# Investigating Signals Leading to B Cell Activation in Sjögren's Disease patients

## CONTEXT

Sjogren's disease (SjD) is a chronic, progressive, and systemic autoimmune disease affecting around 0.05% of the population[1] (*fai2r*). SjD typically originates in exocrine glands but can affect the function of almost any organs in the body and heightens the risk of lymphomas. Extensive dryness of the mouth and the eyes is the consequence of a misunderstood intense lymphoplasmacytic infiltration of the exocrine glands which progressively impairs their functions. While treatments targeting B cells have been shown to restore partially the functionality of salivary glands[4], **there is currently no cure for SjD**. To date, treatments mostly focus on relieving symptoms (*fai2r*). Very recently, promising drugs targeting B cell activation were shown to be effective in small phase 2 trials for decreasing disease activity, and larger phase 3 clinical trials are ongoing[5]. **Restraining B cell hyperactivity is central to treating SjD permanently**. Hence, instead of targeting directly B cells, SjD treatments could also benefit from targeting signals or interactions leading to their activation. To do so, a more detailed and deeper understanding of the cellular and molecular network in SjD is needed to find a relevant therapeutic target.

The main **hypothesis of this project is that treatment should focus on targeting B cell activation driven by Natural Killer (NK) cells**, a cell type (*i*) located within the inflamed tissues; (*ii*) involved in the first line of defense against pathogen; (*iii*) that produce cytokines and chemokines known to act on B cells (BAFF, FLT3, IFNγ), and (*iv*) which could directly interact with B cells.

## OBJECTIVE

In this project we propose to explore the interactions between NK cells and B cells using spectral flow cytometry and in vitro co-culture models. The objectives are the following: (i) identify how NK cells influence B cell activation, and (ii) identify how these interactions influence NK cell activation and function.

## METHODOLOGY

*Cohort*: We will use peripheral blood mononuclear cells (PBMC) from both SjD patients and patients with similar dry symptoms but no signs of autoimmune diseases (referred to as sicca donors) to assess differences in NK cell activation and function.

*Step 1: Panel Optimization Using Spectral Flow Cytometry*

We will design two comprehensive flow cytometry panels using spectral flow cytometry to

- Identify changes in NK cell phenotype in SjD patients compared to sicca donors (panel will include markers of NK cell activation, maturation, inhibitory and activating receptors …)
- Assess B cell changes in activation when in culture with NK cells (panel will include markers of activation, proliferation, differentiation, apoptosis …)

The panels will be validated using PBMCs from healthy donors (from the french blood bank EFS). Once finalized, panel 1 will be tested on selected SjD patients and sicca donors.

*Step 2: Testing Interactions via In Vitro Functional Assays*

We will design a co-culture model between NK cells and B cells. We will optimize co-culture conditions, in particular time of co-culture (long-term co-culture would lead to higher cell death); best media for culturing for each of the receiving cells; and best stimulation conditions. We will assess activation, proliferation, differentiation using Panel 2. Optimization will be done using PBMCs from healthy donors (EFS). When culturing conditions will be optimized, we will compare activation,

proliferation, and maturation of B cells between SjD patients and sicca controls. We will test whether the observed effects are direct or indirect using inserts in co-culture.

## WHY CHOOSING THIS MASTER PROJECT?

Biology – Having a better understanding of the overlooked function of NK cells in driving B cell activation in SjD
Methods – Learning how to develop a spectral flow cytometry panel and optimize in vitro co-culture models

## SKILLS EXPECTED

Cell culture (with human primary cells)
Flow cytometry (basics)
Scientific writing and reading
Team work

## LOCATION/SUPERVISION

*Location*
B Lymphocytes, Autoimmunity and Immunotherapies (LBAI, U1227) – 9 rue Felix le Dantec, 29200 Brest, France

*Supervision*
Marie Frutoso, Postdoctoral fellow (LBAI)

## DURATION/COMPENSATION

Duration: 6 months between January and July 2025

## CONTACT

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