Institutionen för medicin, Huddinge

Toll-like receptor-induced cytokine secretion by human monocytes in healthy donors and septic patients assessed at the single cell level

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Abstract

Following the ligation of Toll-like receptors (TLRs) by bacterial-derived signature molecules, innate immune cells such as monocytes, macrophages and neutrophils initiate the inflammatory process by secreting chemokines and cytokines with a wide range of immunomodulatory effects. Under normal conditions, these mediators work to compartmentalize the inflammatory process and eradicate the intruder, while simultaneously recruiting more immune cells to the site of infection. In sepsis, however, the virtues of this containment are lost as the response against a disseminated bacterial infection becomes systemic with an excessive production of cytokines that can lead to tissue injury, organ dysfunction and ultimately death. Here we have investigated TLR-induced cytokine secretion *in vitro* by monocytes and granulocytes from healthy donors and septic patients using the ELISpot and/or FluoroSpot assays. By looking at the secretion of cytokines directly *ex vivo*, the ELISpot assay offered the potential of being able to better define the immunological status of septic patients. We investigated the lipopolysaccharide (LPS)-induced cytokine secretion by peripheral blood mononuclear cells (PBMC) and granulocytes from healthy donors using the ELISpot assay. Cytokines (IL-1β, IL-6, IL-10, IL-12p40, TNF-α, GM-CSF) and chemokines (IL-8, MIP-1β) important in inflammatory processes were assessed. Granulocytes were found to selectively secrete IL-8 and MIP-1β. Also TNF-α was secreted but by considerably fewer cells. In contrast, PBMCs secreted all evaluated cytokines with $CD14^+$ monocytes being the main source of production. Next, we analyzed the cytokine secretion by enriched monocytes from healthy donors in response to LPS and lipoteichoic acid (LTA). The FluoroSpot technique allowed for the simultaneous analysis of two cytokines from the same population of isolated cells. With this approach, a recurring pattern of cytokine co-secretion was observed, identifying several distinct cytokine-secreting profiles for the TNF-α-, GM-CSF-, IL-10-, IL-12p40-secreting monocytes and those secreting IL-6 or IL-1β. Finally, the spontaneous and LPS-induced cytokine secretion by total leukocytes isolated from septic patients (n=32) and healthy controls (n=30) was evaluated using the ELISpot assay. Surprisingly, we found no increase in the number of constitutively cytokine-secreting cells from any of the septic patients despite significantly increased levels of cytokines (IL-6, IL-1β, TNF-α, GM-CSF, IL-10, IL-12p40) in their plasma. Simultaneously, the LPS-induced *in vitro* capacity revealed a maintained (IL-6, TNF-α) as well as reduced (IL-1β, GM-CSF, IL-10, IL-12p40) number of cytokine-secreting monocytes in the patients compared to normal donors. This selective reduction for some of the cytokines could be correlated with disease severity. In conclusion, our data indicate that circulating monocytes are not the major source of increased cytokine levels in patients with sepsis.

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