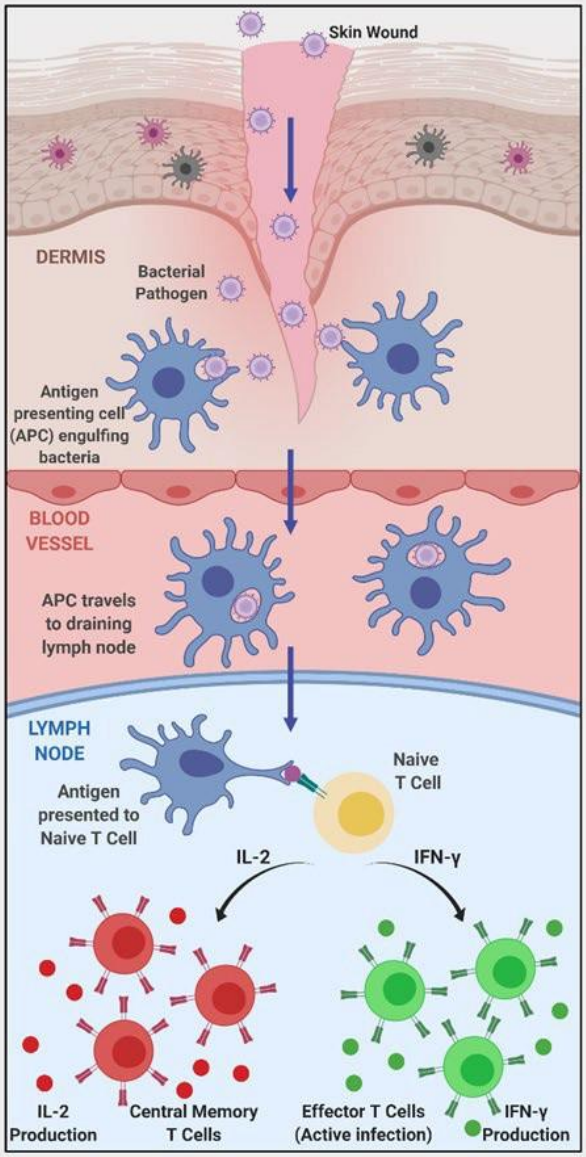
T-CELL SUBSETS IN AN INFECTION & ELISPOT ADVANTAGE



T-cell subsets are generated in the lymph nodes and have varying identities and functions. Maturation of naïve T-cells into antigen experienced subsets is a complex process that depends on the antigen presenting cells, location signals, signal strength, and cellular interactions. There are diverse subsets of antigen specific T-cells such as TH-1 T-cells, TH-2 T-cells, T-regs, and memory T-cells to name just a few[1].

In the presence of an infection the antigen specific TH-1 T-cells are formed early on, followed by the formation of Central memory T- cells in the later stages of the infection[1].

Once a naïve T-cell recognizes an antigen, the so- called cognate antigen, the naïve T-cell matures into an antigen specific T-cell subset. The type of subset is determined by the signals received, the cytokine environment, and any co-stimulatory signals. TH-1 T-cells are known for being active in acute infections and produce Interferon-γ (IFN- γ)[1,2].

When an infection starts to improve and the antigenic burden is reduced, the T-cell may differentiate into a central memory T-cell which produces the cytokine IL-2 upon re-stimulation[2,3].

Figure 1: From skin infection to antigen specific T- cell subsets

ELISPOT TESTING

Elispot is an immunological method to analyze antigen specific T-cells. It was developed in the 1980s and used in research[4].

Since the early 1990s, it has been utilized as a tool to predict viral reactivation prior to liver transplants based on the T-cell subsets identified in the patient's blood. The underlying idea was that if a patient was at a higher risk to develop viral reactivation post-surgery, they would be given antivirals in conjunction with immune suppressants to prevent graft rejection[5,6].

For an ELISPOT, whole blood is required. The peripheral blood mononuclear cells (PBMC's) are isolated and plated on a cell culture dish[7].

The cells are re-stimulated with specific antigens and incubated at 37°C. During this step, everything that is required for a proper antigen presentation to antigen specific T-cells is provided. Antigen presenting cells are amongst the PBMCs and proper culture conditions and factors are provided to ensure antigen presentation in the cell culture dish[7].

Upon re-stimulation, antigen specific TH-1 T-cells will produce Interferon-γ, and Central memory T-cells will produce Interleukin-2. This method enables us to distinguish between the two types of antigen specific T-cell subsets.[2,3].

The released cytokine is stained on the membrane and each spot represents one antigen specific T-cell that responded to their cognate antigen[7].

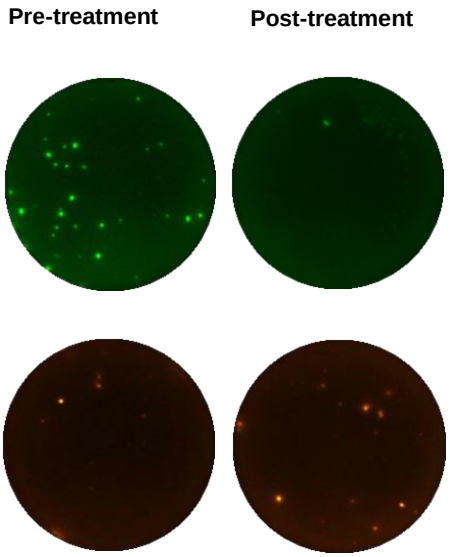


Figure 2: Visualization of Interferon-γ release (green) and Interleukin-2 (amber) release from antigen specific TH-1 and central memory T-cells.

With the ELISPOT system it is possible to enumerate antigen specific TH-1 and Central memory T-cells and based on this, we can track and monitor the immune response changes to a specific pathogen that occurs during an infection.

With these two subsets at hand, it is possible to detect what stage an immune response is at. The T-cell frequency of these two subsets varies based on the antigen exposure in the lymph nodes and represents a function of the antigen load or burden[2,3].

In an acute infection stage, the immune system primarily produces TH-1 T-cells due to the presence of high antigenic load. (Figure 3A). If an infection clears up, either through the immune response or appropriate treatment, the immune response will wind down and skew the T-cell response towards a central memory phenotype (Figure 3B)[2,3].

If the infection develops into a chronic controlled infection, such as a Cytomegalovirus or Epstein-Barr virus infection, the immune system will produce constant TH-1 and central memory T- cells (Figure 3C) [2,3].

3A

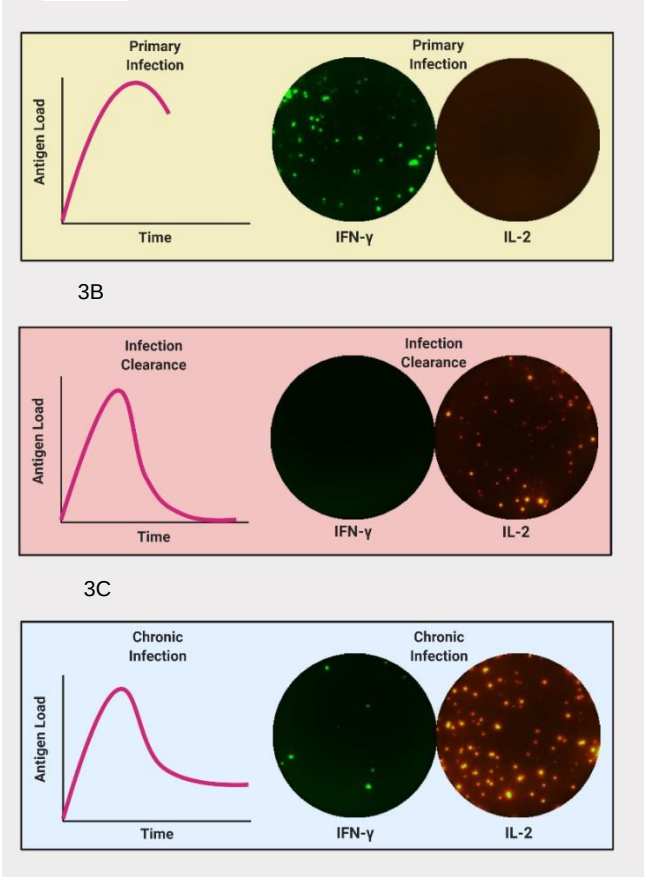


Figure 3: Correlation between antigen load and T-cell frequency of different T-cell subsets.