PROJECT 2

DoS: Matteo Iannacone

Title: In vivo dynamics of antiviral T helper cell responses

Curriculum: BAIO

Link to OSR/UniSR personal page: http://www.hsr.it/research/organization/divisions-centers/division-of-immunology-transplantation-and-infectious-diseases/matteo-iannacone/

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Background. Antibodies are critical for virus control and prevention of re-infection. Their production depends on B cells encountering viral antigens in lymph nodes draining infection sites, getting activated, interacting with different cells, proliferating and differentiating into antibody-secreting cells. Each of these events occur in distinct lymph node sub-compartments, requiring the migration of B cells from niche to niche in a fast and tightly coordinated fashion. Thanks to the recent advent of multiphoton intravital microscopy, several cellular and molecular events by which lymph nodes orchestrate the generation of humoral immune responses to inert, particulate antigens have been clarified. However, how viral infections affect the spatiotemporal dynamics of B cell activation is less well understood. Also, the mechanisms whereby some viruses (e.g. polioviruses in humans and vesicular stomatitis virus [VSV] in mice) induce early, high affinity neutralizing antibody responses whereas other viruses (e.g. hepatitis B and C viruses in humans and lymphocytic choriomeningitis virus [LCMV] in mice) fail to do so remain poorly understood.

Aims. The aims of this project are: 1) To elucidate how neutralizing antibody responses against live lymph-borne viruses are generated in vivo within lymph nodes; and 2) To identify mechanisms whereby certain viruses interfere with the generation of an efficient antiviral humoral response. To this end we will make use of state-of-the-art imaging technology (i.e. multiphoton intravital microscopy), fluorescent replication-competent viruses and dedicated mouse models. Imaging will be complemented by more traditional molecular, cellular and histological approaches (measuring viral titers and the number/function of a variety of Ag-specific and Ag-nonspecific immune cells at time of autopsy), thus characterizing the various steps leading to antibody formation at the molecular-, single cell- and whole animal-level.

Specifically, in *Aim 1* we will systemically examine the steps elicited by VSV to induce humoral immunity in lymph nodes. Using transgenic mice expressing a VSV-specific BCR (VI10Yen) or TCR (tg7) and several replication-competent recombinant fluorescent vesicular stomatitis viruses, we will analyze four sequential steps: 1. Initial B cell antigen encounter; 2. Virus-specific CD4 T cell priming; 3. Follicolar T helper (T_{FH}):B cell interactions; and 4. Germinal center (GC) formation. Together, these experiments will provide the first complete *in vivo* imaging survey of virus-specific B cell activation from the first minutes of viral entry to the eventual generation of high affinity Ab-secreting cells. In *Aim 2* we will investigate how LCMV prevents the generation of an early nAb response. Using transgenic mice expressing a LCMV-specific BCR (KL25) or TCR (SMARTA), replication-competent recombinant fluorescent lymphocytic choriomeningitis viruses, and comparing results with those emerging from Aim 1, we will explore the following non-mutually exclusive reasons underling the failure to efficiently induce nAbs: 1. Antigen handling and initial B cell encounter; 2. Impaired affinity maturation; 3. Induction of abnormal T helper (T_H)-cell function resulting in polyclonal B-cell activation; and 4. The consequence of intranodal immunopathological responses on Ag presentation and B cell activation. Together, these

experiments will identify virus-induced mechanisms interfering with nAb responses. This new knowledge may lead to novel rational vaccine strategies aimed at inducing rapid and long-lived humoral immune responses.

Skills to be acquired by the student: Besides becoming proficient in all the techniques required by the research project, the successful student should develop project management and organization skills, learn how to design and interpret experiments, learn how to set priorities, develop excellent writing and oral communication skills, as well as leadership, networking and interpersonal skills.

References (max. 3)

Sammicheli S*, Kuka M*, Di Lucia P, Jimenez de Oya N, De Giovanni M, Fioravanti J, Cristofani C, Maganuco CG, Fallet B, Ganzer L, Sironi L, Mainetti M, Ostuni R, Larimore K, Greenberg PD, de la Torre JC, Guidotti LG, Iannacone M (2016) Inflammatory monocytes hinder antiviral B cell responses. ***Science Immunology**, 1:eaah6789* (*co-first authors)

Medaglia C*, Giladi A*, Stoler Barak L*, De Giovanni M*, Meir Salame T, Biram A, David E, Shulman Z[#], Amit I[#], Iannacone M[#] (2017) Spatial reconstruction of immune niches by combining photoactivatable fluorescent reporters and single-cell RNA-seq. ***Science**, 358:1622* (*co-first authors; [#]co-last and corresponding authors)

Kuka M and Iannacone M (2018) Viral subversion of B cell responses within secondary lymphoid organs. ***Nature Reviews Immunology**, doi:10.1038/nri.2017.133*