ImmunoTools IT-Box-Cy55M-Award 2013

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The role of transcription factors Stat in microglia activation

Microglia, brain immune cells, respond to injury or infection by initiating the inflammatory response that resembles pathological inflammation in neurological disorders and could be mimicked in vitro by stimulation of microglia with lipopolysacharide (LPS). In my project I performed ChIP-chip using anti Stat1, Stat3 and Stat5 antibodies on primary rat microglia cultures stimulated with LPS. This approach revealed a set of Stat binding sites and combined with expression microarrays identified novel Stat targets. Further experiments using RNA interference and overexpression of constitutively active Stats demonstrated that active Stat1 and Stat3 are crucial and sufficient for microglia activation. Interestingly, ectopic expression of constitutively active Stat1+Stat3 in microglial cells leads to similar changes in gene expression and cytokine production as LPS stimulation). Signals for activation of Stat1 and Stat3 in LPS-stimulated microglia have not been fully characterized. As Stat activation is a rapid process (0.5 h after stimulation). it is not clear if it relies on classical activation by up-regulated cytokines (IFNs, IL-6) or on distinct pathway. I will block protein synthesis in microglia using cyclohexymide to determine if phosphorylation of Stat proteins would be affected and in such case I would like to perform rescue experiments using classical activators of Stat rm IL-6 rm, IFNgamma, rm TNFα. Furthermore stimulation with these cytokines should clarify which genes are activated by active Stats and which more strongly rely on other pathways –such as NF-kappaB.

The role of GM-CSF in activation of microglia by glioma

Many gliomas (especially malignant gliomas) release factors that can activate microglia and turn them into proinvasive cells supporting tumor progression. M-CSF (macrophage colony stimulating factor) was suspected to be one of the main factors responsible for macrophage recruitment in non-brain tumors. Our studies revealed that in a case of murine experimental gliomas not M-CSF but GM-CSF is crucial for microglia activation. Silencing of gm-csf expression in glioma cells decreased tumor invasion and microglia activation. Furthermore, the expression data from patients with gliomas show that the expression of GM-CSF but not

M-CSF is upregulated in malignant gliomas and correlate with patient survival (J.Pathol in revision). I would like to test the effects of recombinant cytokines rm GM-CSF, rm M-CSF to further investigate the role of these proteins in microglia activation. I am planning to compare changes in microglia features: morphology, migration and markers of glioma-associated activation (qPCR) after stimulation with cytokines, glioma conditioned medium and glioma with silenced GM-CSF conditioned medium.

Application of selected recombinant cytokines from the ImmunoTools IT-Box-Cy55M would allow to better understand the processes taking place both in the classically activated microglia and tumor-stimulated microglia.