ImmunoTools *special* Award 2021

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The modification of cell membrane sphingomyelins affects CXCR4 dynamics and functionality

Fine-tuned migration events are fundamental for assuring a correct immune response. Chemokines, a small protein family, primarily described as chemotactic cytokines, need to correctly bind theirs corresponding chemokine receptors. These receptors are seven transmembrane helical domain molecules that are coupled to protein G promoting cellular migration. Retention of chemokines on cell surfaces or matrices is critical for the formation of chemotactic gradients required for directed cell migration.

CXCL12/CXCR4 is key pair of chemokine/chemokine receptor as they are implicated in processes such as lymphocyte trafficking, adaptative and innate immune response, neutrophil homeostasis, bone marrow maintenance and of tumor progression and metastasis among others.

It is described that in Jurkat T cell line and in primary T cells, CXCR4 forms monomers, dimers or even oligomers in the basal state of the cell. Once CXCR4 senses CXCL12 gradient, it needs to oligomerize, that is to create bigger nanoclusters of receptors. This event is required for signal transduction and for further function. Previous research have shown that abolishing F-actin polymerization or the presence of membrane proteins like CD4 alter this oligomerization.

Plasma membrane is composed by phospholipids, sphingolipids and cholesterol. Signaling platforms are packed regions or liquid ordered areas. CXCR4 is located in membrane rafts enriched in long saturated fatty acyl chain phospholipids, cholesterol and sphingomyelin. Sphingomyelin is a structural sphingolipid contained in the outer leaflet of the plasma membrane, formed by the sphingosine base and a polar group.

Degradation of plasma membrane sphingomyelin by using neutral sphingomyelinase causes an increase in the levels of cellular ceramide. This fact generates conformational changes in the receptor that are observed by FRET assays. In addition, not only a decrease in directed cell migration is observed, but also a loss in the oligomerization process once stimulation. However, no apparent alteration in the main signaling pathways activated by CXCL12 has been found. We do observe an

increase in the fluidity of the membrane, which is related with an increase in the number of unsaturations in the new ceramides formed.

On the other hand, CXCL12 is the unique chemokine that is able to stimulate CXCR4, however this chemokine can also bind ACKR3, an atypical chemokine receptor which is described to interact with CXCR4. Atypical chemokine receptors act as scavengers molecules forming and maintaining chemokine gradients and eliminating chemokines during the resolution of inflammatory responses. Apart from being stimulated by CXCL12, ACKR3 can also bind CXCL11. The study of the dynamics of CXCR4/ACKR3 with either CXCL12, CXCL11 or both chemokines remains unknown. rh I-TAC /CXCL11 will be of great help to start performing preliminary assays.

In order to differentiate T lymphoblasts from peripheral blood cells, we will use human recombinant IL2 and anti-human CD3-APC/CD25-FITC/CD69-PE/ CD4-PE/CD8-FITC for its characterization. In addition to measure possible changes in adhesion molecules we will use anti-human: CD11a-FITC/ CD29FITC and CD18-PE.