ImmunoTools *special* Award 2014

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Trypanosoma cruzi and human Toll-like Receptors

What are the mechanisms of human innate response to *Trypanosoma cruzi*?

Previous works have shown that NK cells and monocytes are involved in the primary response to *T.cruzi*. We are looking for the innate receptors involved in recognition of this parasite and that trigger the immune response. We also compare the innate response of human adult blood and human cord blood.

Currently we are looking at different subsets of peripheral blood mononuclear cells (PBMCs) or cord blood mononuclear cells (CBMCs) to determine the presence (or not) of the TLRs on these cells. At the moment we focalize on TLR7 and TLR9 in particular.

We use flow cytometry to characterize the different populations: CD3 for the T cells, CD14 for the monocytes, CD19 for the B cells, CD16 and CD56 for the NK cells. We also analyse the presence of these TLRs on dentritic cells subsets (myeloid and plasmacytoid) with other panels of antibodies (CD1c, CD303, CD19). The TLRs may be found on the cell surface, or in the endosomes. Then we use two types of protocols to see the TLRs: the first without permeabilization, to see the cells expressing the TLRs extracellularly, and the second with cell permeabilization, to see the intracellular presence of the TLRs.

In addition, we are looking at cells stimulated in vitro with *Trypanosoma cruzi*. Briefly, we incubate for 24 hours PBMCs or CBMCs with *T.cruzi* and cytokines (for example rhIL-15 – that maintain NK cells alive and synergize with *T.cruzi* for NK cells'production of IFN-gamma – or rhIL-2 + rhIL-18). After that we look at the modifications in the expression level of the TLRs in the different cell subsets in flow cytometry.

Of course we want to confirm the flow cytometry results by another technique as immunofluorescence microscopy and Western Blot. We also want to look at the mRNA of these receptors and the possible variation after stimulation by *T.cruzi*.

Furthermore, we want to show the effect of *Trypanosoma cruzi* on the production of cytokines via the activation of these TLRs (7 and 9). To show a hypothetic effect, we block these TLRs with specific inhibitors during the stimulation of the PBMCs/CBMCs with *T.cruzi*. These inhibitors are oligonucleotides synthetize to be competitive with the

natural ligands of TLR7 and 9 (RNA and DNA of pathogens). To see the effect of blocking these TLRs we measure different cytokines by ELISA (IL-6, IL-12, TNF-alpha). These cytokines are mainly produced by monocytes so we next isolated this population of the rest of the cells and measure the same cytokines by ELISA in de PBMCs/CBMCs without monocytes. Then we are able to confirm (or not) if the monocytes play a major role in the innate response at *Trypanosoma cruzi* passing by the TLRs 7 and 9.

The next step of the project will be to look at surface TLRs such as TLR2 and TLR4, more described in their role in *Trypanosoma cruzi* infection. We will follow the same way to describe modifications in these receptors 'expression by *T.cruzi*'s stimulation and cytokines' production after blocking. These receptors will be blocked by specific antibodies that hide the ligation site of the ligands.

The comparison between adult and cord blood is a key point in our study. It has been shown that *T. cruzi* have the capacity to trigger a "type one" immune response in early life, overpassing thus immaturity of neonatal immune system. With the study of TLRs in T.cruzi infection, we hope to be able to show some differences or similarities in cord blood's cells innate response against this parasite that lead probably to this "type one" adaptative response.