Abstract

Nucleotide-binding domain and leucine-rich repeat containing proteins (NLRs) contribute essentially to innate immune responses. Many members of the NLR family off pattern recognition receptors have been intensively studied, whereas other members of this protein family remain poorly characterised. NLRP10 has been shown to contribute to cell-autonomous responses against invasive bacteria mediated by NOD1 and been proposed to negatively regulate inflammasome function. However, little is known about the role of NLRP10 in signalling pathways downstream of other pattern recognition receptors. In this study, we aimed to analyse the function of NLRP10 *in vitro* and *in vivo*.

In human cell lines we observed an increase in NLRP10 protein levels after stimulation with the TLR3 agonist poly(I:C) or infection with Sendai virus. This finding was substantiated by increased mRNA expression of NLRP10 in in murine embryonic fibroblasts (MEFs) after stimulation with poly(I:C). Analysis of cytokine secretion after TLR3 activation or infection with Sendai virus in MEFs revealed enhanced secretion of the cytokines CXCL1 and CCL5 in murine *Nlrp10* knock-out cells compared to control fibroblasts. These observations indicate a possible a role for NLRP10 in the negative regulation of inflammatory responses downstream of TLR3 and RIG-I.

As NLRP10 has been proposed to be crucially involved in the migration of dendritic cells (DCs) we asked if NLRP10 might also influence the migration of non-myeloid cells. *In vitro* migration assays showed normal migration in human HeLa and HaCaT cells after siRNA-mediated knock-down of NLRP10 expression. Furthermore, *Nlrp10*$^{-/-}$ murine embryonic fibroblasts showed no defect in migration compared to control cells, arguing against a contribution of NLRP10 to the migration of the cell types analysed.

The main part of this study investigated the role of NLRP10 in inflammatory responses of the skin using two novel *Nlrp10* knock-out mouse strains. NLRP10 is highly expressed in skin and has been genetically linked to susceptibility for atopic dermatitis. We therefore concentrated our efforts on the analysis of NLRP10 in skin inflammatory disease models. Using the Cre-lox system, full-body as well as keratinocyte-specific *Nlrp10* knock-out mice (*Nlrp10*$^{-/-}$ and *Nlrp10*$^{K14\text{-}KO}$, respectively) were generated. Both *Nlrp10* knock-out strains appeared phenotypically normal and both epidermal tissue repair and irritant contact dermatitis responses were not affected in *Nlrp10*$^{-/-}$ mice. In contrast, *Nlrp10*$^{-/-}$ *mice* showed significantly reduced inflammation compared to control animals in a T cell-driven contact hypersensitivity (CHS) model induced by 1-fluoro-2,4-dinitrobenzene (dinitrofluorobenzene/DNFB). Microscopic analysis revealed significantly reduced numbers of

$CD4^+$ and $CD8^+$ T cell infiltrates in animals lacking NLRP10 expression. This was accompanied by a trend towards lower *CXCL1* and *TNF-α* mRNA levels in the inflamed tissue, as well as increased induction of *CCL5* mRNA expression. Interestingly, epidermis-specific deletion of *Nlrp10* was sufficient to account for this phenotype, although T cell recruitment seemed to be unaltered in animals lacking NLRP10 expression in keratinocytes. Reduced infiltration of neutrophils as well as degranulation of mast cells was observed upon elicitation of CHS in ears of both mouse strains. This might also contribute to the reduced inflammation during CHS in those animals.

Taken together, these findings provide evidence that NLRP10 contributes to T cell-mediated inflammatory responses in the skin and highlight a novel physiological role of NLRP10 in epidermal keratinocytes.