ImmunoTools *special* Award 2015

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Re-evaluating the $CD4^+$ T-response to influenza virus (H1N1 and H3N1), at baseline and post-vaccination: assessing the role of HLA-DRB3/4/5 (DR52, DR53 and DR51) in the immune responses.

Infection by influenza virus infection considered a major worldwide public health problem. Seasonal infections with the most common influenza virus strains can usually be resolved, but they still cause a high rate of morbidity and mortality; in 2013, 3,697 deaths and 1.2 deaths per 100,000 population in USA (*http://www.cdc.gov/nchs/fastats/flu.htm*). Factors that influence the outcome of the infection remain unclear and annual variants make compulsory the yearly vaccination. Although immune response can cross-protect from these variants, there is not good tools to evaluate these cellular immune response and select patients who can be immunologically improved.

HLA is a key molecule that defines T-cell immune responses, being HLA class II (DR, DQ and DP) heterodimeric (alpha and beta chains define each HLA-II) presenting molecules responsible of $CD4^+$ helper T-cell responses. The high polymorphism of these molecules (i.e. 1800 DR alleles, mainly defining by ß-chain) that combines thousands of peptides makes very difficult to define specific reagents and methods for evaluate these immune responses.

HLA-DRB1 gene encoding the β chain and is expressed in all individuals; in contrast, "secondary loci", HLA-DRB3 /4/5 loci are present in some individuals giving a second product HLA-DR.

Thus, for each given HLA-DRB1, only a secondary gene DRB3/4/5 may be present. DRB3/4/5 loci are in linkage disequilibrium with the main DRB1, defining the DRB haplotype: DR51, DR52 and DR53 (*Gongora et al., 1996*). The DR51 haplotype defined by the locus DRB1/DRB5 and comprises haplogroup: DRB1*15 and DRB1*16, haplotype DR52 (DRB1/DRB3) belongs haplogroup DRB1*03, DRB1*11, DRB1*12, DRB1*13, DRB1*14 and DR53 (DRB1/DRB4) corresponds haplogroup DRB1*04, DRB1*07 and DRB1 * 09.

Although DRB3 is the most polymorphic in sequence (59 coding variants) of "secondary" gene locus, only 3 alleles show a significant population frequency: DRB3*01:01, *02:02 and *03:01. On the other hand, DRB4 and DRB5 have only one allele with a high significant population frequency, DRB4*01:01 and DRB5*01:01. If we keep in mind the 2 main variants DRB3 together (43% allelic frequency in Caucasians) we can ensure that at least 67.5% of the population expressed DRB3*01:01 or *02:02. Related with DRB4, only one allele, DRB4*01:01 is mainly present in association with HLA-DRB1*04, HLA-DRB1*07 and DRB1*09. Similar situation occurs with DRB5, DRB5*01:01 is the most frequently allele associated with DRB1*15 and DRB1*16.

It is generally accepted that the expression of DRB3 as of DRB4 and DRB5 is significantly lower than DRB1 expression but, it is clear is that DRB3, DRB4 and DRB5 are able to present peptides to $CD4^+$ T cells; several peptides have been defined in relation to this loci from **virus antigens influenza** to NY-ESO-1, HPA-1a, tetanus toxoid. Although in some cases it has been suggested that the presentation could be similar (redundant) between DRB1 and these "secondary loci", some works *Faner R et al 2010, Bioley G et al 2004, Sukati et al 2005* indicate that there is complementarity primarily in presenting these loci on the haplotype.

Our main objective is to improve the knowledge of flu physiopathology by providing tools to detect and better define CD4+ T-cells against influenza antigens, defining epitopes that can be used to determine immune protection beyond annual changes of viral antigenicity. We will use TGEM to determine peptides presented by main "secondary" HLA class II (HLA-II) alleles (HLA- DRB3*01:01, DRB3*02:02, DRB3*03:01, DRB4*01:01 and DRB5*01:01) by defining the major reactive epitopes for each allele and to use and quantify them to measure specific $CD4^+$ T-cell frequency in patients before and after immunization with influenza vaccine. Characterization of these specific T-cells by defining Th1/Th2/Th17 balance should help better understanding flu physiopathology.