Final publishable summary report

-TollLiCoR-

Toll-like receptors (TLRs) are membrane bound receptor proteins that recognize pathogen associated molecular patterns (PAMPs) and are part of the first line of defence against an invading pathogen. Whereas the TLRs for the recognition of bacterial surface molecules localise to the plasma membrane of a cell, the TLR mediated sensing of foreign nucleic acids derived from e.g. bacteria or viruses is taking place in the endosome and is mediated by TLRs 3,7,8 and 9. To broaden our understanding on the mechanisms of TLR action and pathogen recognition we set out to identify novel interactors of endosomal TLRs. To this end we cloned tagged versions of murine endosomal TLRs and transduced them into RAW264.7 murine macrophages. Tandem affinity purification with those receptors has been performed and enriched receptor complexes have been analyzed by mass spectrometry. Using the support of our bioinformatics department seven high confidence interacting proteins were selected for further validation as putative novel interactors of endosomal TLRs.

The first protein we validated was the well known macrophage surface marker and well studied co-factor of TLR4, the innate immune receptor for lipopolysaccharide from gram negative bacteria. Using confocal imaging and immunoprecipitation experiments we were able to confirm the interaction of CD14 with all four endosomal TLRs. In studies using cells that express TLR9 but not CD14 we could show that recombinant CD14 that was added to the cell culture medium was able to multiply the proinflammatory effect in response to the TLR9 ligand (Figure 1A), indicating that CD14 would promote the signaling from endosomal TLRs. In contrast, macrophages and dendritic cells from CD14 deficient mice showed reduced secretion of proinflammatory cytokines (Figure 1B) upon challenge woth the ligands for TLR7/TLR9, confirming the proinflammatory role of CD14 in endosomal TLR signaling.

Figure 1

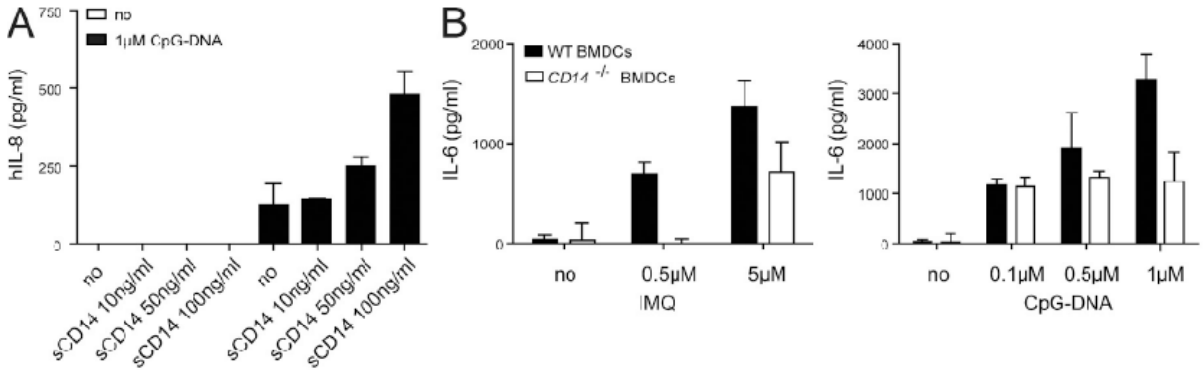

Figure 1. CD14 is required for the proinflammatory response to imiquimod and CpG-DNA. (A) Hek293-TLR9 cells were preincubated with the indicated concentrations of sCD14 30 min before stimulation with 1 µM CpG or mock control (no). Culture supernatants were harvested 8 h later, and the levels of human IL-8 were determined. Data are presented as mean ± SD and are representative of two independent experiments, each performed in biological duplicates. (B) BMDCs from WT or *CD14*□/□ mice were stimulated with CpG-DNA or imiquimod (IMQ) for 6 h. IL-6 in supernatants was measured by ELISA. Data are presented as mean ± SD.

In further studies we embarked on the mechanism of CD14 and found that it is required for the uptake of fluorescently labeled DNA into the macrophages endosome. This reduced delivery of ligand into TLR containing endosome could explain the reduced stimulatory effects of TLRs in CD14 deficient immune cells. However, to further investigate the function of CD14 in the endosome we investigated its role in the uptake of bacteria and viruses and found that – at least for those tested - CD14 was dispensable. Yet, incubation of CD14 deficient cells with TLR7 stimulating viruses (influenza, vesicular stomatitis virus (VSV)) led to a severly reduced proinflammatory response compared to WT cells. Interestingly, VSV-M2, a variant of VSV that is recognized rather in the cytoplasm and not by TLR7, showed comparable levels of proinflammatory cyrokines in CD14 deficient and control cells (Figure 2). This shows that CD14 has 2 roles in the endosome: it promotes the uptake of nucleic acids and it functions as a coreceptor of endosomal TLRs in signaling.

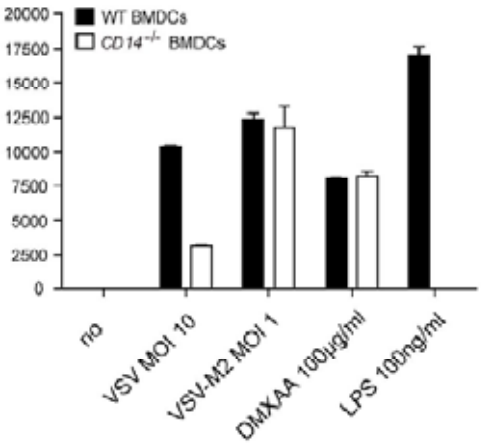

Figure 2. CD14 is dispensable for virus uptake but required for the induction of cytokines in ssRNA virus-infected macrophages. BMDCs from WT and CD14-deficient mice were infected with the indicated amounts of VSV or VSV-M2, a mutant which is mainly recognized in the cytoplasm, or stimulated with LPS or DMXAA. IL-6 accumulation was tested 14 h after stimulation.

Further interactors that we have found in the TollLiCoR screen are under investigation. The project that describes CD14 function in endosomal TLR signaling is of great interest for the scientific and medical community: Toll-like receptors are at the forefront of the recognition of pathogens by the innate immune system. Diseases like sepsis, autoimmune disease, cancer and vaccination are closely linked to these proteins and endosomal TLRs. Cd14 is a protein that might allow to tune immune response e.g. to influenza virus, other pathogens or the immune recognition of self-DNA in autoimmune disorders. We have also shown that soluble recombinant CD14 protein can enhance endosomal TLR activation, in addition, a monoclonal antibody that binds to the ectodomain of CD14 can block such response. These are potential therapheutic approaches in the modulation of endosomal TLR signaling.

The work was carried out at the

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