Lesions

Activated:

- **B cells**
  - autoantibodies

- **T cells**
  - helper/memory
  - LFA.1/HLA-DR+

- **Epithelial:**
  - immunopathology

*Skopouli et al, J Rheumatol. 1991*
*Moutsopoulos et al, Clin Imm Immunop. 1994*
Sjögren's Syndrome – Autoimmune Epithelitis
Salivary Gland - Immunopathology

*In situ:* ↑ expression of molecules implicated in initiation & perpetuation of immune responses:

- **Ag-presentation**
  - MHC molecules *(Moutsopoulos et al, Ann Rheum Dis. 1986)*
  - B7 costimulatory molecules *(Manoussakis et al, Arthritis Rheum 1999)*
  - nuclear autoantigens → translocation to cell surface *(Yiannopoulos et al, J Clin Immunol 1992)*

- **Lymphocyte recruitment/homing/expansion**
  - Proinflammatory cytokines *(IL-1, IL-6, TNFα)* *(Boumba et al., Br J Rheumatol., 1995)*
  - Lympho-attractant chemokines *(Xanthou et al, Arthritis Rheum 2001)*

- **Apoptosis-related molecules**
  - Fas, FasL *(Abu-Helu et al, J Autoimmun 2001; 17:141)*
Sjögren's Syndrome – Autoimmune Epithelitis
Salivary Gland Epithelial Cells

**In situ** activation:

- epiphenomenon (cytokine/cell-mediated)
- intrinsic processes
Sjögren's Syndrome – Autoimmune Epithelitis
Salivary Gland Epithelial Cells

- MSG lobule
- obtained during diagnostic biopsy
- long-term cultured MSG cell lines
  (non-neoplastic)

Sjögren's Syndrome – Autoimmune Epithelitis
Salivary Gland Epithelial Cells

- Role of epithelial cells in:
  - Acquired immunity
  - Innate immunity
  - Antigen release
Expression of MHC molecules

- HLA-ABC (Class I): constitutive (↑ in SS)
- HLA-DR (Class II): cytokine treatment

Manoussakis et al., Arthritis Rheum. 1999
Sjögren's Syndrome – Autoimmune Epithelitis
Cultured Salivary Gland Epithelial Cells

↑ expression of B7 costimulatory molecules

Manoussakis et al., Arthritis Rheum. 1999
Sjögren's Syndrome – Autoimmune Epithelitis

Cultured Salivary Gland Epithelial Cells

- B7.2 molecules of salivary gland epithelial cells:
  - surface
  - soluble

Costimulation Assay

**Signal 1**: anti-CD3

**Signal 2**: CD28

CD4+ T cell proliferation
(3H-thymidine incorporation)

Cytokine production
(ELISA, blast proliferation)

SGEC: fixed with paraformaldehyde

CD4+ blocking reagents
Sjögren's Syndrome – Autoimmune Epithelitis
Cultured Salivary Gland Epithelial Cells

- B7.2 (CD86) molecules:
  - Costimulate CD4⁺ T cell proliferation

Sjögren's Syndrome – Autoimmune Epithelitis
Cultured Salivary Gland Epithelial Cells

- B7.2 (CD86) molecules display distinctive binding properties:
  - interact with the CD28 receptor
  - reduced binding to CTLA4 (negative regulator)

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Sjögren's Syndrome – Autoimmune Epithelitis
Salivary Gland Epithelial Cells

- Role of epithelial cells in:
  - Acquired immunity
  - Innate immunity
  - Antigen release
Innate Immunity: Toll-Like Receptors (TLRs)

- Homologue to the TOLL receptor of *Drosophila melanogaster*
- Mammalian TLR family: TLR1 – TLR11
- Expression in various types of cells:
  - Lymphoid/monocytoid
  - Other (*epithelial, endothelial, fibroblasts*)
Innate Immunity: Toll-Like Receptors (TLRs)

Pattern Recognition Receptors (PRPs)

Recognize:

- Pathogen-Associated Molecular Patterns (PAMPs) specific for microbes
  - Bacterial lipoproteins & gram(+) peptidoglycan (TLR2)
  - Viral dsRNA (TLR3)
  - Gram(-) bacterial lipopolysaccharide (TLR4)
  - Bacterial flagelin (TLR5)
  - Bacterial CpG DNA (TLR9)

- Endogenous ligands
  - Heat-shock proteins hsp60, hsp70 (TLR2, TLR4)

*Takeda et al. Annu Rev Immunol 2003*
Toll-Like Receptors (TLR)

- Induction of inflammatory responses & activation of acquired immunity

  - Activation of transcription factors (*NFkB, AP-1, IRFs*)

  - Gene induction
    - Cytokine and chemokine secretion
    - Expression of adhesion and costimulatory molecules
    - Expression of Toll-like receptors

 Akira et al. Immunol Let 2003
Sjögren's Syndrome – Autoimmune Epithelitis
Cultured salivary gland epithelial cells
Toll-Like Receptors (TLR) expression

Real-time PCR

Surface protein expression

Spachidou et al, submitted
Stimulation of SGEC lines \((n=4)\) with analogues of TLRs ligands

<table>
<thead>
<tr>
<th>Molecules</th>
<th>PGN  ((\text{TLR-2})) ((100\mu\text{g/ml}))</th>
<th>PolyI:C ((\text{TLR-3})) ((5\mu\text{g/ml}))</th>
<th>LPS ((\text{TLR-4})) ((1\mu\text{g/ml}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A,B,C</td>
<td>0.6 ± 0.01</td>
<td>5.1 ± 0.60</td>
<td>0.6 ± 0.01</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>ICAM.1 (CD54)</td>
<td>0.6 ± 0.20</td>
<td>9.4 ± 4.10</td>
<td>1.0 ± 0.30</td>
</tr>
<tr>
<td>B7.2 (CD86)</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>CD40</td>
<td>0.3 ± 0.08</td>
<td>0.9 ± 0.04</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>Fas (CD95)</td>
<td>0.2 ± 0.06</td>
<td>0.5 ± 0.02</td>
<td>No effect</td>
</tr>
<tr>
<td>FasL (CD154)</td>
<td>No effect</td>
<td>No effect</td>
<td>No effect</td>
</tr>
</tbody>
</table>

Spachidou et al, submitted
Constitutive mRNA expression of TLR-1, -2, -3 and -4 receptors

- ↑ in SS-SGEC

Functional:

- Their ligands up-regulate the expression of:
  - HLA-A,B,C (MHC-I)
  - ICAM.1
  - CD40
  - Fas

_Spachidou et al, submitted_
Sjögren's Syndrome – Autoimmune Epithelitis
Salivary Gland Epithelial Cells

- Role of epithelial cells in:
  - Acquired immunity
  - Innate immunity
  - Antigen release in vesicles:
    - Apoptosis
    - Exosomes
Antigen Release

Apoptosis

- **Induced:**
  - Fas/Fas L interaction
  - Cytotoxic T cells:
    - perforin
    - Granzymes

- **Results:**
  - release of apoptotic blebs (*autoantigens*)
    - *Bachmann et al., Autoimmunity 1991*
  - externalization of cellular components ➔ immune response

- **Inhibited by:**
  - Bcl-2
Sjögren's Syndrome – Autoimmune Epithelitis

Apoptosis

- **In situ:** ↑ epithelial apoptotic cell death

- **Salivary Gland Epithelial Cells:**
  - ↑ expression of Fas & FasL
  - Operation of anti-apoptotic mechanisms
    - Fas ligation (anti-Fas) only → no apoptotic effect
    - Fas ligation + protein synthesis inhibition → apoptosis
  - Sensitive to IFNγ-induced cell death

Abu-Helu et al, J Autoimmun 2001*
Exosomes

- Small vesicles (30-100 nm)
- Endosomal origin
- Released by variety of cell types
  - Fusion of multi-vesicular late-endosomes/lysosomes with the plasma membrane
Exosomes

- **Contain:**
  - Cytoskeletal proteins (*actin, tubulin*)
  - Chaperones, heat shock proteins
  - Proteins that mediate membrane transport and fusion
  - Molecules involved in antigen presentation (*MHC, costimulatory, adhesion*)

- **Physiological role:**
  - intercellular communication/exchange of cellular material
  - antigen presentation/regulation of immune responses:
    - immunogenic
    - tolerogenic
Salivary Gland Epithelial Cells
Exosomes

- Isolation by ultracentrifugation \((10^5 g)\) of cell-free culture supernatants
  - Analysis by:
    - electron microscopy
    - western blot (WB)
    - immunoprecipitation/WB
- 50-100 nm
- Express:
  - epithelial-specific cytoskeletal components \(\textit{cytokeratins}\)
  - MHC-class I molecules \(\textit{on the surface}\)

*Kapsogeorgou et al., Arthritis Rheum, 2005*
Salivary Gland Epithelial Cells
Exosomes: contain autoantigenic ribonucleoproteins

Kapsogeorgou et al., Arthritis Rheum, 2005
Sjögren's Syndrome – Autoimmune Epithelitis
Salivary Gland Epithelial Cells

- Capacity to participate in the induction & maintenance of inflammatory reactions

- Evidence for intrinsic activation ➔ Viral Infection?
Chronic viral sieladenitis mimicking Sjögren's syndrome

**Viruses:**
- **Hepatitis C (14-50%)**  \(\text{Mariette et al. Arthritis Rheum, 1993}\)
- **HIV**  \(\text{Kordossis et al. Brit J Rheum 1998}\)
- **HTLV-1**  \(\text{Terada K, et al. Lancet. 1994}\)

**Differences:**
- Infiltrating phenotype
- Lack of autoantibodies
- Lack of sex (♀) preference
Sjögren's Syndrome – Autoimmune Epithelitis

Viruses

- Cytomegalovirus (CMV)  
  Shillitoe et al., Arthritis Rheum. 1982

- Epstein-Barr virus (EBV)  
  Fox et al., J. Immunol. 1986

- Human herpes virus type 6 (HHV6)  
  Fox et al., J. Autoimmunity 1989

- Retroviruses  
  Talal et al., Arthritis Rheum. 1990
Sjögren's Syndrome – Autoimmune Epithelitis
Coxsackie viruses

**Differential Display**
- **LMSG tissues:**
  - Sjögren's Syndrome patient
  - control
- Band isolated, cloned and sequenced
- 94 bp fragment homologous to CoxB4 (*P2-A protein*)

_Triantafyllopoulou et al., Arthritis Rheum, 2004_
Coxsackie Viruses

- Small non-enveloped enteroviruses (*Picornaviruses*)
- Classification based on serotype
  - A: 1-24 (w/o 23)
  - B: 1-6
- Single-stranded RNA

![Polyprotein CB4]

Polyprotein CB4

7395 nucleotides
Coxsackie Viruses Infection

- Mainly cytolytic
  - Broad clinical spectrum
- Persistent (not lytic viruses)
  - Viral determinants
    - Viral strains
  - Host determinants
    - Cellular receptors
    - Cell-cycle
Coxsackie Viruses
Autoimmune Diseases

- Persistent infection has been associated with the development of autoimmune diseases

- Diabetes type I  
  Graves et al., Diabetes, 1997  
  Horwitz et al., Nat Med, 1998

- Dilated cardiomyopathy  
  Muir et al., Lancet, 1989  
  Klinger et al., Proc Natl Acad Sci USA, 1992

- Myositis  
  Archard et al., Biochem Soc Symp.; 1987
Sjögren's Syndrome – Autoimmune Epithelitis

Coxsackie Viruses in MSG biopsy samples

- RNA from Minor Salivary Gland biopsies
- Primers designed to amplify the 5’ untranslated region (5’UTR)

**Sequence Alignment**

- 4 products showed 98-99% identity with **Coxsackie B4**
- 3 products showed 97-99% identity with **Coxsackie A13**

**5’-UTR Sequences of CV**

<table>
<thead>
<tr>
<th></th>
<th># positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary SS</td>
<td>7/8</td>
</tr>
<tr>
<td>Secondary SS</td>
<td>0/9</td>
</tr>
<tr>
<td>Controls</td>
<td>0/8</td>
</tr>
</tbody>
</table>

Triantafyllopoulou et al., Arthritis Rheum, 2004
Coxsackie Viruses
Receptors

- Coxsackievirus & adenovirus (CAR)
- Decay-accelerating factor (DAF/CD55)
- Intercellular Adhesion Molecule-1 (ICAM.1/CD54)
- Phagocyte glycoprotein-1 (Pgp-1/CD44)
- Vitronectin receptor (Integrin αvβ3/CD51)
Sjögren's Syndrome – Autoimmune Epithelitis
Coxsackievirus-Adenovirus Receptor (CAR)

- Salivary gland biopsy specimens
  - Ductal epithelial cells
  - Infiltrating mononuclear cells
  - SS/Controls: similar expression

- Cultured Salivary Gland Epithelial Cells
  - SS/Controls: similar expression

IB: Isotype control anti-CAR

HeLa HeLa SS Control

52 kDa 40 kDa
Sjögren's Syndrome – Autoimmune Epithelitis
Decay-accelerating factor (DAF/CD55)

- Salivary gland biopsy specimens
  - Infiltrating mononuclear cells

![Image of salivary gland biopsy specimens comparing Sjögren's Syndrome (SS) and control samples.](image-url)
Sjögren's Syndrome – Autoimmune Epithelitis

Other receptors

- **ICAM-1 (CD54):**
  - Ductal epithelial cells (*in-situ & long-term cultured*)
  - Infiltrating mononuclear cells
  - SS: ↑ epithelial cell expression
  
  *Kapsogeorgou et al, Clin Exp Immunol. 2001*

- **CD44:**
  - Cultured Salivary Gland Epithelial Cells
  - SS/Controls: similar expression
  
  *Kapsogeorgou et al, unpublished data*
Sjögren's Syndrome - Autoimmune Epithelitis
Coxsackie Viruses

Future Directions

- Characterization of these viral sequences
  - full-length
- Persistent infection
- Role in activation of epithelial cells
Autoimmune Epithelitis

Endocrine

EPITHELIUM

EXOSOMES

APOPTOSIS

Ag-Release

Ag-Presentation

DC

MHC-I

CD40

FasL

B7

ICAM-1

CK receptors

Cytokines/Chemokines

B

T
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