ImmunoTools IT-Box-Cy55M-Award 2013

Maria Trovato

PhD Supervisor: Dr. Piergiuseppe De Berardinis

Institute of Protein Biochemistry,
CNR, Via P. Castellino 111, 80131, Naples

E2 scaffold as HIV-1 candidate vaccine

In our laboratory we have been exploring the advantages of an antigen display system that is based on the E2 protein of the pyruvate dehydrogenase complex from *Bacillus stearothermophilus*. The E2 monomer self assembles into trimers which aggregate to generate a 60 chain scaffold with icosahedral symmetry capable of displaying up to 60 copies of an antigen. We have recently demonstrated that co-immunization with HIV-1 Envelope gp160 plasmid DNA and E2 scaffolds displaying the V3 loop of gp120 was able to rapidly generate autologous neutralizing antibodies (NAbs) in rabbits and V3-specific $CD8^+$ T cells producing IFN-◦in mice (1). One strategy for HIV-vaccine design is to target conserved regions of Envelope know to induce broad NAbs, such as the membrane proximal external region (MPER) of gp41. To focus the immune response on this region we have generated multimeric E2 scaffolds displaying the MPER region. It is widely believed that for an effective HIV-vaccine immunogen should stimulate both $CD8^+$ T cells for viremia controlling and $CD4^+$ T cells for sustaining neutralizing antibody response. To investigate the $CD4^+$ T cell response and the cytokine polarization induced by MPER-E2 particles, purified $CD4^+$ splenocytes from immunize mice need to be activated in presence of rm IL-2 and antigen-presenting cells, derived from bone marrow in medium supplemented with rm GM-CSF, loaded with MPER-E2 antigen. The Th1, Th2, Th17 phenotypes of antigen-activated $CD4^+$ T cells can be further defined with intracellular cytokine detection by flow cytometry using antibodies specific for the cytokines of interest. Additional murine recombinant cytokines such as rm IL-2, rm IFNgamma, rm IL4, rm IL-6, rm IL-21, are necessary to derive $CD4^+$ T cells producing Th1, Th2, Th17-type cytokines, that serve as positive controls for the staining with cytokine antibodies in the intracellular cytokine assay. Furthermore cytokine levels can be measured on the supernatants from stimulated $CD4^+$ T cells by using ELISA assay.

1. Jaworski JP, Krebs SJ, Trovato M, Kovarik DN, Brower Z, Sutton WF, Waagmeester G, Sartorius R, D'Apice L, Caivano A, Doria-Rose NA, Malherbe D, Montefiori DC, Barnett S, De Berardinis P, Haigwood NL. *Co-immunization with multimeric scaffolds and DNA rapidly induces potent autologous HIV-1 neutralizing antibodies and CD8+ T cells*. PLoS One. 2012;7(2):e31464.

ImmunoTools IT-Box-Cy55M for **Maria Trovato**
includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9,

rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1α / CXCL12a, rm SDF-1β / CXCL12b, rm TNFα, rm TPO, rm VEGF DETAILS