POSTER 18: Functional impact ofWaldenstriim's Macroglobulinemia SPil mutation Q226E on B lymphopoiesis

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B cell malignancies essentially result from somatic mutations appearing during the germinal center reaction (GC). The GC reaction allows the selection of B cells expressing high affinity BCR which then differentiate into plasma cells or memory B cells. During this reaction the activated B cells acquire somatic mutation in immunoglobulin genes and undergo important chromatin remodeling. Waldenstrom's Macroglobulinemia (WM) is defined as an IgM-secreting and bone marrow-infiltrating lymphoplasmacytic lymphoma, derived from post-GC compartment, and presumed to be related to memory B-cells. We identified a Q to E mutation in the DNA binding domain of the SPII gene (a transcription factor of the class III of the ETS family) in 6% of WM. The mutation changes the DNA binding of the mutant protein from classical SPil to ETS 1-like sequences, shifting the balance from enhancer binding to promoter regions. Increased binding by mutant SP11 at promoters activates gene expression of intracellular signaling pathways typically promoted by other ETS factor family members. Additional analyzes are required to understand the consequences of the SPil mutation on B cell maturation, and more specifically on GC reaction and cellular fate.

To do so, we developed a conditional knock in allele allowing to express the mutant Spi1. Then we analyzed the adaptive immune response to sheep red blood cells and NP-COG. Spi1QE mice are able to mount T-dependent immunization 12 days after immunization but with higher % of activated B cells (Gir) and plasma cells (CDl38+) compared to wild-type mice. Regarding specificity, we observed a 25% decrease in the percentage of antigen-specific plasma cells (NP +, CDl38+). In vitro stimulation experiments of follicular (Fo) and marginal zone (MZ) B cells, with CD40, IL4 and with LPS, ILS, IL2 respectively showed a faster and stronger differentiation into plasma cells, assessed by the expression of the CDl38 marker, in Spi1-QE cells compared to wild type cells. Transcription profiling confirmed these observations: indeed, the analysis ofa first series ofRNA-seq comparing LPS, IL5, IL2 stimulation of Spi1QE and wild type MZs confirms this observation.

We have shown an abnormality of plasma cell differentiation in vitro and in vivo, which is characterized by an increase in plasma cell differentiation, associated with a defect in the specificity and the maturation of the immune response. We now have to specify the phenotype and identify the mechanisms at its origin.