Institutionen för medicin, Huddinge

Toll-like Receptor Activation Induced Changes in Dendritic Cells

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ABSTRACT

Dendritic cells (DC) are professional antigen-presenting cells that act as a “bridge” between innate and adaptive immunity by the induction and subsequent orchestration of immune responses. The ligation of Toll-like receptors (TLR) and other innate receptors on DC determines their immune-stimulating capacity. In the studies included in this thesis, TLR activation of DC and the different aspects of such activation were investigated. In paper I, we explored how low physiological temperatures, commonly found in the skin where DC reside, affect DC activation and function. We found that several cellular functions, including macropinocytosis, phagocytosis, podosome formation, migration, and antigen processing, were similar for unstimulated DC at 28°C and 37°C. However, when DC were stimulated with the TLR agonist LPS at 28°C the kinetics of macropinocytosis and TNF production were delayed. These altered responses are most likely explained by the observed delay in the kinetics of TLR signalling, e.g., via the MAPK signalling pathway at 28°C. In addition, other functions of DC were more severely affected by the low temperature, including a reduction in NO production, CD40 receptor upregulation, and degradation of the extracellular matrix by podosomes. Also, the capacity of DC to activate T-cells was reduced after TLR activation at 28°C. These data provide new insights into an area of DC biology with potential relevance for vaccine development.

Cellular migration involves as series of events including the formation of podosomes, which are highly dynamic actin-filament scaffolds. In paper II, we examined the role of the actin-severing and capping protein gelsolin for podosom formation and function in DC. For this purpose, DC from mice deficient in gelsolin were used. In contrast to what was previously shown for osteoclasts, we found that DC form podosomes independently of gelsolin. Moreover, the formation and disassembly dynamics of podosomes are normal in DC deficient in gelsolin, as is their matrix-degrading function. Furthermore, we found that gelsolin is not required for TLR4-induced podosome disassembly. The actin cytoskeleton of podosomes involved in DC extracellular matrix degradation thus appears to be regulated in a different manner to the cytoskeleton in osteoclast podosomes that mediate bone resorption.

In order to ingest particulate material via phagocytosis, for example apoptotic cells and microbes, DC depend on rearrangement of the actin cytoskeleton. It is known that upon pathogen recognition by TLR, DC undergo rapid actin cytoskeleton rearrangements. However, most studies on TLR stimulation and phagocytosis have focused on posttranscriptional effects, i.e., the upregulation of receptors involved in phagocytosis, rather than how the process of phagocytosis is affected directly after TLR activation. In paper III, we report that the stimulation of DC using soluble TLR ligands increased their capacity to phagocytose various substrates within minutes. These included polystyrene beads, sheep red blood cells, and apoptotic lymphoma B cells. We also found that signalling through both of the TLR4 adaptor molecules, MyD88 and TRIF, was necessary for optimal LPS-stimulated phagocytosis. Furthermore, we confirmed that stimulated phagocytic uptake proceeds independently of gene transcription, as actinomycin D, which blocks gene transcription, had no effect on the stimulated uptake. In summary, our data suggest that soluble TLR ligands induce enhanced phagocytic uptake, proximal to gene transcription. Thus, our study provides new information about the role of TLR engagement in modulating the phagocytic capacity of DC.

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