4.3 Assessing cellular immunity in the lab

Hello. Although it is not an issue strictly of Virology, let's review briefly the complex process by which acquired immune responses are generated when there is a viral infection. T-lymphocytes, activated after recognising viral antigen in the membrane of cells, proliferate and differentiate into two different populations: T-helper lymphocytes or Th, that cooperate with other cells so that immune responses progress, and T-cytotoxic lymphocytes or Tc, that kill the infected cells. As these processes progress, lymphocytes secrete molecules, which are called cytokines, that serve as communication between the cells of the immune system and prompt them to respond or to do their function.

Well, to check if the cellular response to the virus is correct different points can be analyzed such as cell multiplication after antigenic stimulation; the destruction of infected cells, by a test that evaluates the cytotoxicity ability of Tc-lymphocytes; or the secretion of cytokines, for example using the ELISA that we saw in the previous video. In this video we will see how to determine the multiplication of lymphocytes, or lymphoproliferation.

Lymphoproliferation is a technique that measures the proliferation of T-helper lymphocytes in response to viral antigens presented by antigen-presenting cells. This happens only if the patient has been previously exposed to the virus, i.e., if he has recovered from an infection or if he has been vaccinated, always with that same virus.

The first thing is to isolate the lymphocytes. The easiest to get are those from the peripheral blood, collected with anticoagulant. Blood is diluted with culture medium and placed carefully on top of a dense substance called Ficoll. After spinning, erythrocytes and granulocytes penetrate the Ficoll and they settle in the bottom of the tube, but the lymphocytes and some monocytes stay at the interface between the Ficoll and the plasma and culture medium. We collect them carefully and count them under the microscope. We will call these cells PBMCs.

We put 100,000 PBMCs in each well of a 96-well plate and add a virus suspension or viral antigen into each well, and we allow to incubate at 37°C for 4-6 days. During this time, antigen-presenting cells process the virus and present it to T-lymphocytes. If the patient has been previously exposed to the same virus, there will be T-lymphocytes that recognize the virus and proliferate.

To determine how much they have proliferated, we add thymidine, which you know that it is a nucleotide that forms part of the DNA, labeled with tritium, which is radioactive hydrogen, and we incubate 6 more hours. This tritiated thymidine is incorporated into the DNA of the new cells, so when we collect the cells and we measure the amount of radioactivity in a scintillation counter, it indicates the degree of cell multiplication. As always, we must take precautions, such as do each sample in triplicate and add PBMCs of a normal non-infected individual, as well as the PBMCs of the patient without stimulating with the virus, to be used as a control.

The results can be expressed as stimulation index, which is the ratio between the radioactivity measured in the stimulated cultures and the controls without stimulating from the same individual.

The proliferation of lymphocytes may be also assessed by kits that require no radioactivity. They measure the amount of cytoplasmic ATP in metabolically active cells. Therefore, the greater the amount of cells, the higher the levels of ATP. ATP is used in a luminescence reaction that can be measured with a luminometer.

As you can see there are different techniques to detect the cellular immune response after the viral infection. I have only mentioned a few. There are many more, and many others are being developed. Enjoy discovering them. Thank you for your attention.