Cigarette Smoke: Study of Immune system dysregulation in the ProgeNia cohort.

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Introduction
Cigarette smoking is a significant health problem that is thought to cause the death of more than 8 million people a year worldwide, mainly triggering different types of cancer, heart and respiratory disease, and stroke. More than 7 million of those deaths result from direct tobacco use, while around 1.2 million results from non-smokers exposed to second-hand smoke (1).

It has been shown that cigarette smoking has some effects on the immune system, like leukocytosis and immune cell function impairment (2,3), but many aspects of its effects on the immune system and diseases, such as autoimmune maladies, remain unclear. To fill this knowledge gap, we systematically analyzed the effects of smoking on the immune system using a large cohort of ~6,000 individuals of the general population deeply characterized for hundreds of immune variables and providing precise information about the smoking status over life.

Methods
In the peripheral blood of 6,000 volunteers of the ProgeNIA cohort, we quantified the levels of 731 immunophenotypes by flow cytometry and 12 related serum proteins by ELISA, as well as PCR and VES (4).

Furthermore, in 47 heavily smokers vs. 46 never smokers, we profiled the transcriptome of Treg secreting, Treg activated, and IgD-CD38 B cells by RNAseq. DESeq2 software was used to identify differentially expressed genes (DEGs), then g:profiler, GSEA and Enrichment map software were used to perform pathways enrichment analysis.

In the same individuals, we evaluated whether CS impaired Treg function by performing Treg suppression assays.

Results
CS causes a general increase in the main leukocyte subsets. The most significant rise was observed in the late memory B cell subset defined as IgD-CD38- (p-value=2.12x10^-86) and in the memory subsets of regulatory T cells (secreting and activated) (p-value=2.71x10^-72).

All serum protein levels were found dysregulated. In particular, the primary B cell activation factor BAFF serum protein level was increased in smokers (p-value=1.55x10^-14) and, despite the overall increase of B-cells (p-value=9.07x10^-29) including plasma cells (p-value=7.27x10^-35), we found that serum protein levels of immunoglobulins were reduced.

RNAseq has, for the first time, profiled the three cell-types mostly related to smoking. Overall, we found 3,850 differentially expressed genes between heavy smokers and never smokers (3,406 in IgD-CD38-, 424 in Treg activated, and 348 in Treg secreting). In both Treg activated and secreting, pathways enrichment analysis indicated a decrease of suppressive activity subsequently confirmed by functional assays.

By combining public genome Wide Association Studies (GWAS) and expression quantitative trait loci (eQTLs), we found that differentially expressed genes were enriched in autoimmune diseases and smoking-related traits.
Conclusions
The comprehensive dissection of circulating immune cells, combined with the analysis of transcriptional profiling and functional assays on specific cell subsets, will provide new insights about how cigarette smoking could cause immune dysfunction and health perturbations.

References:
3) Sopori et al., Immunotoxicology and Immunopharmacology 1994.

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