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SJÖGREN'S SYNROME

Recent Advances in Pathogenesis

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Sjögren's Syndrome
Recent Advances in Pathogenesis

Outline

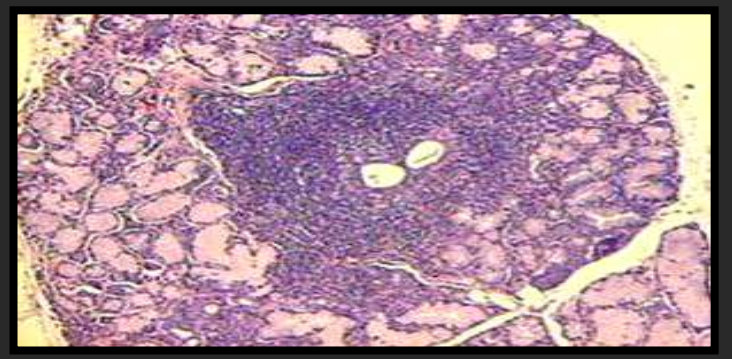
- Pathogenesis Overview
  - Innate Immunity
    - IFNs
  - Acquired Immunity
    - B lymphocytes and biology of ectopic germinal centres (eGCs)
    - T lymphocytes
  - Epithelial cells
- Recent Advances

Sjögren syndrome – Autoimmune epithelitis

Chronic systemic inflammatory disease

- Female disease (15♀ : 1 ♂)
- 0.1% of the general population

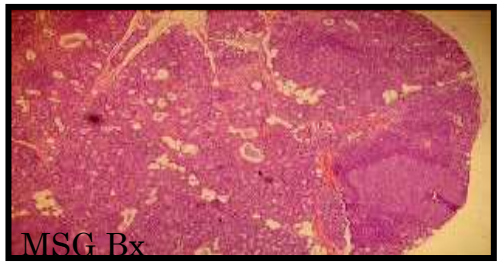
- Dysfunction & damage of exocrine glands
- Polymorphic disorder
  - exocrinopathy, systemic disease, B lymphoma development



- Autoimmune reactivities
- Chronic inflammatory reactions in affected tissues
- Interplay of genetic/epigenetic/enviromental factors

Sjögren's Syndrome
Pathogenesis

- Peri-epithelial lymphocytic infiltrations
  - CD4+ T-lymphocytes
  - B lymphocytes
  - CD8+ T-lymphocytes
  - Macrophages – Dendritic cells



Infiltration by activated T- & B-lymphocytes

- Systemic B cell hyperactivity
  - Hypergammaglobulinemia
  - Autoantibodies
  - Monoclonal antibodies
  - Low C3 and C4 complement levels

- Active role of epithelium (autoimmune epithelitis)
  - Expression of MCH I, II molecules
  - Expression of co-stimulatory molecules
  - Production of cytokines and chemokines
  - Increased apoptosis

Moutsopoulos et al, Ann Rheum Dis. 1986, Manoussakis et al, Arthritis Rheum 1999, Yiannopoulos et al, J Clin Immunol 1992, Boumba et al., Br J Rheumatol., 1995, Xanthou et al, Arthritis Rheum 2001, Groom et al, J Clin Invest., 2002, Abu-Helu et al, J Autoimmun 2001, Polichronis et al, Clin Exp Immunol 1998, Kapsogeorgou et al. Clin Exp Immun. 2001

Sjögren's syndrome (SS) - Autoimmune epithelitis

- **Activation of adaptive immunity**
  - ✓ **B cell hyperactivity**
    - Wide spectrum of autoantibodies
    - RF positivity
    - Predominance of B cells with progression of disease
    - Autoreactive B cells and plasma cells
    - Presence of germinal center-like structures
    - Hypergammaglobulinemia
    - Elevated levels of free light chains and b2-microglobulin

- **Activation of innate immunity**
  - ✓ Epithelial cells: active pathogenetic role.
  - ✓ CD68+ MΦs: ↑ in severe lesions,
  - ✓ Aberrant clearance of apoptotic cells
  - ✓ Defective degradation of remnants of necrotic cells
  - ✓ Type I IFN pathway is activated

Sjögren's Syndrome
Pathogenesis-Innate Immunity

IFNs

- Salivary Glands
  - Increased expression of IFN-stimulated genes (IFNSGs)

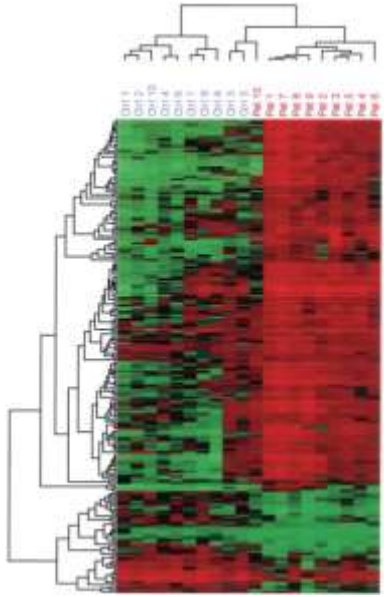
    Hjelmervik et al. Arthritis Rheum 2005
    Gottenberg et al. Proc Natl Acad Sci USA 2006
    Wakamatsu et al. Arthritis Rheum 2007

  - Presence of pDCs at tissue level

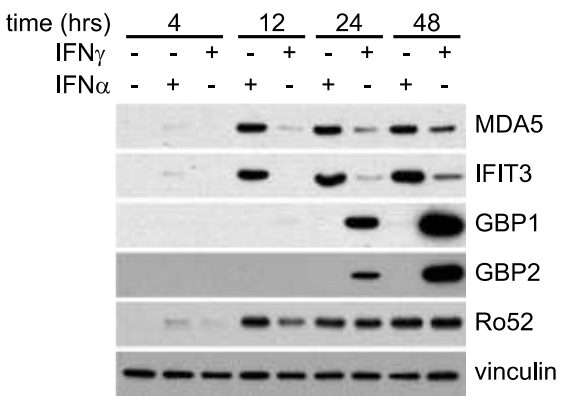
    Bave et al. Arthritis Rheum 2005
    Gottenberg et al. Proc Natl Acad Sci USA 2006

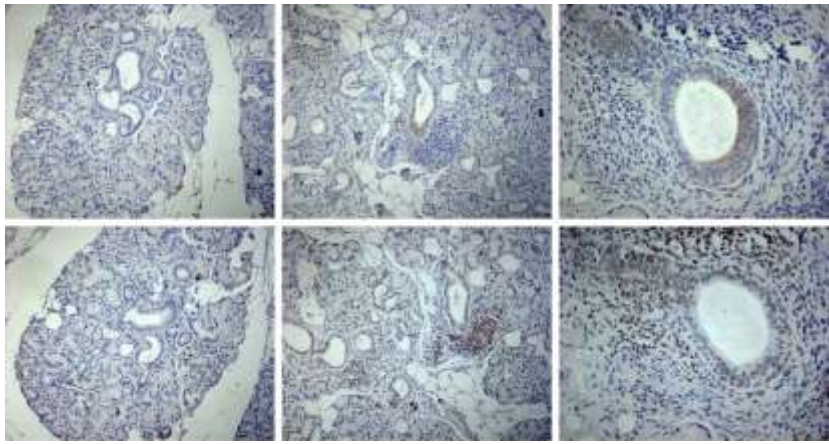
  - Type I (IFIT3) and II (GBP2) IFNs signature at the protein level in inflammatory lesions by epithelial and immune cells respectively

    Hall et al. Proc Natl Acad Sci USA 2012



Gene name	GenBank ID	Gene symbol	Gene group/function	T score
Interferon-stimulated transcription factor 3γ	BF525953	ISGF3G†	Virus response	−5.72
Proteoglycan 1 secretory granule	BG489803	PRG1	Secretory granule core protein	−5.41
Guanylate binding protein 2	M55543	GBP2†	Immune response	−5.40
CD53 antigen	AW575081	CD53	Leukocyte signaling	−5.22
CD74 antigen	AL543515	CD74	Sorting of MHC molecules	−5.20
Killer cell lectin–like receptor subfamily C	NM_002259	KLRC1	NK cells/virus defense	−5.13
CD8 antigen α-polypeptide (p32)	M12824	CD8A	T cell development	−5.07
Interferon-induced protein 41, 30 kd	AI796501	SP110†	Transcription factor	−5.05
Human interferon-inducible protein 9–27	J04164	IFITM1†	Signal transduction	−4.98
Human interferon regulatory factor 1	BC009483	IRF1†	IFNα and IFNβ transcription	−4.88
Interleukin-13	NM_002188	Il13	B cell maturation	−4.79
Human transcription factor	M97935	ISGF3	Signal transduction	−4.79
Interferon-induced, hepatitis C virus–associated	NM_006417	IFI44†	Virus response	−4.74
Interferon sequence-binding protein 1	AW964220	ICSBP1†	Transcription	−4.40
Interferon-induced transmembrane protein 3	BE886918	IFITM3†	Signal transduction	−4.36
Interferon-α–inducible protein, 15 kd	AI739106	GIP2†	Cell–cell signalling	−4.22
Lysosome-associated membrane protein 1	BC007845	LAMP1	Antigen presentation	5.77
Human carbonic anhydrase II mRNA	J03037	CA2	Secretion	5.34
Bcl-2–like 2	D87461	BCL2L2	Regulation of apoptosis	4.85
Androgen-regulated serine protease	AF270487	TMPRSS2	Protease activity	4.40
LIV-1 protein, estrogen regulated	U41060	LIV1	Metal ion transport	4.31





Sjögren's Syndrome
Pathogenesis-Innate Immunity

IFNs

- Systemic and peripheral expression
  - Increased serum/plasma levels of type I IFNs and expression of IFNSGs by PBMCs, Β λεμφοκύτταρα και μονοκύτταρα

    Bave et al. Arthritis Rheum 2005
    Widenberg et al. Eur J Immunol 2008
    Emanian et al. Genes Immun 2009
    Imgerberg-Kreuz Scand J Immunol 2018

  - Peripheral blood vs salivary glands : type I>II
  - IFNγ/IFNα mRNA ratio in MSG tissues showed the best discrimination for lymphoma development

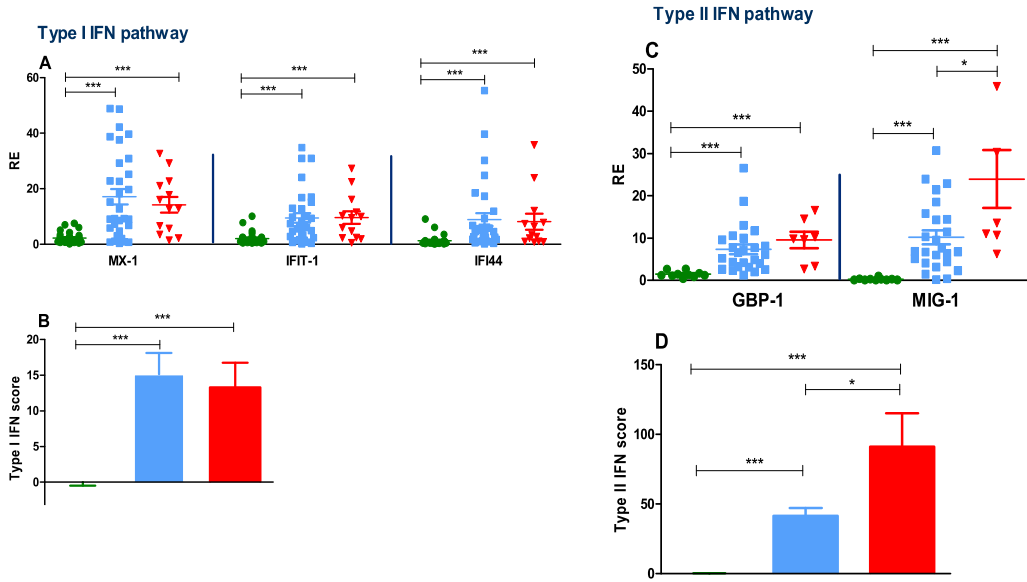
    Nezos et al. J Autoimmun 2015

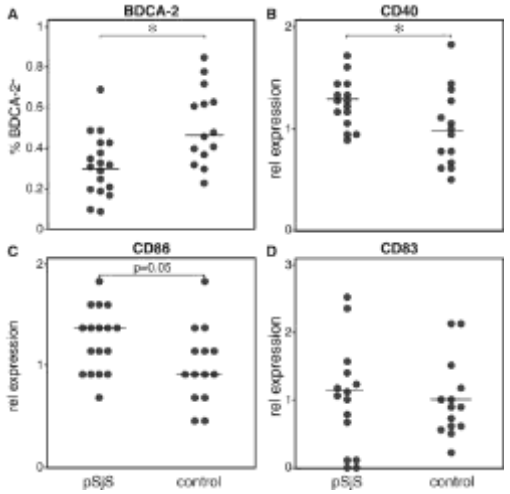
  - Peripheral blood: no signature, type I or type I & II

    Bodewes et al. Rheumatology 2018

  - ↑pDCs in salivary glands and reduced numbers in blood (activated)

    Gottenberg et al. Proc Natl Acad Sci USA 2006
    Widenberg et al. Eur J Immunol 2008





Activation of innate immunity in Sjögren's syndrome (SS) – Role of macrophages

- **Peripheral blood**
  - **Aberrant clearance of early apoptotic cells (efferocytosis)**
    - ✓ **Inhibitory IgG autoantibodies**
    - ✓ **Macrophages' (MΦs) dysfunction**

Manoussakis et al, PLoS One, 2014

  - **Defective degradation of remnants of necrotic cells**
    - ✓ **impaired DNA degradation (low DNase1 activity).**
    - ✓ **increased serum levels of circulating nucleosomes and cell-free genomic DNA (CF-DNA)**

Fragoulis et al, J Autoim, 2015

- **Minor Salivary Gland (SG) lesions**
  - **CD68+ MΦs: ↑ in severe lesions,**
    - **cryoglobulinemia**
    - **lymphoma**

❖ **↑ IL-18 expression by CD68+ MΦ correlates:**
Advanced lesions:
- **↑ biopsy focus score**
- **SG enlargement**
- **↓ C4 levels**

Manoussakis et al, Arthritis Rheum, 2007,
Christodoulou et al., J Autoim., 2010

The fate of apoptotic & necrotic cells

Increased apoptosis & necrosis:
- cell damage
- dysfunctional autophagy
- cell-penetrating autoabs

Healthy

efficient clearance by phagocytes

efficient degradation by serum factors

apoptotic cells

secondary necrotic cells

release of modified antigens (DAMPs)

inflammatory innate immunity reaction

SLE & SS

impaired clearance

- inhibitory IgG autoabs
- lack of IgM opsonins

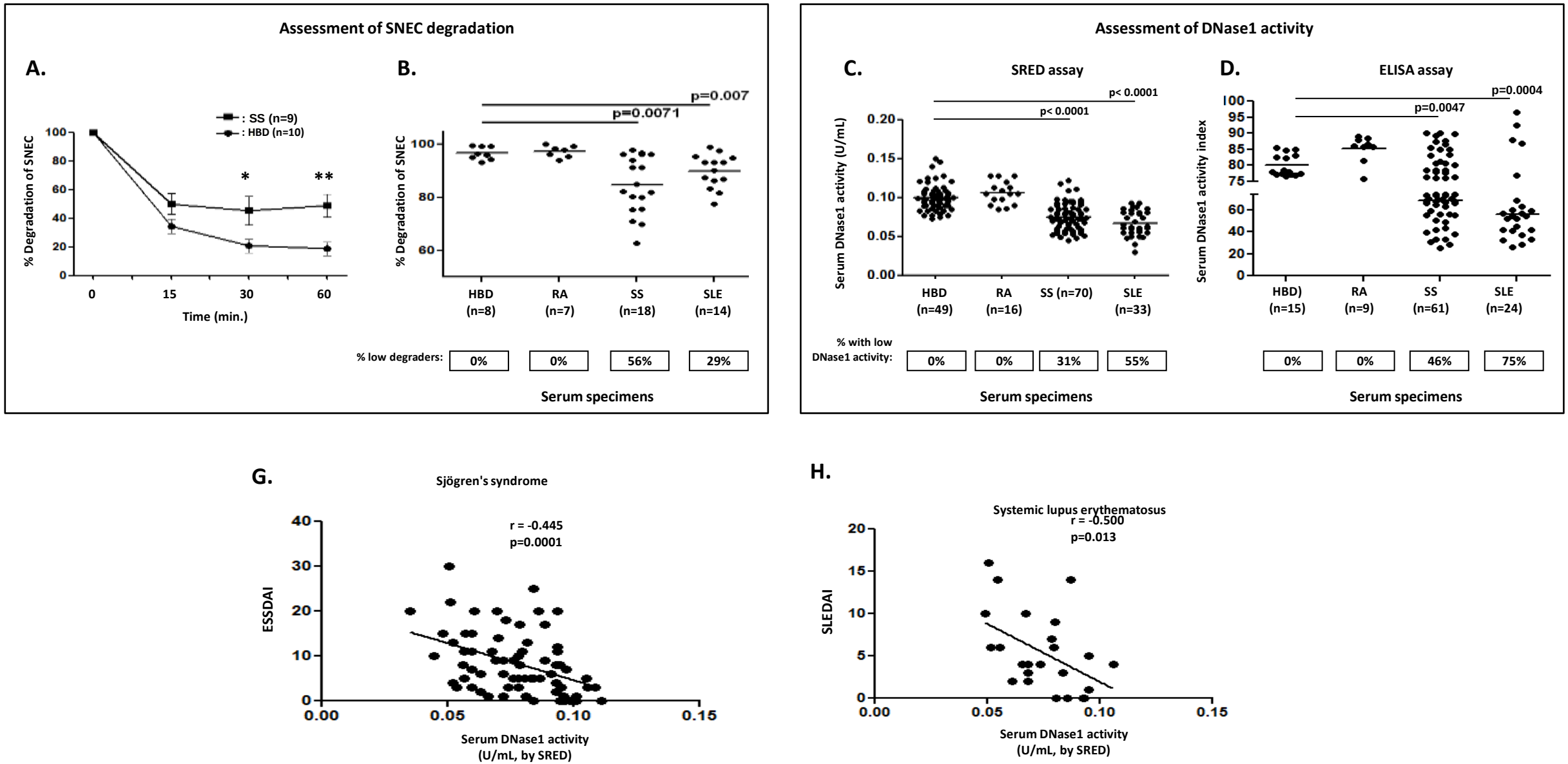
impaired degradation

- "shielding" autoabs

- increased intrinsic DAMP sensing

Manoussakis et al, PLoS One, 2014
Fragoulis et al, J Autoimm. 2014

Defective degradation of remnants of necrotic cells in SS and SLE: impaired DNA degradation (low DNase1 activity)

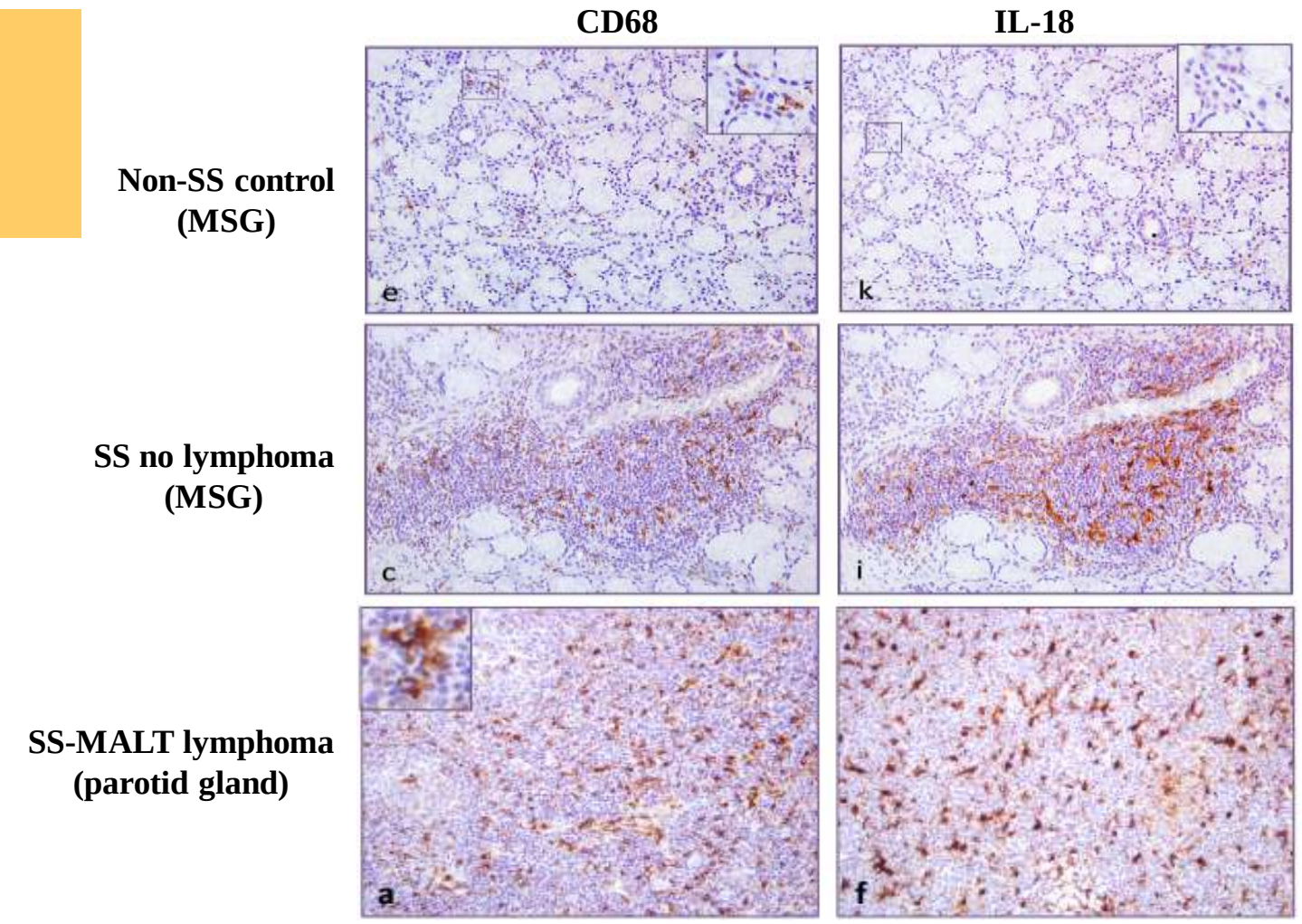


Fragoulis et al, J Autoimm. 2014

Macrophages in the SG lesions of Sjögren's syndrome

High rates of IL-18-expressing CD68+ macrophages

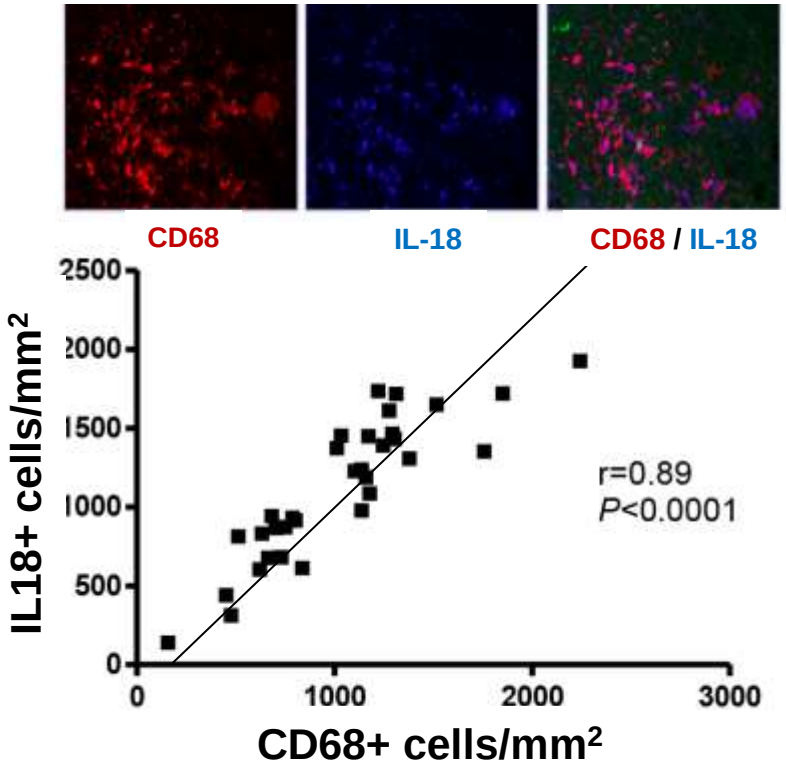
- in lymphoepithelial lesions of SS patients
- also in MALT lymphoma tissues

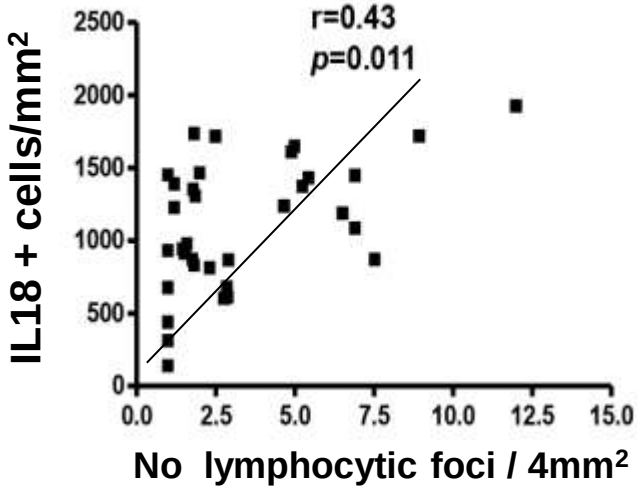


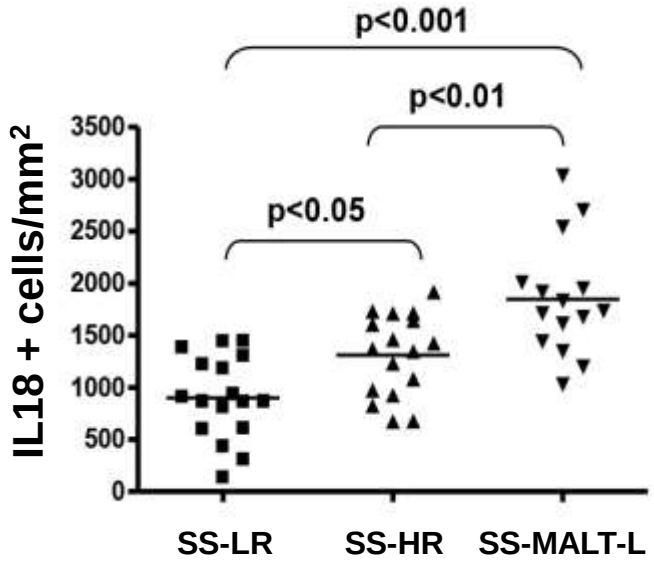
Representative images

IL-18 expression:

- Highly significant positive correlation with the intensity of CD68+ macrophagic infiltrates
- positive correlation with the intensity of mononuclear infiltrates
- significantly increased in SS-HR and SS-MALT-L



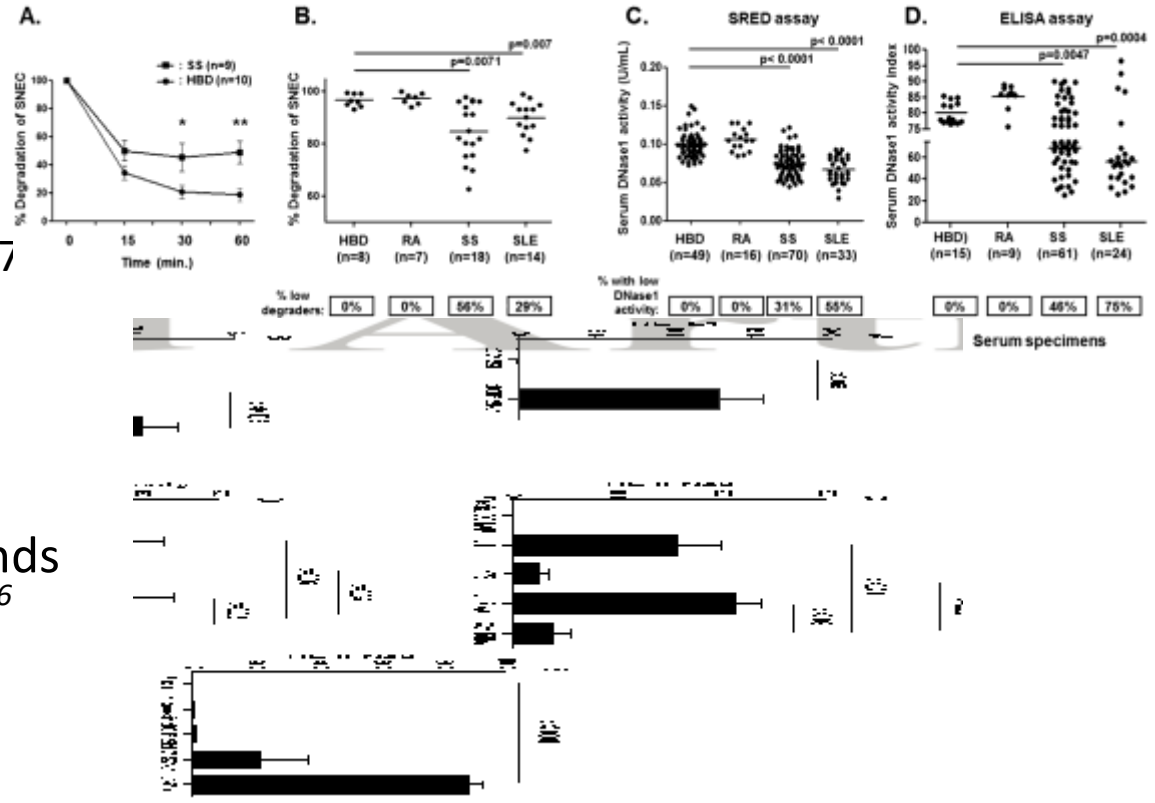




Sjögren's Syndrome
Pathogenesis-Innate Immunity

IFNs

- Induction of type I IFNs
  - Transposon (LINE-1) elements
    - Hyper-expression in salivary glands (RNA/protein) and correlation with L1 and IFN I (mRNA) and compensatory increase of DNA methyltransferase and APOBC3A
    - Hypomethylation of L1 promoter in salivary glands
    - IFNα production by pDCs after transfection with L1 (TLR7



- IFN III
  - Increased expression at the protein level in salivary glands

Mavragani et al. Arthritis & Rheumatol 2016
Mavragani et al. J Autoimmun 2017
Mavragani et al. Ann Rheum Dis 2019

Apostolou et al. J Autoimmun 2016

Sjögren's Syndrome Pathogenesis
B lymphocytes

B-lymphocytes and plasma cells

- Increased number of circulating CD38++IgD+ (pre-GC) and plasmablasts and reduced number of memory CD27+ cells

Bohnhorst et al. J Immunol 2001
Hansen et al. J Arthritis Rheum 2004
Roberts et al. Arthritis Rheumatol 2014

- Plasma cells within the salivary glands bear the long-lived phenotype

Mingueneau et al. J Allergy Cin Immunol 2016
Halliley et al. Immunity 2015

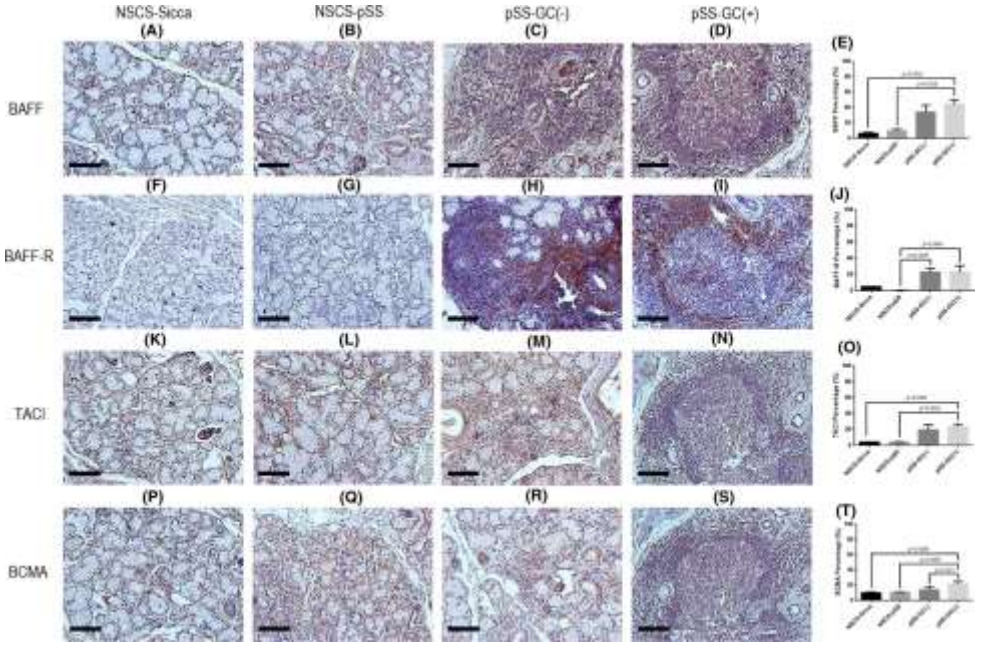
- B regulatory cells
  - B+10: no loss of their regulatory capacity upon Th1
  - Blood imbalance of IL-12/IL-35
  - Increased IL-35 serum levels are associated with reduced disease activity

Candando et al. Immunuol Rev. 2014
Menon et al. Immunity 2016
Fogel et al. J Clin Allergy Immunol 2017

Sjögren's Syndrome
Pathogenesis-B lymphocytes

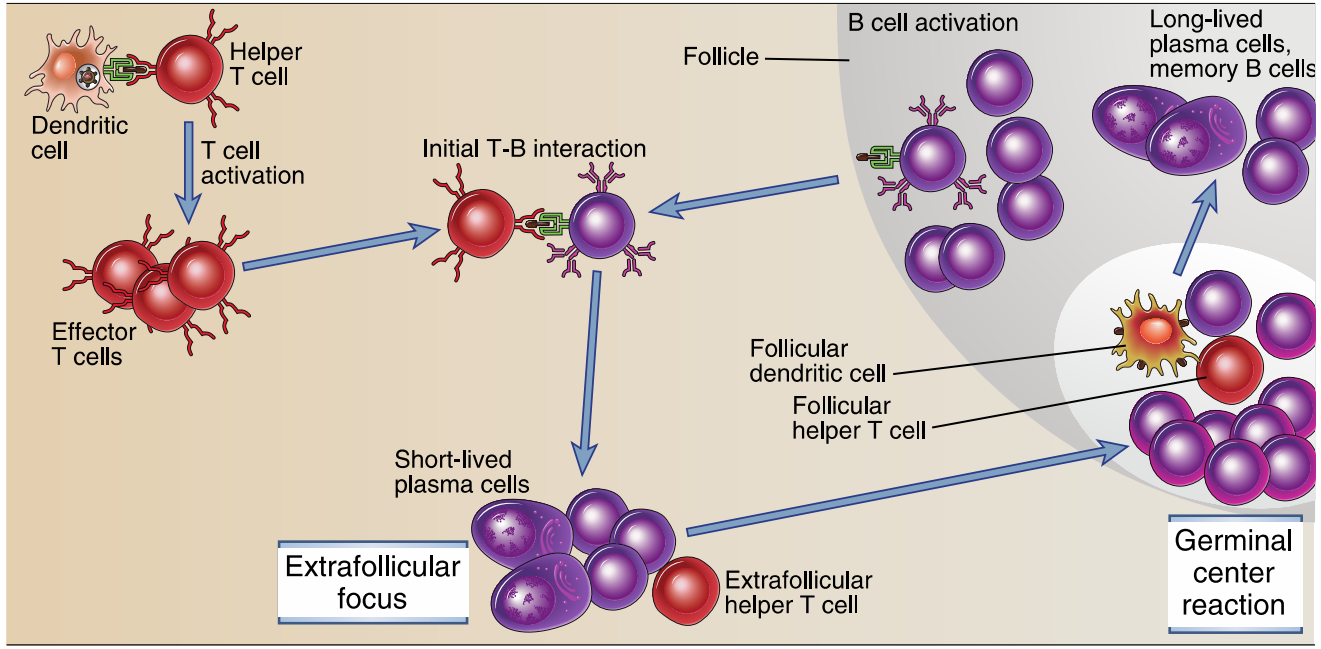
B-lymphocytes and plasma cells

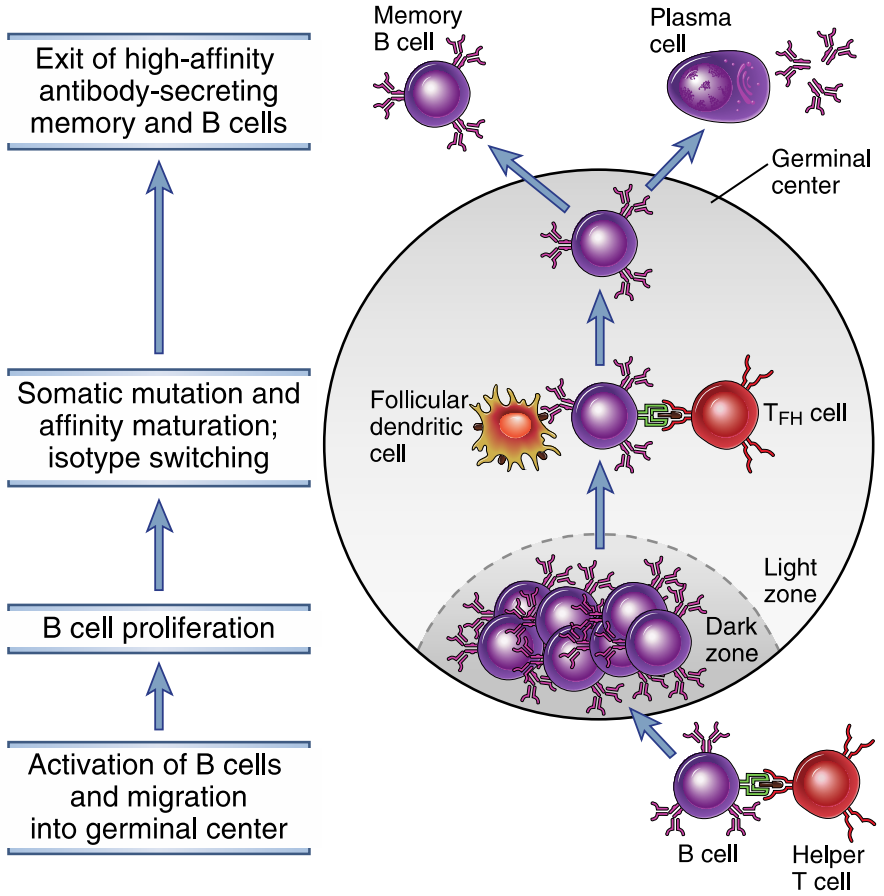
- BAFF (B-cell activating factor)
  - Increased levels in serum and salivary glands
  - Secreted by epithelial cells under the action of IFNα
  - Promotes B cell differentiation and organization of eGCs



Mariette et al. J Autoimmun 2003
Lavie et al. J pathology 2004
Ittah et al. Arthritis Res Thres 2006
Groom et al. J Clin Invest 2002
Jonsson et al. 2005
Lahiri et al. J Autoimmun 2014
Carillo-Ballesteros et al. Clin Exp Med 2019

Sjögren's Syndrome
Germinal Center Reaction in lymph nodes





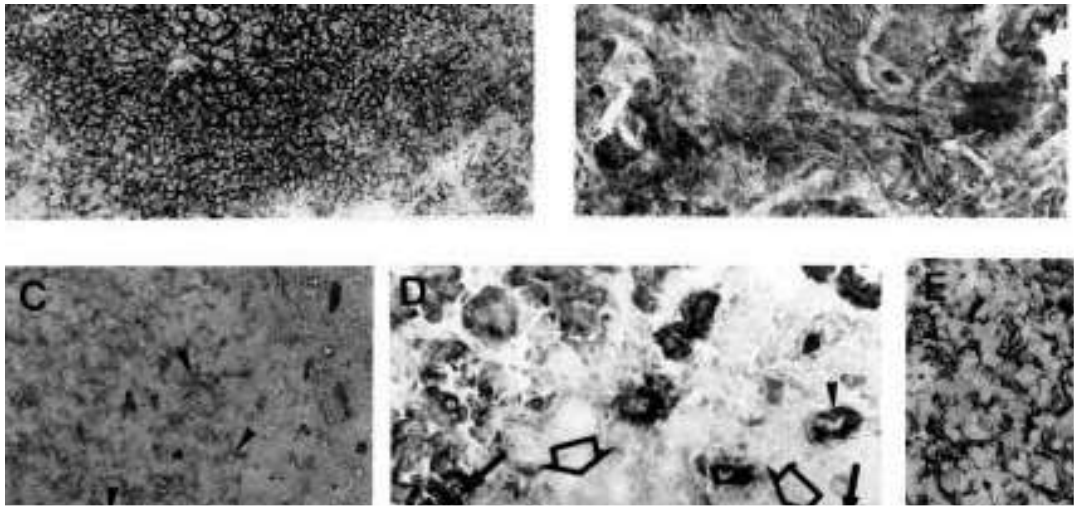
B cell activation and antibody production. In Abbas et al: Cellular and molecular Immunology 7th edition, 2011

Sjögren's Syndrome
Ectopic Germinal Centers

Ectopic GC

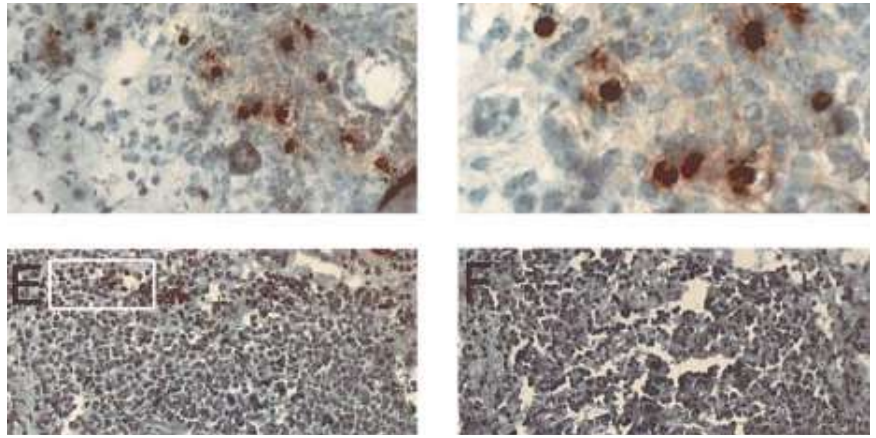
- Presence of eGC within salivary glands
- Prognostic event for lymphoma development

Aziz et al. Ann Rheum Dis 1997
Stott et al. J Clin Invest 1998
Amft et al. Arthritis Rheum 2001
Xanthou et al. Arthritis Rheum 2001



- Functional eGC
  - B and T cell proliferation
  - Follicular dendritic cells and endothelial cells
  - Plasma cells producing autoantibodies (biotinylated autoantigens)

Solomonsson et al. Arthritis Rheum 2003



- Simple B cell aggregates vs eGCs
  - Follicular dendritic cells

Bombardieri et al. J Immunol 2007
Le Pottier et al. J Immunol 2009

Sjögren's Syndrome - Ectopic Germinal Centers

Increased formation of eGCs

- Increased CXCL13 levels (B cell-attracting chemokine) in serum and within salivary glands
- Production by epithelial, endothelial and stromal cells

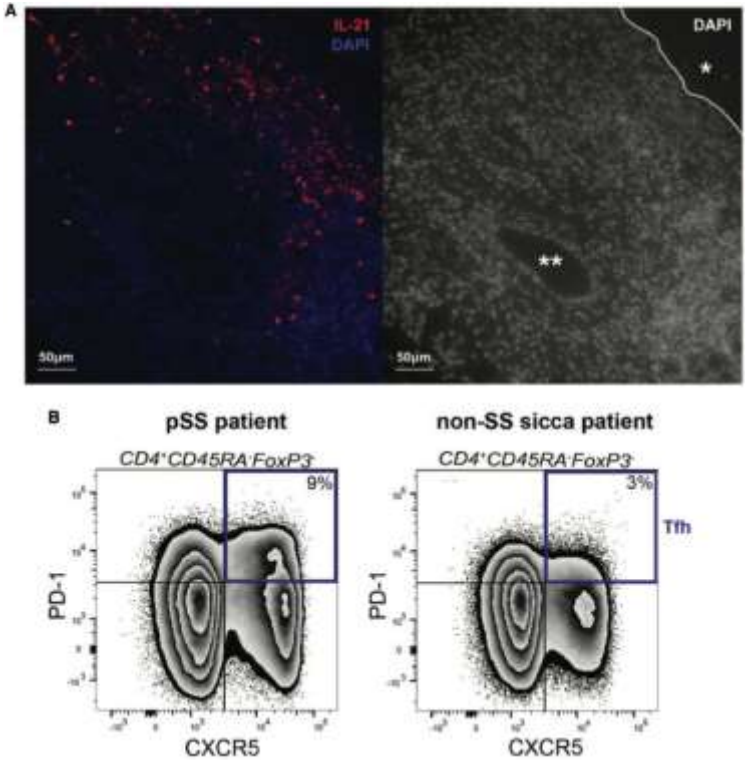
Barone et al. J Immunol 2008
Nocturne et al. Arthritis Rheumatol 2014
Bombardieri et al. Nat Rev Rheumatol 2017

- T follicular helper cells (Tfh)
  - essential for germinal center formation, affinity B-cell maturation
  - 9% of CD4+ cells in salivary glands
  - IL-6 levels in serum and salivary glands (induction of Tfh differentiation)
  - SGEC in vitro may induce the differentiation into Tfh

Fonseca et al. Arthritis Rheumatol 2018
Boumbas et al. Br J Rheumatol 1995
Pollard et al. Ann Rheum Dis 2013
Gong et al. J Autoimmunity
Fonseca et al. Arthritis Rheumatol 2017

- Tfh-like
  - CCR9 and CCL25
  - Increased numbers in blood and salivary glands
  - IL-21 production and induction of IgG from B cells

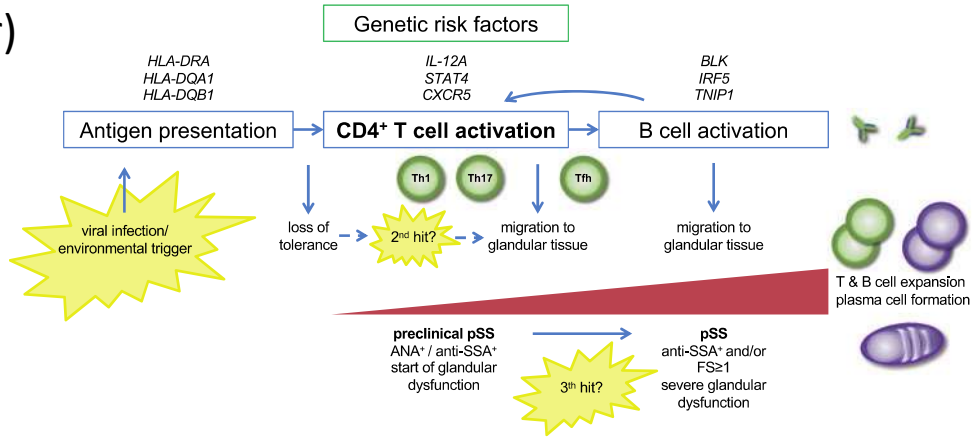
Blokland et al. Arthritis Rheumatol 2017
McGuire et al. Immunity 2011



Sjögren's Syndrome
T lymphocytes

- Increased Th1 cells
  - Protein level: ↑IFNγ+ (salivary glands)
  - Recruitment through CXCL9/10 of epithelium (via CXCR3 receptor)
  - mRNA transcripts: majority of SS patients without eGCs
  - Limited TCR repertoire

Van Woerkom et al. Ann Rheum Dis 2005
Hall et al. Arthritis Rheumatol 2015
Ogawa et al. Arthrits Rheumatol 2002
Maehara et al. Clin Exp Immunol 2012
Joachims et al. JCI insights 2015
Voight et al. Clin Immunol 2018



Verstappen et al. Rheumatology 2019

- ~ Th2 κύτταρα
  - Protein level: IL-4 + with no difference (salivary glands)
  - mRNA transcripts: where B cells and eGC are present

Van Woerkom et al. Ann Rheum Dis 2005
Ohyama et al Arthritis Rheum 1995
Joachims et al. JCI insights 2015
Voight et al. Clin Immunol 2018

- ~ Th17 (salivary glands)
  - CD161+
  - IL-17+ CD4-CD8-

Alunno et al. Ann Rheum Dis
Zhao et al. Rheumatology 2017

Primary Sjogren's syndrome
INTRINSICALLY ACTIVATED DUCTAL SALIVARY EPITHELIAL CELLS

- **Long-term cultured non-neoplastic SGEC cell lines (ductal type)**
  - **Evidence of intrinsic activation**

    constitutively aberrant expression of various molecules (compared to control cell lines)

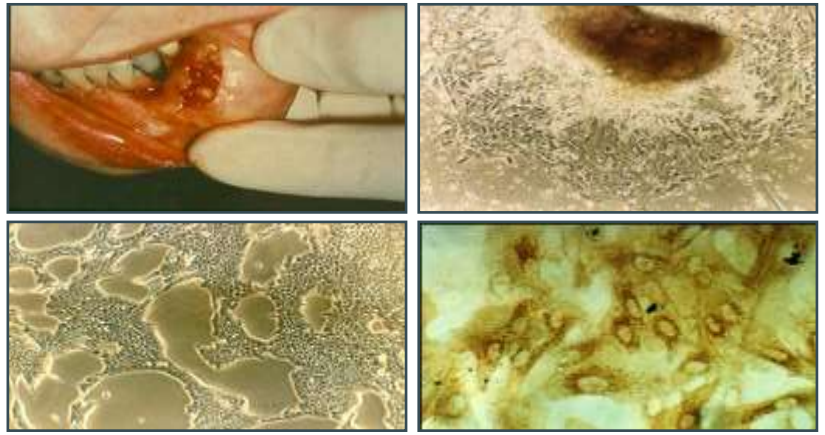
    – high IL-1β production

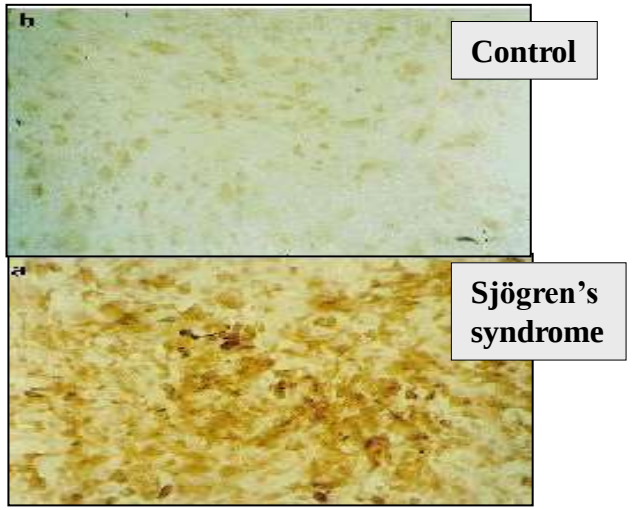
    – activated NF-κB pathway

    – impaired expression of immunoregulatory PPARγ

    – aberrant miRNA expression

    – various dysregulated inflammatory and metabolic pathways (microarray transcriptome analyses)





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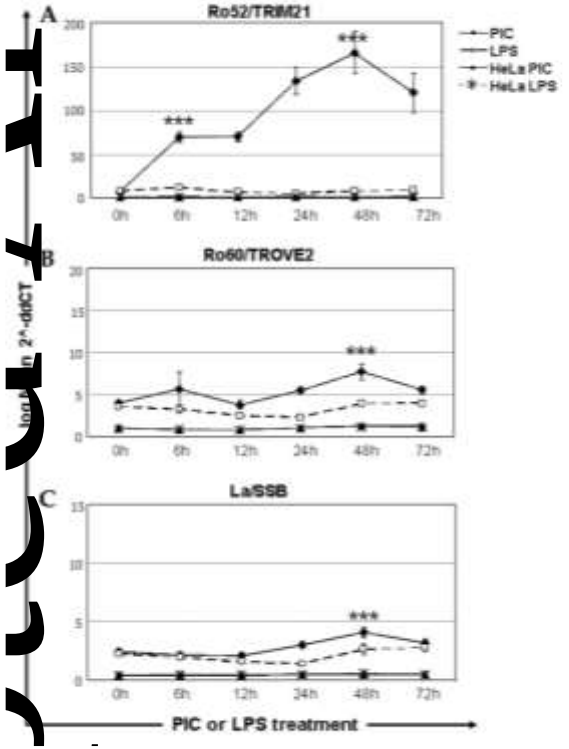
Manoussakis & Kapsogeorgou, J Autoimmunity 2010

Sjögren's Syndrome
Epithelial Cells

- Expression of TLRs and especially TLR3
  - Hypersensitivity to TLR3-mediated apoptosis
  - Cytokines (e.g. BAFF)
  - Autoantigens redistribution and presentation

Spachidou et al. Clin Exp Immunol 2007
Bourazopoulou et al. J Autoimmunity 2009
Manoussakis et al. J Autoimmunity 2010
Ittah et al. Euro J Immunol 2010
Kyriakidis et al. Clin Exp Immunol 2016

- Activated
  - Through IFNs
  - MSG: DNA hypomethylation (IIF)
  - Epigenome EWAS in SGEC: 2560 genes with differential methylation level (e.g. IFN-stimulated genes)
  - Hypomethylation of La/SSB promoter και overexpression of La/SSB by SGEC after treatment with azacytidine (demethylation agent)



Kyriakidis et al. Clin Exp Immunol 2016

Hjelmervik et al. Arthritis Rheum 2005
Gottenberg et al. Proc Natl Acad Sci USA 2006
Hall et al. Proc Natl Acad Sci USA 2012
Konsta et al J Autoimmun 2016
Charras et al. Ann Dis Rheum 2017

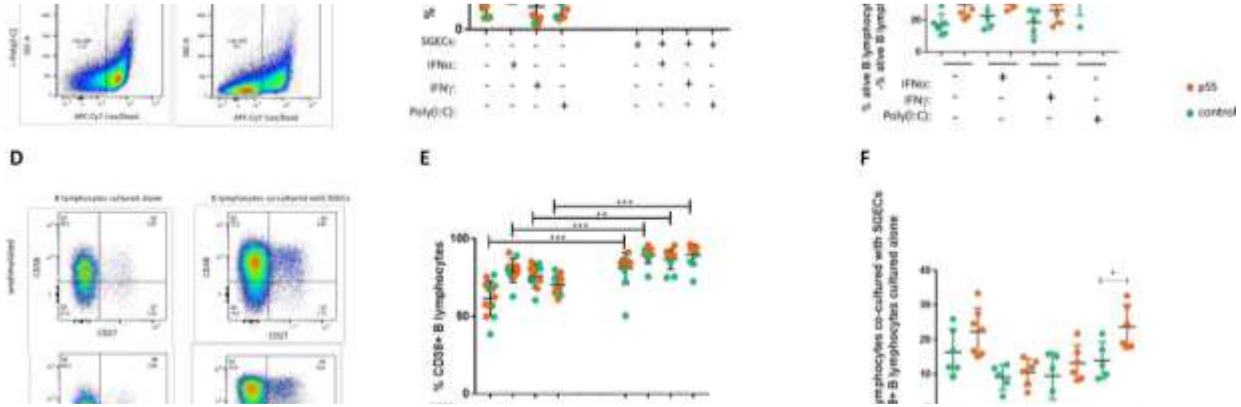
Sjögren's Syndrome
Epithelial Cells

- Apoptosis
  - Induction of apoptosis by cytokine stimulus (IFNγ)
  - induction of anoikis by TLR3 activation
  - Apoptotic blebs with autoantigens

Ohlsson et al. Scad J Immunol 2002
Ping et al. Atrhtis Rheum 2005
Cha et al. Scad J Immunol 2004
Wang et al. Arch Oral Biol 2009
Manoussakis et al. J Autoimmun 2010

- Exosome release
  - Ro/SSA, La/SSB, URNP, Sm

Kapsogeorgou et al. Arthritis Rheum 2005



- SGEC and B cells
  - Trascriptomics/MSG: SGEC→↑IFSGs, IL-7 and BAFF
  - Trascriptomics/MSG: B cells→↑CD40 και CD48
  - Co-cultures with or without poly I:C : increased survival of B cells, especially after poly I:C through soluble factors
  - Supernatant from co-cultures→ cytokines and chemokines

Riviere et al. Ann Reum Dis 2020

Sjögren's Syndrome-intrinsic activation of ductal salivary epithelial cells

- NF-kB και IL-1β pathways
  - ↑ expression in ductal epithelia in the salivary glands of SS patients
  - ↑ constitutively activated in SS-SGEC lines and following activation of normal SGEC lines

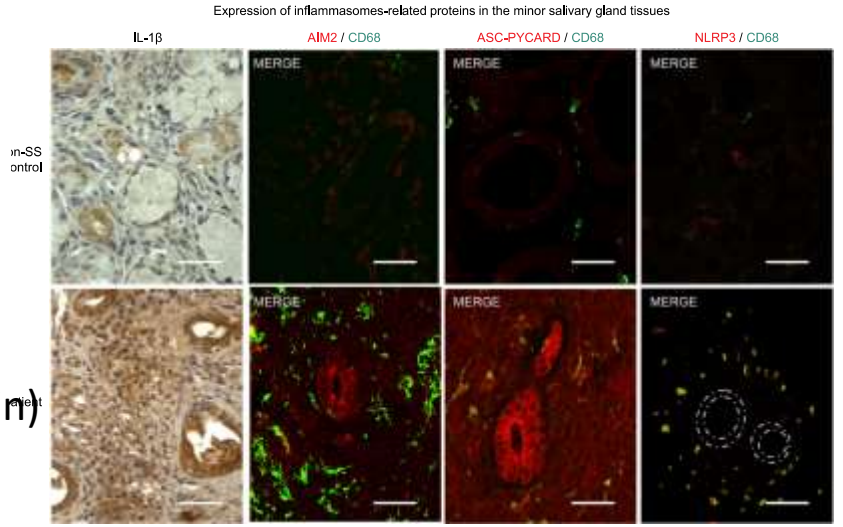
Vakrakou et al. J Autoimmun 2016

- PPARγ (anti-inflammatory/Peroxisome proliferator-activated receptor-gamma)
  - ↓ in ductal epithelia in salivary glands
  - ↓ constitutive expression and function in SS-SGEC lines and following activation of normal SGEC lines
  - Correlation with endogenous activation of NF-kB και IL-1β pathways
  - Stimulation with PPARγ agonists limits the endogenous NFkB/IL-1β activation

Vakrakou et al. J Autoimmun 2017

- AIM2 inflammasome
  - ↑ AIM2 expression in ductal salivary epithelial cells of patients (RNA/protein)
  - ↑ AIM2 expression in SS-SGEC lines
  - Correlation with ↑ deposits of cytoplasmic damaged DNA
  - Decreased DNase 1 expression correlates with AIM2 activation
  - In normal SGEC: TLR3 stimulation→ decreased DNase 1

Vakrakou et al. J Autoimmun 2019

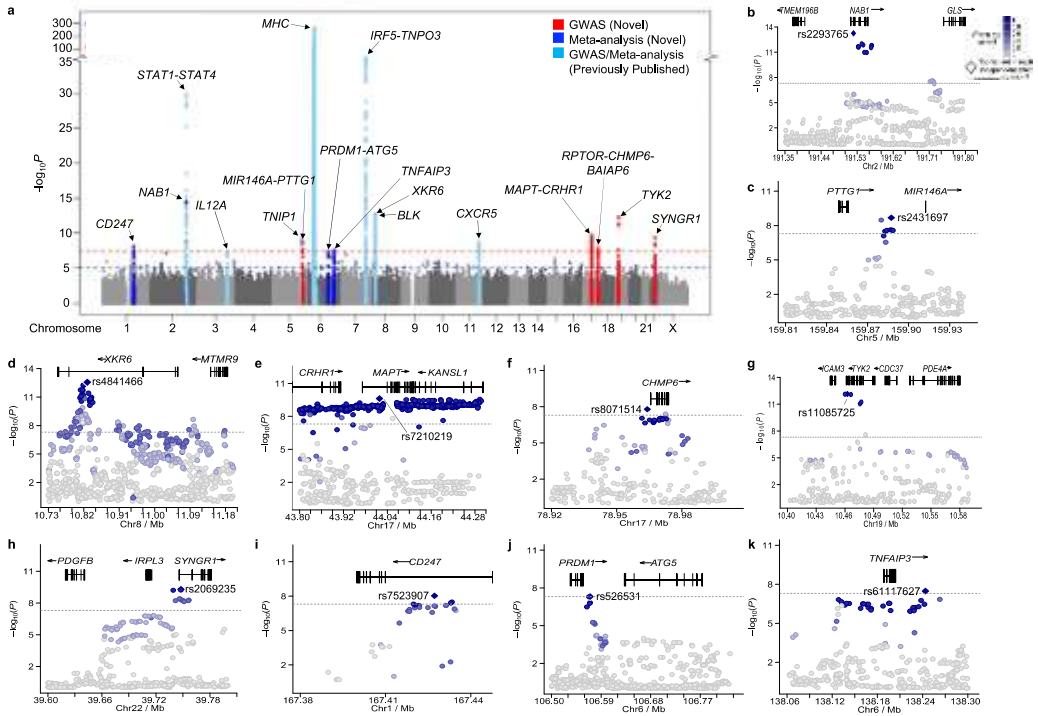


Sjögren's Syndrome

Recent Advances in Pathogenesis
Genetics/Epigenetics

- 3232 SS patients vs 17481 population controls of European ancestry
  - GWAS
  - Polygenic risk score analysis
  - SS-SNPs enriched in epigenetics and expression data (cell type and tissue)
  - Meta-analysis and refinement of novel associations
  - Functional analysis in enhancers and promoters

- Results
  - 10 novel risk loci
    - immune cell function (CD247, NAB1, MIR146A, PRDM1, TNFAIP3, TYK2)
    - inflammatory signaling (TNFAIP3, CRHR1, TYK2)
    - cell survival and proliferation (CD247, MIR146A, PRDM1, TNFAIP3, TYK2)
    - cell stress (ATG5, CHMP6)
  - Interplay between immune cells and salivary glands

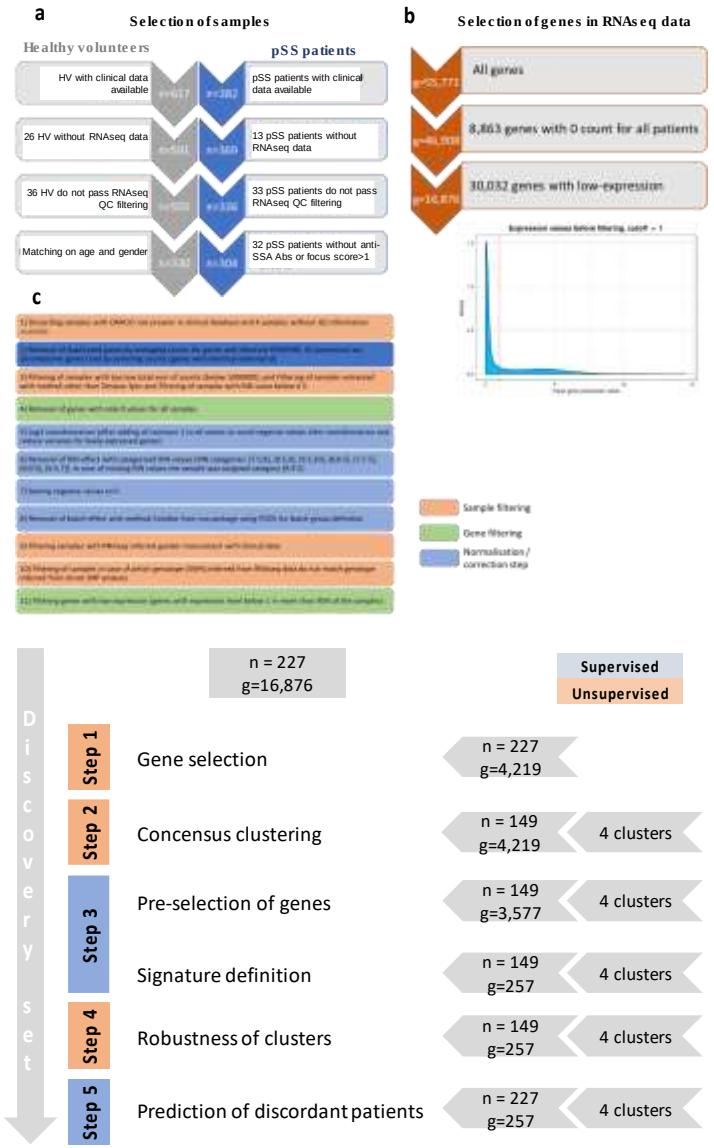


Kharti et al. Nat Commun 2022

Sjögren's Syndrome
Recent Advances in Pathogenesis-Molecular Stratification

- **Patients and Methods**
  - 304 SS patients
    (227 discovery set + 77 validation)
  - 330 Healthy Volunteers (HV)
  - Whole blood RNA (RNA seq, methylation, genotyping, flow cytometry)
  - Serum (cytokines, chemokines, antibodies)
  - Machine learing algorithms

  - Molecular signature (clustering)
  - Prognostic gene profiling
  - Associations



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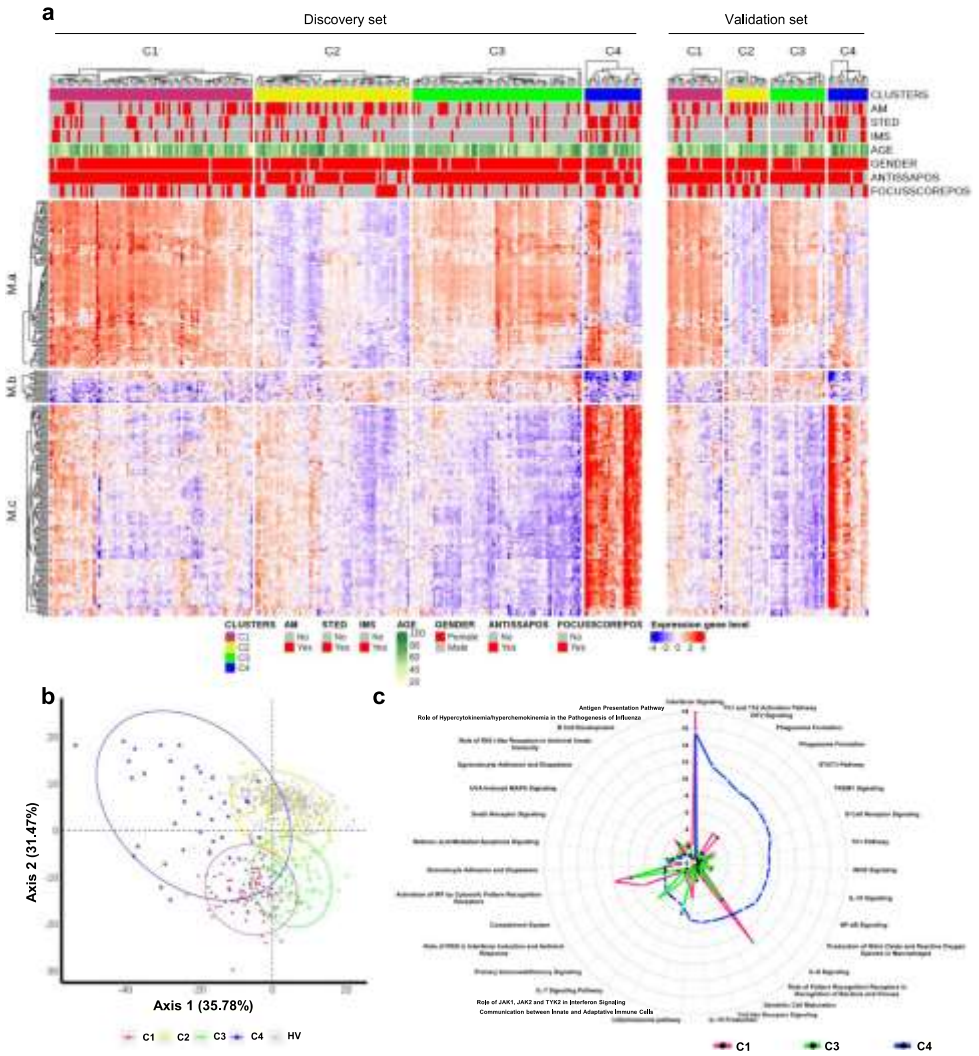
Sjögren's Syndrome

Recent Advances in Pathogenesis-Molecular Stratification

- Heatmap of 4 clusters
  - Clusters and functional modules (3) after normalization
- Scatter plot: HV assigned by distancing (PCA analysis)
- Radar plot: top 20 canonical pathways for each cluster
  - No annotation for C2



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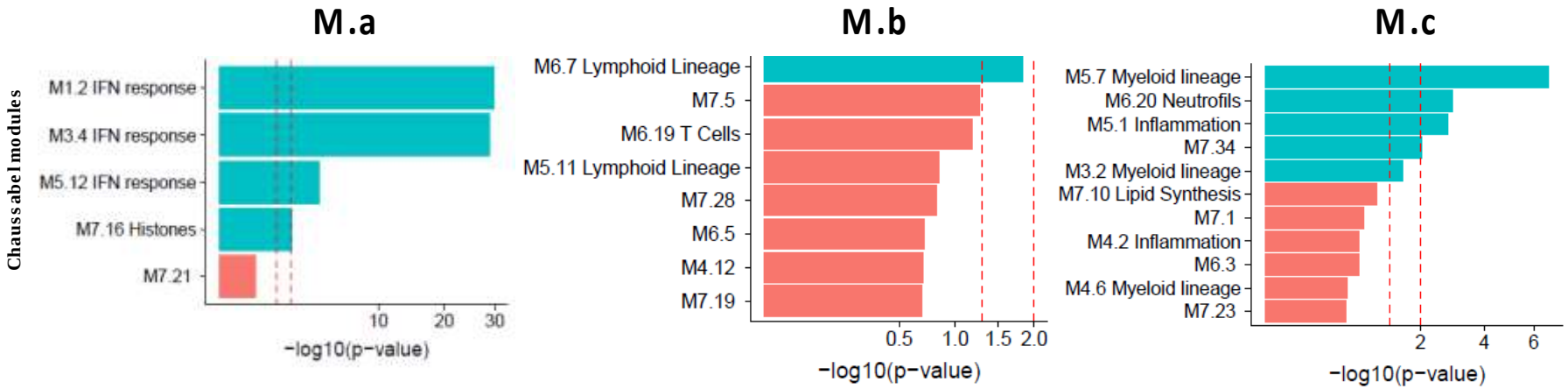
Recent Advances in Pathogenesis-Molecular Stratification

The 257-gene signature



- M.a (105 genes)
  - IFN signature
- M.b (20 genes)
  - Lymphoid lineage pathways
- M.c (132 genes)
  - Inflammatory and myeloid pathways

- C1:101 (33.2%), C2:77 (25.3%), C3:88 (28.9%), C4:38 (12.5%)
- HV (n=304): 93% C2
- C1>C3: M.a
- C2: M.b, M.c
- C4: M.c

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Recent Advances in Pathogenesis-Molecular Stratification

DEGs compared to HV: 20 top canonical pathways of each cluster

Patients (n=304)

HV (n=330)

- C1: 284 DEG (IFN, PRR bacteria/viruses)
- C2: none
- C3: 301 DEG (adaptive/B cells)
- C4: 1686 DEG (adaptive B cells, Th1, Th2, inflammation)

Supplementary Data 2: Top 20 canonical pathways of each DEG signature in the various pSS clusters. Ingenuity pathway analysis was applied (NA : not applicable). Statistical significance was determined by Fisher's exact test.

	Ingenuity Canonical Pathways	-log(p-value)	z-score
C1	Interferon Signaling	17,9	3,051
	Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	11,6	3,162
	Activation of IRF by Cytosolic Pattern Recognition Receptors	9,9	1,508
	Complement System	6,75	0,816
	Systemic Lupus Erythematosus In B Cell Signaling Pathway	4,75	3,606
	Inflammasome pathway	4,16	2
	Salvage Pathways of Pyrimidine Deoxyribonucleotides	4,1	NA
	Phagosome Formation	4,02	NA
	Pathogenesis of Multiple Sclerosis	3,93	NA
	Role of RIG1-like Receptors in Antiviral Innate Immunity	3,85	1,342
	Retinoic acid Mediated Apoptosis Signaling	3,22	2,236
	Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	2,84	NA
	TREM1 Signaling	2,78	2,236
	Role of JAK1, JAK2 and TYK2 in Interferon Signaling	2,6	NA
	Death Receptor Signaling	2,41	2,236
	Communication between Innate and Adaptive Immune Cells	2,31	NA
	Dendritic Cell Maturation	2,29	1,89
	UVA-Induced MAPK Signaling	2,27	NA
	Antigen Presentation Pathway	2	NA
	Role of PKR in Interferon Induction and Antiviral Response	1,94	NA
C3	Interferon Signaling	11,7	3,162
	Activation of IRF by Cytosolic Pattern Recognition Receptors	7,86	1,667
	Communication between Innate and Adaptive Immune Cells	6,24	NA
	Primary Immunodeficiency Signaling	6,17	NA
	Systemic Lupus Erythematosus In B Cell Signaling Pathway	6,05	3,742
	Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	5,4	2,236
	Hematopoiesis from Pluripotent Stem Cells	5,01	NA
	Pathogenesis of Multiple Sclerosis	4,09	NA
	Retinoic acid Mediated Apoptosis Signaling	3,45	1,342
	Iron homeostasis signaling pathway	3,3	NA
	Phenylethylamine Degradation I	3,22	NA
	Role of RIG1-like Receptors in Antiviral Innate Immunity	3	1
	IL-7 Signaling Pathway	2,93	2
	LXR/RXR Activation	2,84	0,447
	Granulocyte Adhesion and Diapedesis	2,63	NA
	B Cell Receptor Signaling	2,55	NA
	Hepatic Fibrosis / Hepatic Stellate Cell Activation	2,54	NA
	Agranulocyte Adhesion and Diapedesis	2,46	NA
	IL-10 Signaling	2,28	NA
	B Cell Development	2,24	NA
C4	Interferon Signaling	15,5	4,472
	Th1 and Th2 Activation Pathway	11,6	NA
	EIF2 Signaling	10,6	-3,053
	Phagosome Formation	9,87	NA
	STAT3 Pathway	9,71	3,273
	Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	9,55	NA
	TREM1 Signaling	9,53	4,796
	B Cell Receptor Signaling	9,37	3,536
	Th1 Pathway	8,81	0,784
	iNOS Signaling	8,79	3,873
	IL-10 Signaling	8,7	NA
	NF-κB Signaling	8,65	3,212
	Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	8,6	4,667
	Tec Kinase Signaling	7,99	3,024
	IL-6 Signaling	7,92	4,536
	Type I Diabetes Mellitus Signaling	7,9	3
	Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	7,54	3,9
	Dendritic Cell Maturation	7,31	3,656
	Toll-like Receptor Signaling	7,13	3,873
	IL-15 Production	7,09	NA

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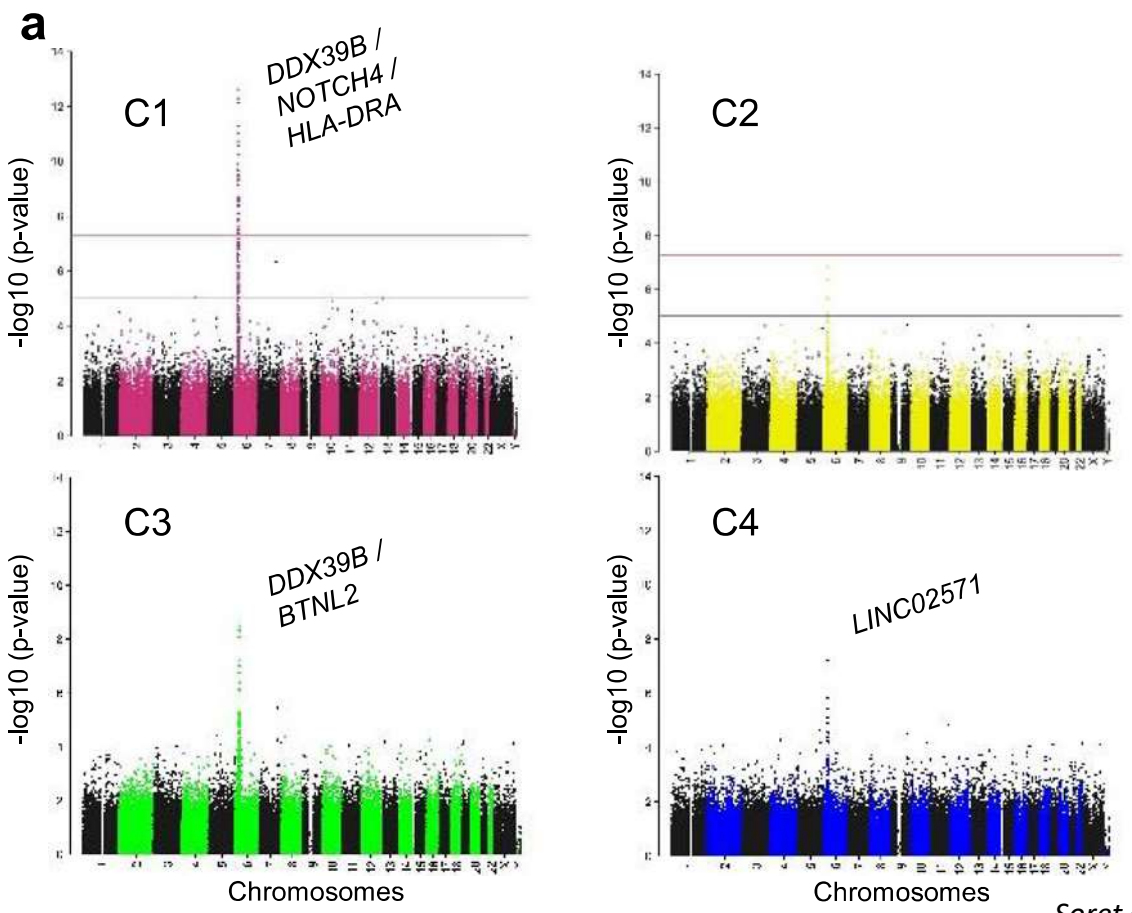
Sjögren's Syndrome

Recent Advances in Pathogenesis-Molecular Stratification

GWAS analysis-Single nucleotide polymorphisms

- C1: 35 SNPs
  - Immune system
  - Signal transduction
  - Developmetal
  - Gene expression
  - Cell cycle
- C2: 0 SNPs
- C3: 6 SNPs
- C4: 1 SNP



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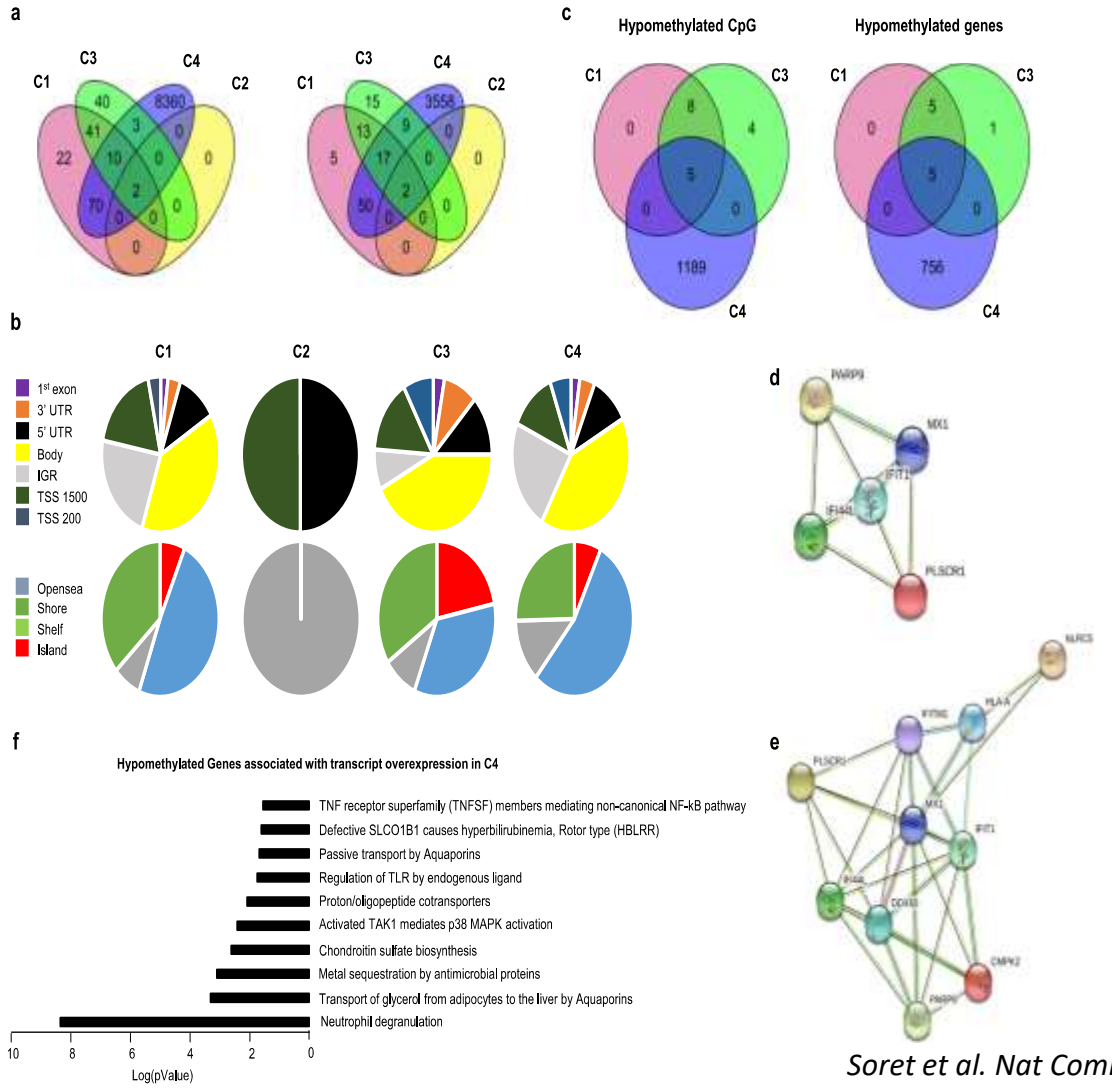
Sjögren's Syndrome

Recent Advances in Pathogenesis-Molecular Stratification

DNA methylation analysis vs HV

- DMP and genes in all clusters
- DMPs in promoter and CpG islands
- DMPs/Genes: 145/87 (C1), 96/56 (C3), 8445/3636 (C4)
- Global hypomethylation
  - IFN related genes (C1/C3)
  - hypomethylation of neutrophil degranulation pathway
- Hypomethylated CpGs and genes among all clusters (n=5)
- Hypomythelated CpGs in C1 and C3 (n=10)



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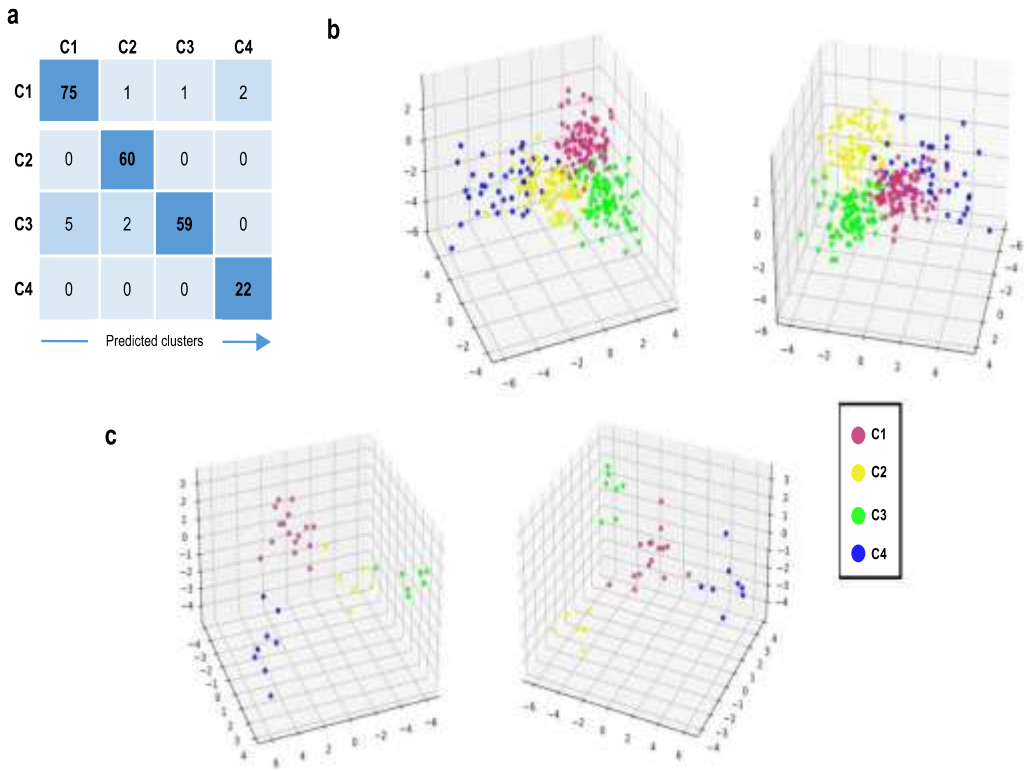
Recent Advances in Pathogenesis-Molecular Stratification Prediction Model

Composite prediction model

- DEGs based strategy with 1154 common genes of 14.240 derived from combination of clusters (n=227)
- Boruta algorithm for gene selection
- 2 step strategy
  - XG boost binary (C4 vs C1/C2/C3)(255 genes)
  - Random Forest (C1 vs C2 vs C3)(597)
- 10 genes for step 1 and 30 genes for step 2



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Sjögren's Syndrome

Recent Advances in Pathogenesis-Molecular Stratification

Overall Analysis

- Clustering of SS patients based on 2 molecular signatures with 257 και 41 DEGs

- 4 clusters
  - C1: strong type I and II IFN signature and cytokines (IFN-γ, pro-inflammatory and CXCL13, BAFF, RFs, low C4)
  - C2: similar to HV
  - C3: type I IFN and B cell activation (CXCL13, BAFF)
  - C4: high activity, inflammatory component, neutrophils

- Global hypomethylation (especially in IFN-related genes)

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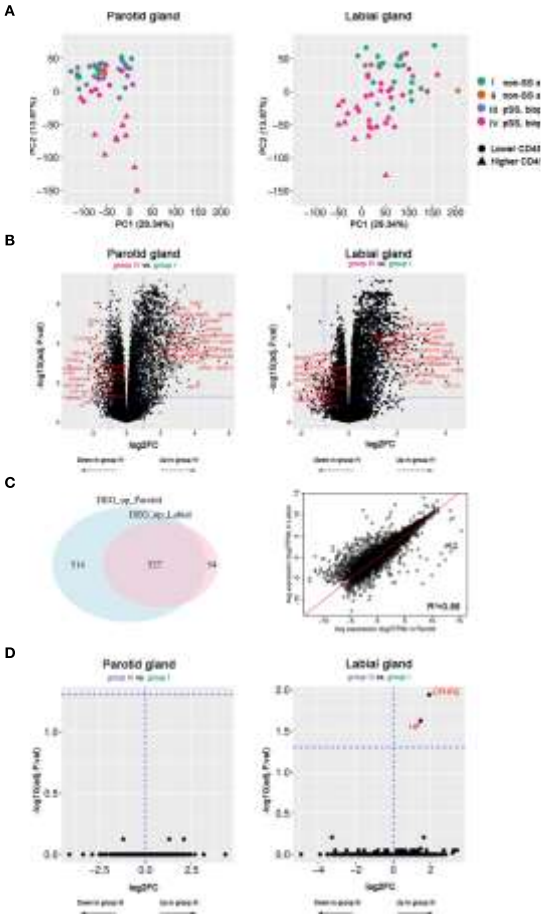
Sjögren's Syndrome

Recent Advances in Pathogenesis-Molecular Stratification

- 34 SS patients vs 17 sicca controls
- 52 PG and 57 LG (51 paired) analyzed
  - Whole blood, PBMCs, parotids and minor salivary glands RNA
  - PCA for transcriptomics comparison

- Results
  - In both PG and LGS : aberrations only in SS+ (vs sicca controls)
  - strong DEGs overlap between PG and LG
  - mainly related to B and T cells and IFNs
  - IFN-a signaling, IL-12/IL-18 signaling, CD3/CD28 T-cell activation, CD40 signaling in B-cells, DN2 B-cells, and FcRL4+ B-cells
  - PBMCs reflected SG type I IFN



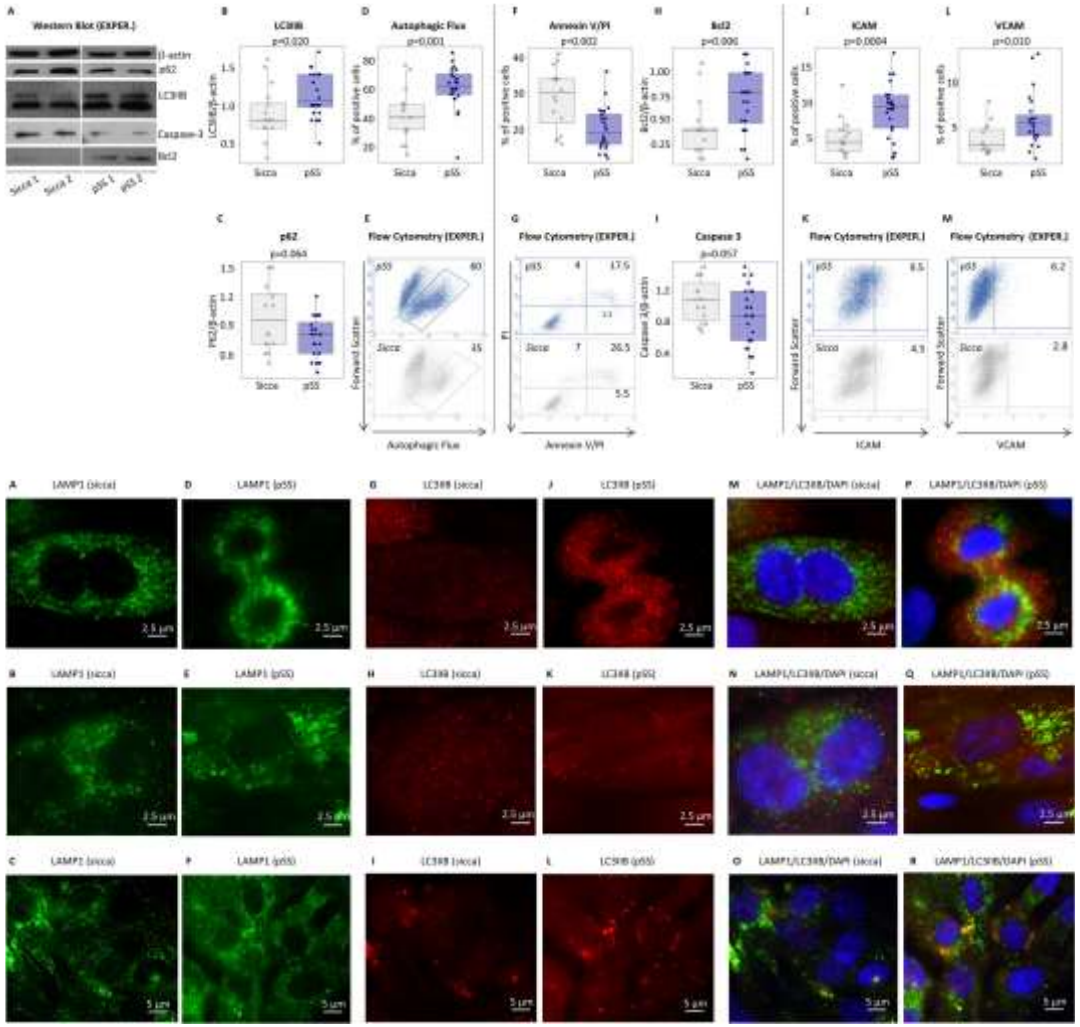
Vertsappenet al. Front Immunol 2022

Sjögren's Syndrome

Recent Advances in Pathogenesis-Epithelium Autophagy

- 24 SS patients vs 16 sicca controls
  - SGEC lines: autophagy, apoptosis and activation (WB, flow and IIFs)

- Results
  - Increased autophagy (pro-survival recycling of metabolic and anabolic substrates) and evidence of activation
  - Autophagy correlated with histologic disease severity
  - Decreased apoptosis and increased anti-apoptotic molecules
  - Induction of autophagy and activation of HSGs by PBMCs and serum of SS patients (vs HC)

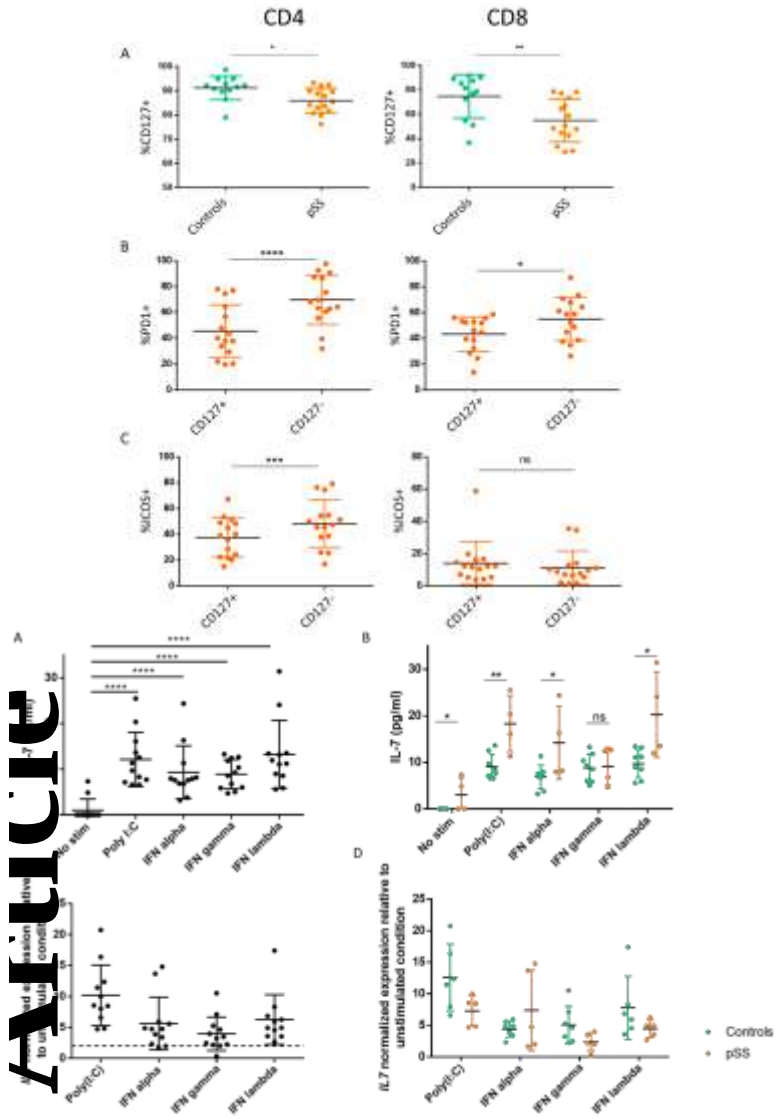


Colafrancesco et al. Arthritis Rheumatol 2022

Sjögren's Syndrome

Recent Advances in Pathogenesis-The IL-7/IFN axis

- 395 SS patients vs 73 healthy controls
  - IL-7 serum levels
  - Stimulated SGECs cultures (poly I:C, IFNα,γ, λ)
  - IFN signature and IFNγ in peripheral blood T cells
- Results
  - IL-7 is increased and IL-7R reduced in CD4+ and CD8+ of blood lymphocytes (downregulation)
  - SGECs from SS patients produce IL-7 after stimulation compared to controls
  - RNA profile analysis: IFN signaling and IFNγ are upregulated in peripheral blood T cells of pSS compared to controls
  - IL-7R inhibition in salivary explants decreased IFN signature by explant and pellet cells



Reviere et al. Arthritis Rheumatol 2021

Sjögren's Syndrome- Molecular Pathogenesis: conclusions-I

- **Peripheral blood**
  - IFN signature (increased IFNγ)
  - IL-7/IFN axis activation in PB T cells
  - Increased BAFF levels in serum
  - GWAS: novel risk loci
  - Global gene hypomethylation (especially in IFN-related genes)
  - Molecular Stratification reveals distinct clustering of SS patients (4 clusters)

Sjögren's Syndrome- Molecular Pathogenesis: conclusions-II

- **In whole SG analysis:**
  - differential gene activation: T-cell and B-cell genes
  - activation of IFNa signaling (increased expression of IFN-stimulated genes)
  - activation of IL-12/IL-18 signaling
  - global gene hypomethylation (including IFN-stimulated genes)

Sjögren's Syndrome- Molecular Pathogenesis: conclusions-III

- **Ductal salivary gland epitheliaI cells: intrinsic activation**
  - cell-autonomous activation of NF-kβ και IL-1β pathways
  - constitutively high expression of HLA-I, costimulatory and adhesion molecules
  - increased BAFF expression
  - constitutive activation of AIM2 inflammasome associated with increased cytoplasmic DNA deposits
  - constitutively low PPARγ expression (anti-inflammatory)
  - increased autophagy and anti-apoptotic molecules
  - activation of IL-7/IFN axis
  - global gene hypomethylation (especially in IFN-related genes)