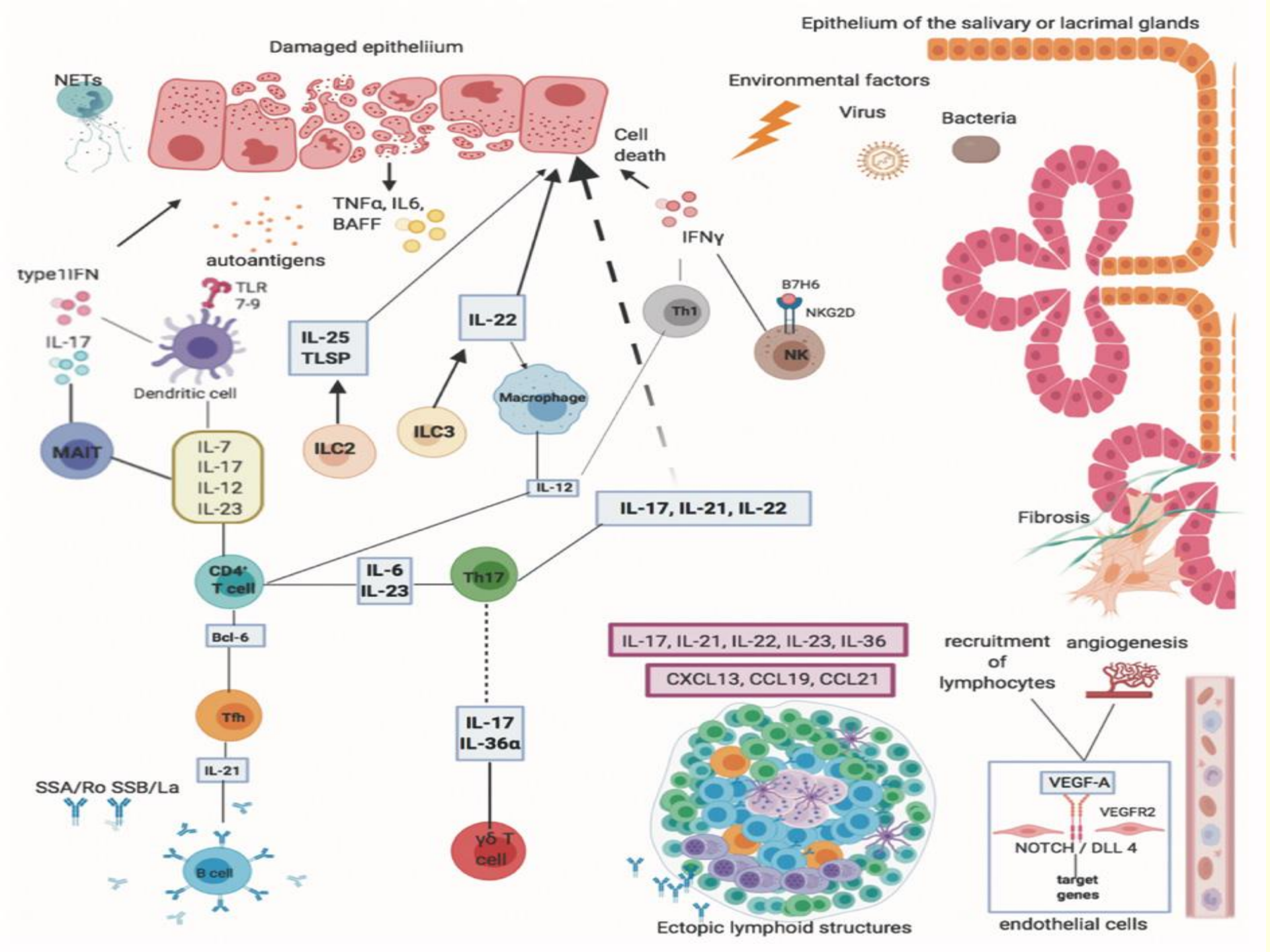
Complement fixation Test

Practical Immunology

Lect- 6

Sjogren's syndrome (Sicca syndrome)

- **Sjögren syndrome** is a **chronic** **pleomorphic clinical autoimmune disease** in which the white blood cells destroy the **exocrine glands,** specifically the **salivary causing** xerostomia (dry mouth) **and lacrimal glands leading to** keratoconjunctivitis sicca (dry eyes). Also it association with lymphocytic infiltration of the glands leads to damages or destroys the glands. Sjogren's syndrome can be defined as primary or secondary, depending on whether it occurs alone or in association with other systemic autoimmune diseases, respectively.


Epithelium of the salivary or lacrimal glands
Damaged epitheliium
NETs
Environmental factors
Virus
Bacteria
Cell death
TNFα, IL6, BAFF
IFNγ
autoantigens
type1IFN
TLR 7-9
IL-17
Dendritic cell
IL-25 TLSP
IL-22
Th1
B7H6
NKG2D
NK
Macrophage
ILC3
ILC2
MAIT
IL-7 IL-17 IL-12 IL-23
IL-12
IL-17, IL-21, IL-22
Fibrosis
CD4+ T cell
IL-6 IL-23
Th17
Bcl-6
Tfh
IL-21
IL-17 IL-36α
SSA/Ro SSB/La
B cell
γδ T cell
IL-17, IL-21, IL-22, IL-23, IL-36
CXCL13, CCL19, CCL21
Ectopic lymphoid structures
recruitment of lymphocytes
angiogenesis
VEGF-A
VEGFR2
NOTCH / DLL 4
target genes
endothelial cells

Diagnosing of Sjogren's syndrome

- **Blood tests :**

1. Complete blood count (CBC) (showed low platelets & WBCs, ESR is elevated in 80%) of patients.
2. **Antinuclear antibody (ANA)**
   - **Typical Sjogren's syndrome ANA patterns are SSA/Ro (Anti-Sjögren's-syndrome-related antigen A, also called anti-Ro) and SSB/La,** SSA/Ro is associated with numerous other autoimmune conditions, but are often present in SS. **SSB/La is more specific to SS.**
   - **These tests done by ELISA or by Indirect immunofluorescent technique.**
1. **IgM Rheumatoid factor** RF (because SS frequently occurs secondary to RA)

Other tests:

a- Schirmer test : Measures the production of tears. a strip of filter paper is held inside the lower eyelid for **five minutes,. The amount of moisture is measured. <5 mm in 5 min is positive.**

b- The rose bengal test: measures state and function of the lacrimal glands. using nontoxic dye rose Bengal on the eyes.

c- lip/salivary gland biopsy can reveal lymphocytes clustered around salivary glands, and damage to these glands due to inflammation.

scalogram, a special X-ray to see if any blockage in the salivary **gland ducts**.

Introduction:

- The complement fixation test (CFT) was extensively used in syphilis serology after being introduced by Wasserman in 1909.
- Complement is a protein (globulin) present in normal serum.
- Whole complement system is made up of nine components: C1 to C9
- Complement proteins are heat labile and are destroyed by heating at 56°C for 20 – 30 minutes.
- Complement binds to Ag-Ab complex
- When the Ag is an RBC it causes lysis of RBC's.

Complement Fixation

- When antigen and antibodies of the IgM or the IgG classes are mixed, complement is “fixed” to the antigen-antibody aggregate. If this occurs on the surface of a red blood cell, the complement cascade will be activated and hemolysis will occur.

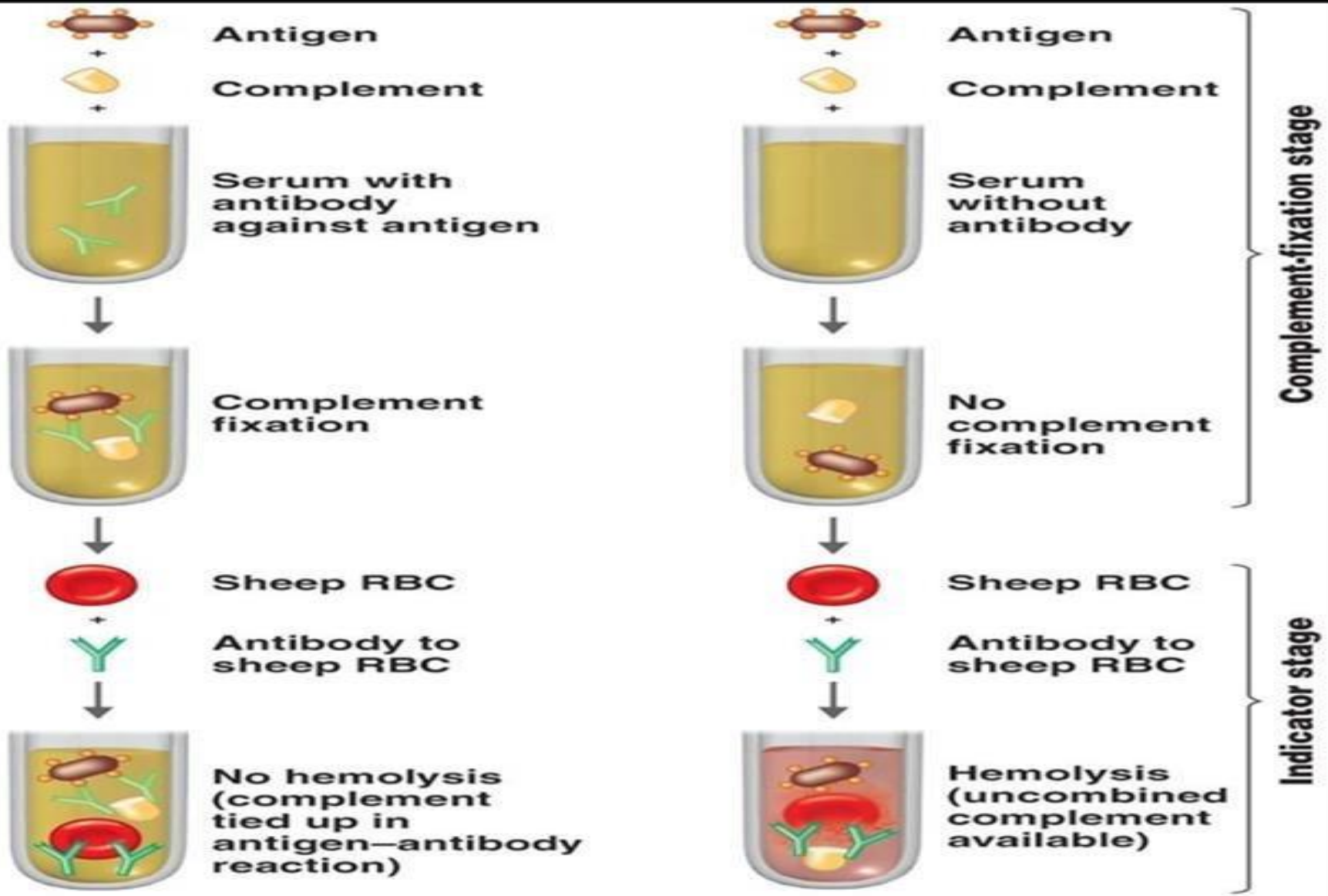
- This method used for detection of specific Ab in patients serum.

The method involves two reactions:
A- indicator system.
B-test system (antigen-antibody complement systems).

- **a. The indicator system consists of red blood cells that have been preincubated with a specific anti-red cell antibody, in concentrations that do not cause agglutination, and in the absence of complement to avoid hemolysis; these are designated as "sensitized" red cells.**

- **b. In the test system, patient's serum is first heated to 56°C to inactivate the native complement and adsorbed with washed sheep RBC to eliminate broadly cross reactive anti-red-cell antibodies (also known as Forssman-type antibodies) which could interfere with the assay.**

- **Then the serum is mixed with purified antigen and with a dilution of fresh guinea pig serum, used as a controlled source of complement. The mixture is incubated for 30 minutes at 37°C to allow any antibody in the patient's serum to form complexes with the antigen and fix complement. "Sensitized" red cells are then added to the mixture.**



(a) Positive test. All available complement is fixed by the antigen–antibody reaction; no hemolysis occurs, so the test is positive for the presence of antibodies.

(b) Negative test. No antigen–antibody reaction occurs. The complement remains, and the red blood cells are lysed in the indicator stage, so the test is negative.

Positive Test

- **Step 1:**

Antigen + Antibody + Complement (from serum) —(At 37°C, 1 Hour)→ Complement gets fixed

- **Step 2:**

Fixed Complement complex + Haemolytic system —(At 37°C, 1 Hour)→ No Haemolysis (Test Positive)

Negative Test

- **Step 1:**

Antigen + Antibody absent + Complement —(At 37°C, 1 Hour)→ Complement not fixed

- **Step 2:**

Free Complement + Haemolytic system —(At 37°C, 1 Hour)→ Haemolysis (Test Negative)

Applications

- **Complement fixation has the advantage of being widely applicable to the detection of antibodies to almost any antigen. Thus, complement fixation reactions have been widely used in a large number of tests designed to assist in the diagnosis of specific infections, such as the Wassermann test for syphilis and tests for antibodies to *Mycoplasma pneumoniae*, *Bordetella pertussis*, many different viruses, and to fungi such as Cryptococcus, Histoplasma, and *Coccidioides immitis*.**

Limitations

- **Complement fixation tests are riddled with technical difficulties and have been progressively replaced by other methods.**