ImmunoTools *special* Award 2014

Griselda Moreno, PhD

LISIN- Facultad de Ciencias Exactas- UNLP. La Plata, Argentina

Immune Response elicited by fusion protein between Flagellin from Salmonella and Lumazine Synthetase From Brucella (Bls).

Increasing understanding on microbial biology, pathogenesis and host immunobiology, in combination with development of powerful screening tools and predictive bioinformatics have shifted vaccine development from exclusively empirical approaches towards rational design. Initial vaccines were mainly based on the use of attenuated or inactivated whole microorganisms. A new generation of vaccines based on recombinant components has followed, moving towards chemically defined vaccines. The vaccine optimization ideal vaccine should keep the highest protective capacity eliciting as few as possible adverse effects. Chemically defined vaccines have the best safety profiles, but they have to face the challenge of eliciting protection using just one or a few components of a pathogen. Immunogenicity of single proteins or peptides is much lower than whole microorganism. To override this disadvantage, the selection of the best immunogen(s) for each pathogen as well as combining it with powerful adjuvants becomes crucial.

In recent years, the discovery of several families of innate response receptors has refreshed the adjuvant field, providing a rational background for the selection of compounds with adjuvant properties. Although at present just a few adjuvants are licensed to be used in human vaccines, it is expected that new generation of adjuvants will have an impact in vaccine development.

The improved knowledge on host immunobiology is also shaping immunization strategies. Among this advances, there is a rising awareness that using the mucosal route for immunization allows the achievement of a better protective response in mucosal surfaces. Since there is a large amount of pathogens that have evolved to use the mucosa as the entry site of infection, a great interest is placed in developing strategies to improve mucosal responses. One aspect that is shared by different mucosal sites is the present of important commensal populations that interplay with host immune system. Coexistence of local mucosal microbiota in close proximity with immune effectors is achieved by strategic distribution of innate receptors and immune cell populations and the establishment of tolerance/regulatory circuits that modulate mucosal immune responses.

Along the intestine, continuous sampling of lumenal content for immunosurveillance takes place. Intestinal dendritic cell (DC) populations are critical in this aspect. Most intestinal DCs are poor responders to different innate activators, like toll like receptor (TLRs) ligands. One exception seems to be TLR5, that is functionally expressed in the major intestinal DC subsets. Flagellin is the ligand of TLR5 and is one of the few protein agonist of innate response, being also recognised by intracytosolic innate receptors of the NOD-like receptor (NLR) family. The widespread distribution of TLR5 along mucosal surfaces, especially among mucosal DCs and the possibility of using protein engineering tools for combining its capacity to enhance immune response with candidate immunogens makes flagellin an interesting candidate for mucosal vaccine design.

Brucella spp. lumazine sintetase (BLS) is a bacterial protein that is highly immunogenic and has a compact decameric structure. It has been shown that fusion of peptides to the N-terminus does not affect oligomerization of BLS allowing its use as carrier for vaccinal antigens. Strong immunogenicity of BLS is due, at least in part, to its capacity to trigger TLR4 signalling. It has been shown that the immunization with BLS-peptide fusion is able to induce cytotoxic T cell response in a TLR4 dependent way. The capacity to function as vaccine carrier achieving protection has been tested in different systems, such as rotavirus, brucella, Taenia solium and influenza infection models. It has been shown to induce protective responses delivered by systemic or oral routes.

The rationale of this Project is that flagelin oligomerization on this protein scaffold would generate a new vaccine platform with enhanced capacity to activate mucosal immune response.

Actually, we are studying the TLR5 dependent activity of BLS-peptide using in vivo assays on C3H/HeJ mice. We used the capacity to recruit cells to BAL or peritoneal cavity after i.n. or i.p. delivery of the protein. In both assay formats we could evidence the capacity of recruit cells (mainly neutrophils), presumably due to TLR5 triggering.

For that we want to extend the characterization of recruited cell phenotype by analyzing more surface markers such as $CD11b^+$, $CD11c^+$, $GR1^+$, $Ly6G^+$.