

4.4 Markers of immune deficiency

Some viruses infect cells of the immune system and destroy them, so they cannot exercise their function of defence. As we have said, the immune response is highly complex, and any of the cells involved can be victim of the virus. In this video we will see how to evaluate the number of T-lymphocytes, but the same principle applies to other cells.

Immune cells have on their surface molecules that identify them, that are called CD (or cluster of differentiation). The function of all of them is not known but we know that they are present always on certain cells or are expressed associated with certain states of cell activation. For example, T-lymphocytes are characterised by CD3, associated to their receptor; T-helper or Th cells are also CD4+ and T cytotoxic CD8+; B-lymphocytes are CD19+ and when activated, they are also CD23+; and all of these are CD45+, which defines them as leukocytes. It seems complex, but you'll see how useful it is to know these molecules.

Determining CD4 and CD8 markers, which define Th and Tc lymphocytes, respectively, although not exclusively, is often used to evaluate the immune status of an individual, especially the relationship or ratio CD4:CD8 cells. This relationship may be affected by viruses that infect cells of the immune system. This is the case of the infection by HIV, or by the virus of the infectious mononucleosis in people; or in cats by the feline immunodeficiency or leukaemia viruses. In these infections the ratio CD4:CD8 is often used to determine the progress of the infection and the success of the antiretroviral therapy. Ratios greater than 2 indicate a normal immune system and below 1, immunodeficiency.

To determine the number of cells that have a certain CD monoclonal antibodies are used. They are very specific and are marked with a fluorochrome. When we want to determine several populations, for example CD4+ and CD8+, two different fluorochromes can be used. As sample we can use whole blood, previously removing red blood cells by lysing them with a specific solution. We add fluorochrome-labelled anti-CD and after a brief incubation in darkness we analyze the cells in the flow cytometer.

The flow cytometer is a very sensitive instrument. It has a flow through system in which cells are analysed individually. Thus, each cell is crossed by a laser beam, activating the fluorescence in cells that have been "tagged" with the specific antibody. The cytometer recognizes fluorescence in the surface of the specific cells, and diverts the cells, collecting them in individual containers. Thus, a heterogeneous population of cells can be purified separating specific subpopulations that have different CDs.

In the case of the T-helper and T-cytotoxic cells, the result is expressed in a graph in which complexity and size are first analysed. The lymphocytes are separated this way. These are small leukocytes with low complexity. In a second stage, they are classified according to the intensity of the fluorescence of their respective fluorochromes. Take a look at the graph on the right. Note that the scale is logarithmic, and the three main populations of cells: the CD4 and CD8 negative; and the positive to one of the two markers.

Don't forget to do the recommended exercises to determine if the patients who we show are immunodeficient or not. Thank you very much for your attention.