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Imprint of mechanical forces on antibody affinity maturation in B cell immune responses

Lead supervisor: Pierre Milpied, PhD, Centre d'Immunologie de Marseille-Luminy (CIML)
Co-supervisor: Philippe Robert, MD, PhD, Laboratoire Adhésion et Inflammation (LAI)

Abstract: Antibodies are lifesaving biomolecules – major effectors of vaccines efficacy and powerful therapeutics for cancer treatment. In natural immunity, highly potent antibodies are produced by the descendants of a single antigen-specific B cell after complex processes of cellular and molecular maturation. Affinity maturation of antigen-specific B cells, in which the affinity of the antibodies increases from the µM to the nM range, involves iterative cycles of (i) *de novo* mutations in the antibody coding genes followed by (ii) selection of the B cells expressing mutated antibody proteins with competitive antigen binding, in a process reminiscent of Darwinian evolution. Despite some situations where antibody function may occur in solution (e.g. blocking), most antibody functions occur at cell-cell interfaces. In particular, the selection of antibody-expressing B cells during affinity maturation requires the uptake of membrane antibody-bound antigen from lymph node stromal cells. At 2D cell-cell interfaces, antibody binding properties are under the influence of disruptive mechanical forces exerted by cells, and 3D characterization of antibody binding is no longer relevant. Yet it is currently unknown whether affinity maturation specifically improves antibodies binding under force, or which mechanical properties of antibodies may be selected in immune responses *in vivo*. Here we propose to measure *in vitro* binding properties of antibodies at various stages of *in vivo* affinity maturation, using state-of-the-art biophysical methods adapted to characterize antibody-antigen bonds in 2D and in response to mechanical force. Our project will solve whether mechanical constraints on antibodies exert selective pressure during B cell affinity maturation in immune responses.

Keywords: antibody, affinity maturation, B lymphocyte, force, single bond

Objectives: The study aims to explore the selection mechanisms driving affinity maturation in B lymphocytes during *in vivo* immune responses. We hypothesize that antibodies are selected on the basis of the response of their antigen binding capacity under 2D mechanical force, rather than on their affinity in solution. In our project, we will: (objective 1) characterize antibody-antigen bonds at the single-molecule level in 2D in response to mechanical force for large collections of antibodies derived from B cells at various stages of affinity maturation *in vivo*; (objective 2) define how maturation-induced amino acid substitutions in antibody heavy and light chains impact on mechanical properties of antibody-antigen bonds; (objective 3) investigate the impact on *in vivo* B cell differentiation of amino acid substitutions which modify the mechanical properties of antibody-antigen bonds.

Proposed approach (experimental / theoretical / computational)

In mice intentionally vaccinated with the model antigen chicken ovalbumin (OVA), we will sort antigen-specific B cells undergoing affinity maturation for parallel analysis of transcriptome and antibody genes sequences at single-cell resolution. We will focus on B cell lineages (cells derived from a single antigen-specific founder B cell) to produce recombinant antibodies derived from B cells at various stages of affinity maturation.

For several B cell lineages, a dozen of maturation steps in each lineage will be selected, and antibody-antigen binding properties will be analyzed for the corresponding recombinant antibodies. Binding properties will be measured at the single molecule level in 2D and under forces between 5 to 150 pN, *i.e.* in conditions relevant for an interaction occurring at cell-cell interface when a cell pulls on a receptor.

Relationships between B cell differentiation, maturation stage, antibody structural features and binding properties for each lineage of antibodies will ultimately be formalized.

Interdisciplinarity

The project gathers two CenTuri teams with complimentary expertise.

The team of Pierre Milpied at CIML specializes in the biology of B cell responses using single-cell genomics tools, and has the know-how to select maturing B cells, produce their antibodies, and characterize them genetically and structurally. The team also has experience in quantitative analysis of B cell differentiation dynamics from single-cell gene expression data.

The team of Philippe Robert at LAI specializes in the development of the laminar flow chamber for measurement of binding properties of TCR to pMHC and antibodies to antigens. The high level of automation of the method allows measurement of large collections of ligand-receptors interactions. LAI was a pionner in use of the method for force measurements but also in the formalization of binding interactions between proteins, providing theoretical ground to describe the physical properties of the bonds.

Expected profile

Tasks will consist in producing antibodies, preparing reactive surfaces for flow chamber experiments, performing flow chamber experiments, extracting and compiling binding data through dedicated software and discussing results.

The candidate must either be a cellular biologist or immunologist with a strong interest in biophysics, or a physicist with a strong interest in biology and immunology.