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The influence of danger and resolution signals on innate immune memory in human primary monocytes and in M1 or M2 polarized macrophages

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Immunological memory was traditionally thought to be developed in adaptive system, now it has been put into a much broader context which also includes the innate immune system. Early studies reached the conclusion that exposure of monocytes to LPS alters the ability of these cells to interact with, and consequently respond to, LPS. Also in vivo LPS tolerance was noticed and defined as the transient period after initial LPS exposure during which a normally responsive individual is rendered hypo-responsive. Hence, the first question we were addressed is to 1) test the hypothesis that different danger signals can initiate innate immune memory in monocytes and polarized macrophages; and 2) do monocytes and polarized macrophages differ with respect to their response to secondary LPS stimulations? It has been known that in stimulated macrophages (i.e. by LPS and other TLR ligands or cytokines like type I and type II interferons), itaconate, a crucial intermediate able to block succinate dehydrogenase (SDH) and executing a variety of anti-inflammatory actions, is produced from cis-aconitate. The enzyme responsible for the conversion of cis-aconitate to itaconate is aconitate decarboxylase 1 (ACOD1), also known as immune-response gene 1 protein (IRG-1). Hence, we would assess 3) whether additional dimethyl itaconate (DMI), a membrane permeable derivate of itaconate affect immune paralysis? Next, we would assess 4) whether inflammation resolution signals like omega 3 fatty acid, DHA influence immune paralysis in monocytes and polarized macrophages? As itaconate allows nuclear Nrf2 translocation subsequently HO-1 expression and inhibits IκBζ in an ATF3 dependent manner subsequently inhibits IL-6 production. Also, TNF-α is the first response upon to LPS. Therefore, IRG-1, HO-1 expression and TNF-α, IL-6 production was obtained as read-out in our immune memory assay. The studies described in the present thesis aimed to test the hypothesis that different danger signals, but not cytokines, can initiate innate immune memory in monocytes and polarized macrophages. Innate immune memory is reflected by failure to express IRG-1, TNF-α and IL-6 upon re-challenge with LPS. Danger signals that interact with different PRR may partly overlap and cross-talk. Resolution signals do not affect innate immune memory. The results of this study can be summarized as follows: 1) activation of TLR4 by LPS results in immune paralysis in monocytes upon a second challenge with LPS. Although prior activation of TLR1/2 by Pam3sck4 significantly diminishes cytokine expression (TNF-α and IL-6) upon a subsequent challenge with LPS, immune paralysis mediated by prior TLR4 activation differs from that mediated by prior TLR 1/2 activation for induction of IRG-1 and inhibition of HO-1. 2) Also, in M1 or in M2 polarized macrophages immune paralysis occurs, as compared to M1 macrophages M2 polarized cells have a higher expression of IRG-1 and HO-1. 3) Prior TNF-α stimulation does not affect immune paralysis, in fact it increases cytokine expression upon a subsequent LPS challenge provided that the cells were not exposed to LPS before. 4) DMI inhibits LPS mediated IRG-1 expression, yet failure of LPS mediated IRG-1 expression is not necessarily is associated with immune paralysis. In polarized macrophages DMI also inhibits IRG-1 expression. While in M2 but not in M1, polarized macrophages TNF-α production is significantly increased in the presence of DMI, IL-6 production is differentially influenced by DMI in these cells (down-regulated in M1 vs up-regulated in M2). 5) Although DHA inhibits LPS mediated IRG-1 expression, it does not affect innate immune memory in monocytes and polarized macrophages. DHA increases the expression of HO-1, which is downregulated in the presence of LPS. In conclusion, our experiments applied the interventions of dangerous and resolution signals in the vitro immune memory model, providing new findings and conclusions to the studies of sepsis, mainly indicating the DMI dose dependently inhibits IRG-1, but not pro-inflammatory cytokines, HO-1 was induced by DMI and DHA treatment, whereas the immune paralysis cannot be affected by them. It is obvious that the signaling pathway and immunometabolism

related to sepsis are extremely complex, while our experiments were conducted in vitro monocytes and macrophages that did not involve various tissues and organs, therefore the results cannot yet be equally applied to clinical treatments.