induction upon viral infection, further indicating that its kinase activity is required for tamping IFNs induction. Strikingly, blockade of CK2 activity alone was sufficient to activate TBK1 and IRF3, resulting in type I IFN induction in the absence of PRR stimuli. More importantly, blocking CK2 activity was able to efficiently suppress the infection and replication of VSV and HCV, suggesting a new strategy to overcome viral immune evasion mechanisms which target signal molecules upstream of TBK1. Mechanistically, CK2 regulates the activation of TBK1 and IRF3 through its substrate protein phosphatase PP2A. Indeed, PP2A, but not CK2 forms complexes with TBK1 and IRF3, and was also able to diminish TBK1- and IRF3-induced IFN exprssion. Taken together, our results not only identify CK2 as a novel regulator of TBK1 and IRF3 in PRR signals, but also reveal a new strategy to contain viruses which are capable of interfering type I interferon induction by PRRs.

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Interferon regulator factor (IRF) 8 regulates the microglial response to neuronal injury

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The transcription factor interferon regulatory factor (IRF) 8 has a key role in the cellular response to IFN-γ and is involved in myeloid cell differentiation. We have previously identified IRF8 to be a constitutive and IFN-γ-stimulated nuclear factor that regulates the homeostatic properties of microglia. Our study aimed to determine the role of IRF8 in the microglial response to sterile neuronal injury.

Facial nerve axotomy (FNA) was performed in wildtype (WT) and IRF8$^{-/-}$ (KO) mice and the brains removed at different times post-lesion. A subset of mice was injected with bromodeoxyuridine (BrdU) prior to retrieval of the brain. Changes in the facial nucleus (FN) were examined by immunohistochemistry and histochemistry.

In brains from IRF8KO mice, nucleoside diphosphatase (NDPase) hisotchemistry revealed gross alterations in the morphology of microglia, which were stunted and hypertrophied. After FNA in WT mice, a progressive increase in microglial activation was observed in the lesioned FN peaking at day 7 and was accompanied by dense staining for Iba1, lectin, NDPase and CD11b. By contrast, in IRF8KO mice, the microglial response was markedly attenuated with little staining for Iba1, while the density of staining for lectin and NDPase was reduced significantly. The attenuated microglial response in IRF8KO mice was paralleled by a significant decrease at day 3 post-lesion in proliferation as monitored by BrdU, phosphohistone 3 and Ki67-positive cells. Furthermore, a decrease in PU.1-positive cells was observed in the FN of IRF8KO mice compared with WT. The wrapping of individual motor neuron cell bodies by microglia involved in synaptic stripping and phagocytosis was incomplete in the absences of IRF8 in the axotomised FN. Quantitative analysis showed that in IRF8KO mice, the degeneration of axotomised motor neurons was significantly increased.

These studies extend on our previous finding that IRF8 is a key homeostatic transcription regulator of microglial cell function in the healthy brain, and moreover, has a crucial role in the regulating the response to microglia to neuronal injury.

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Endogenous matrix metalloproteinase (MMP)-9, and not MMP-2, promotes rheumatoid synovial fibroblast survival, inflammation and cartilage degradation

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Aim: To investigate the effect of endogenous matrix metalloproteinase (MMP)-2 and MMP-9 on the invasive characteristics of rheumatoid synovial fibroblasts (SF).

Methods: SF isolated from patients with rheumatoid arthritis (RA) or osteoarthritis (OA) were treated with MMP siRNAs, inhibitors and recombinant (r) proteins or TNF-a, with or without cartilage explants. Cell viability and proliferation were measured by MTT and BrdU proliferation assays, respectively; apoptosis by an in situ cell death detection kit; migration and invasion by CytoSelectTM invasion assay, scratch migration and collagen gel assays; cartilage degradation by 1,9-dimethylmethylene blue assay; inflammatory mediators and MMPs by ELISA, western blot and zymography.

Results: MMP-2 was expressed by both OASF and RASF, whereas only RASF expressed MMP-9. Suppressing MMP-2 or MMP-9 reduced RASF proliferation equally. However, MMP-9 siRNA had greater effects, compared to MMP-2 siRNA, on promoting apoptosis and suppressing RASF viability, migration and invasion. Suppression/inhibition of MMP-9 also decreased the production of IL-1β, IL-6, IL-8 and TNF-α, inactivated nuclear factor (NF)-κB, ERK and JNK and suppressed RASF-mediated cartilage degradation. In contrast, Suppression/inhibition of MMP-2 stimulated TNF-a and IL-17 secretion and activated NF-κB, while rMMP-2 inactivated NF-κB and suppressed RASF-mediated cartilage degradation. Results using specific inhibitors and rMMPs provided supportive evidence for the siRNA results.

Conclusions: Endogenous MMP-2 or MMP-9 contribute to RASF survival, proliferation, migration and invasion, with MMP-9 having more potent effects. Additionally, MMP-9 stimulates RASF-mediated inflammation and degradation of cartilage, whereas MMP-2 inhibits these parameters. Overall, our data indicate that MMP-9 derived from RASF may directly contribute to joint destruction in RA.

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Regulatory effect of interleukin-4 in the innate immune and inflammatory responses to *Rhodococcus aurantiacus* infection in mice

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Aim: Inoculation of wild-type (WT) mice with gram-positive bacterium *Rhodococcus aurantiacus* induces Th1-type granulomatous inflammation, in which interleukin (IL)-4 promotes the regression of granulomas during the late phase of infection. As IL-4 plays diverse roles in disease pathogenesis, we investigated the effect of IL-4 in the innate response to this bacterium infection by using IL-4-deficient mice.

Methods: After intravenous inoculation with 1×10^8 CFU of bacteria, mouse survival rates, bacteria load in organs, local and systemic cytokine production, and morphological changes in liver were assessed. Following stimulation with heat-killed *R. aurantiacus*, cytokine production in mouse peritoneal macrophages was also examined.

Results: Compared to WT mice, IL-4-deficient mice showed the decreased production of TNF-α and IL-6 in the spleen, liver and blood during the early phase of infection, as well as low bacterial load in the liver and improvement in survival rate. IL-4-deficient mice also showed diminished IL-10 secretion in the spleen and blood, whereas their hepatic IL-10 levels were similar to those observed in WT mice, which were concomitant with the augmented hepatic IFN-γ production. Moreover, histological studies revealed reduction in hepatic granuloma formation at day 14 post-infection in IL-4-deficient mice. Upon stimulation with heat-killed *R. aurantiacus*, macrophages from IL-4-deficient mice showed lower expression of TNF-α, IL-6, and IL-10 at both the gene and protein levels than WT mouse cells.

Conclusions: These findings indicate that IL-4 deficit attenuates cytokine responses in macrophages but augments the IFN-γ response during the initial stage of *R. aurantiacus* infection, which together contribute to amelioration of mouse survival, rapid bacterial elimination in liver at the early phase, and reduction of granulomatous inflammation. We previously demonstrated that the early IFN-γ response to *R. aurantiacus* is induced in mouse natural killer cells. This study thus identifies the essential effect of IL-4 on regulating the activation and function of immunocompetent cells, including macrophages and natural killer cells, during the initial phase of systemic *R. aurantiacus* infection.

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A role for protein kinase R in regulating NLRP3 inflammasome assembly

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Aim: NLR family pyrin domain containing-3 (NLRP3) has been associated with inflammatory disorders. In response to exogenous and endogenous molecules, NLRP3 assemble an inflammasome containing the apoptosis-associated speck like protein containing a CARD (ASC) for activating caspase-1, inducing pyroptosis and processing interleukin (IL)-1β and IL-18. A recent model proposes that the assembly of the inflammasome is dependent on the cytoskeleton. As the protein kinase R (PKR) has been shown to regulate actin dynamics, we examined whether PKR regulates inflammasome activity.