ImmunoTools *special* Award 2025

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Accelerated co-cultured dendritic cell (acDC)-based assays:

Optimization for antigen discovery and application to CD4$^+$ T-cell epitope identification in Yersinia spp

Yersinia spp bacteria are significant pathogens that cause severe enteric infections, particularly in infants and young children. As part of their pathogenetic strategies, the Yersinia have evolved numerous ways to invade host tissues, gain essential nutrients, and evade host immunity. Our earlier studies collectively highlight the sophisticated mechanisms employed by various Yersinia species to evade the host immune system, particularly through interactions with complement regulatory proteins. Yersinia pestis, Y. pseudotuberculosis, and Y. enterocolitica utilize outer membrane proteins, notably Ail (Attachment invasion locus), to recruit complement regulators such as C4b-binding protein (C4BP) and factor H [1-6]. These interactions lead to the inactivation of complement components like C4b, effectively protecting the bacteria from complement-mediated killing[1]. The ability to bind these host

regulatory proteins is not unique to Yersinia, as evidenced by similar mechanisms in Salmonella, suggesting a conserved strategy among pathogenic bacteria for immune evasion.

The detailed characterization of these interactions, including functional mapping of protein binding sites, underscores the complexity and importance of these mechanisms in bacterial pathogenesis and survival within the host. In addition to the immune evasion of these bacteria, they increasingly exhibit resistance to antibiotics[7, 8]. Antimicrobial resistance (AMR) in Yersinia species, particularly Yersinia pestis, Yersinia enterocolitica and Yersinia pseudotuberculosis, poses a significant public health threat. Resistance can arise from intrinsic mechanisms or through horizontal gene transfer via plasmids, leading to multidrug-resistant strains [9, 10]. Y. pestis has shown alarming resistance patterns, especially to antibiotics like streptomycin and doxycycline, while Y. enterocolitica and Y. pseudotuberculosis exhibit resistance to tetracyclines and sulfonamides. Accordingly, the development and provision of an effective vaccine against Y. pestis is really on demand.

Recent research has shown promising results in vaccine development. The anti-yersinial vaccines examined include Killed Whole-Cell Vaccines (KWCVs), Live Whole-Cell Vaccines (LWCVs), subunit and peptide vaccines [11, 12].

In our previous publication, we used a new method that is called accelerated co-cultured dendritic cell (acDC), (Figure 1)[13]. This method could be a fascinating means to present antigen to T cells. It has several advantages compared to conventional methods. It reduces the differentiation time of DCs from 7 days to 2–3 days. Moreover, purification steps were not required, and the volume of blood needed for acDC induction was much lower than in previous methods. In this method, lymphocytes are in direct contact with differentiating DCs and the antigens are loaded on DCs in real-time [14].

In this project, we will evaluate the immunostimulatory potential of Yersinia-derived peptides. These peptides have been designed using immunoinformatics tools, which employ computational methods to predict potentially effective immune system targets. We will assess these peptides within the framework of the acDC methodology. Specifically, our aims are to:

1. Use the acDC approach, which involves preparing dendritic cells from monocytes that exist in the PBMCs
2. Determine the effectiveness of these peptides in triggering T cell responses when presented by dendritic cells

Materials and methods:

1. Peptide design:

First, we will select Yersinia proteins that are sufficiently immunogenic, based on literature. Next, we will design immunodominant peptides using immunoinformatics tools. These peptides will be restricted to the most prevalent HLA-DR alleles.

2. **acDC stimulation on human PBMCs:**

PBMCs will be isolated from infected patients using Ficoll and on day 0, PBMCs will plated (10^6 /100) in 96-well flat-bottomed plates in AIM-V medium supplemented with 1000 U/mL of GM-CSF, 500 U/mL of IL-4 (ImmunoTools, Germany). After 24 hours (day 1), maturation stimuli will be added, comprising the following reagents; 100 µl of fresh media supplemented with maturation-inducing factors including tumor necrosis factor (TNF)-α, IL-1β, and IL-7 (all from ImmunoTools, Germany), 1 µM prostaglandin (PG)E_2 and peptides will be added to each well. On day 2 (48 hours after start of culture), non-adherent cells will be collected, washed, and analyzed.

3. **DC characterization:**

Adherent cells will be collected to evaluate the phenotypes of acDCs using monoclonal antibodies (mAbs) specific for HLA-DR (PerCP), CD80 (APC), CD86 (APC), and CD11c (PE) (all from ImmunoTools, Germany). For linage negative we will use CD14, CD19, CD16, and CD3 (all FITC labeled, all from ImmunoTools, Germany).

4. **Checking the specific response against the peptides using CD137, CD154 staining and IFN-gamma secretion**

To evaluate the specific response against the peptides, we will perform intracellular staining for IFN-γ in $CD4^+$ T cells. Briefly, peripheral blood mononuclear cells (PBMCs) will be recovered from 48-hour acDC cultures. The cells will first be stained for the surface marker CD4 (APC) and CD8 (PerCP) (ImmunoTools, Germany), followed by fixation and permeabilization. Golgi Stop will then be added to block cytokine secretion, and the cells will subsequently be stained for intracellular IFN-γ (PE) (ImmunoTools, Germany). In addition to IFN-γ, the expression of CD154 and CD137 will be assessed on $CD4^+$ T cells to evaluate antigen specificity. These three markers are used to evaluate the specificity of T cells against the antigen of interest [15].

5. **Checking the frequency of Trege cells in the acDc setting:**

Non-adherent cells will be collected to evaluate the phenotypes of T reg using monoclonal antibodies (mAbs) specific for CD4 (APC), CD25 (PE), and CD127 (FITC) (all from ImmunoTools, Germany).

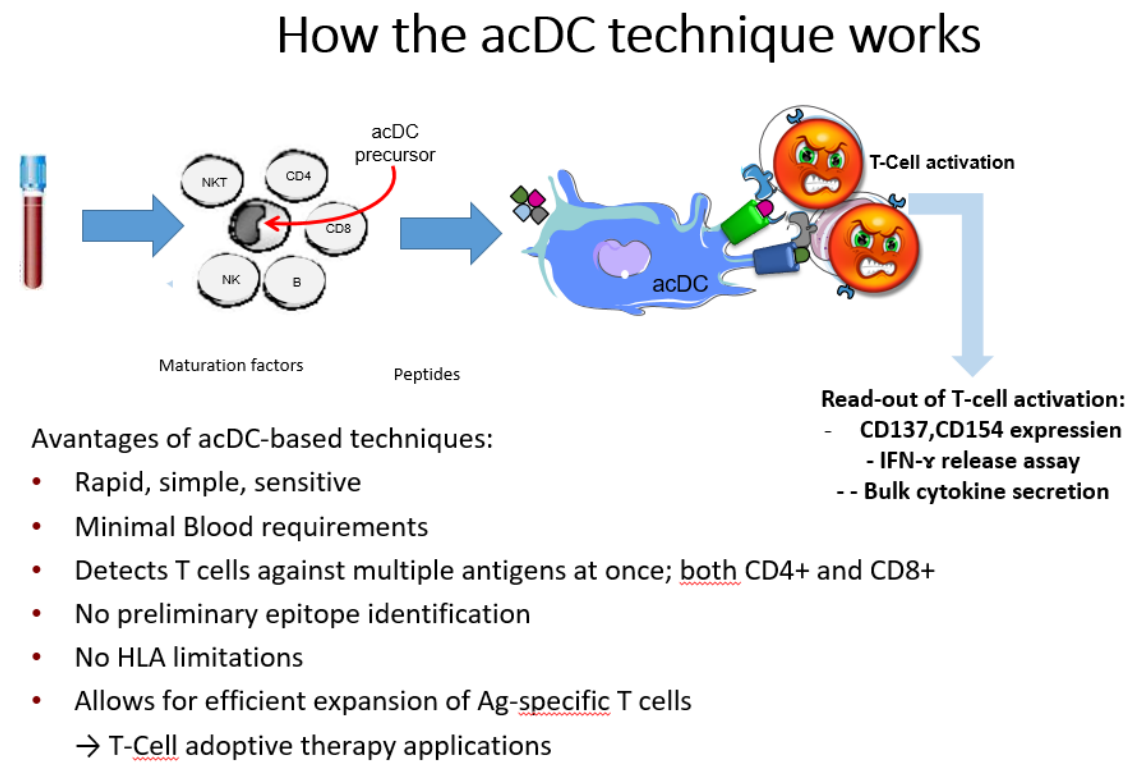

Figure1: Preparing of acDC and read-out of T-cell activation

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