Differentially Methylation Positions After IL-6 in the Endothelium

Ramon Bossardi Ramos, Nina Martino, Alejandro Adam

Introduction: Interleukin 6 (IL-6) is a major mediator of the septic cytokine storm, leading to multiorgan dysfunction. While some direct actions of this cytokine in acute inflammation are being elucidated, little is known about long-term effects that may lead to post-intensive care syndrome (PICS). IL-6 activates JAK kinases to phosphorylate the transcription factor STAT3. We hypothesize that STAT3-mediated endotheliopathy promotes changes in DNA methylation leading to long-lasting effects. Here, we sought to determine if the IL-6/STAT3 signaling axis altered the DNA methylation in the endothelium. **Methods:** DNA methylation was profiled in HUVEC and enrichment endothelial cells from kidneys in mice treated with LPS. To induce IL-6 signaling, cells were treated with a combination of IL-6 and sIL-6Rα (IL-6+R) for 6h and 72h. DNA was isolated and was bisulfite converted and applied to the Infinium Methylation EPIC arrays. Raw methylation data were analyzed using the ChAMP package in R, with the support of several auxiliary scripts. Gene expression was measured by RT-qPCR. **Results:** Our data suggest that an IL-6+R treatment resulted in significant modifications to the human methylome in endothelial cells. Unbiased clustering showed a separation between treated and control cells after 72h, with no significant changes after 6h. 360 CpG sites were significantly modified (FDR-adjusted p=<0.05), where 329 CpG sites were hypomethylated versus 31 CpG sites that were hypermethylated. This result shows that the time after exposure to IL6 is critical for the alteration of DNA methylation. Gene ontology analysis of the differentially methylated genes showed enrichment of pathways directly linked with the platelet and leukocyte interaction, cell proliferation and angiogenesis, and signaling. The annotated genes with a difference with hypomethylation between groups greater than 30% included 46 Differentially methylation positions (DMP). Among the 46 DMPs, 26 mapped to known genes, and 20 were located in intergenic regions. The annotated genes included genes involved in response to IFNα and inflammatory response (LAMP3), TNFα signaling via NFKβ (ABCA1 and RCAN1), regulation of cell division (MACC1) and TGFβ signaling (RAB31). 7 DMR with greater than 15% increase in methylation mapped to annotated genes associated with immune response (MYO10, ADAM19).

Consistent with the demethylation of LAMP3 locus, IL-6+R induced 72h post-treatment a dramatic increase in LAMP3 expression. The same treatment induced an inhibition of ADAM19 and MYO10 gene expression, consistent with their hypermethylation status. Mice data showed DMP between LPS and control. **Conclusions:** Persistent activation of IL-6 leads to endothelial DNA methylation changes and corresponding gene expression differences. In mice, we're increasing the sample size. These differences may regulate pathways that can be associated with reduced long-term survival and prolonged organ damage as observed in PICS.