ImmunoTools IT-Box-Cy55M-Award 2013

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Small, non-coding RNAs (microRNAs) in the differentiation of autoreactive T helper cells (Th) - importance for the pathogenesis of autoimmune demyelination

Short non-coding RNAs (microRNA, miRNA) are an emerging group of non-classical gene products that exert their function through interference with protein coding mRNA. Number of reports suggest a critical role of miRNA in regulation of development and differentiation of various cell populations. So far little is now with regard to the potential role of miRNA in development of autoimmune demyelination. We believe that miRNA changes in Th cells control the development of the major autoagressive Th population during autoimmune demyelination – Th17 type cells. In particular we would like to investigate on the mechanisms of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), These conditions are believed to result from imbalanced proportion between proinflammatory subsets of T helper cells, Th1 and Th17 and anti-inflammatory Th2. Therefore, we aim to investigate the role of miRNAs in a regulation of T helper cells (Th) differentiation. We hope that our findings are likely to result in better understanding of the pathogenesis of major human autoimmune demyelinating disease – multiple sclerosis (MS).

$CD4^+62L^+$ T cells derived from spleen will be cultured in IMDM supplemented with IL-6 (25ng/ml), TGFb (2ng/ml), IL-1b (20ng/ml), IL-23 (20ng/ml), under stimulation with anti-CD3 (5ug/ml) and anti-CD28 (10ul/ml) in the presence of anti-IL-4(10ug/ml) and anti-IFNg (10ug/ml) we will receive Th17 cells. In case of T regs, $CD4^+$CD62L+ T cells will be cultured in IMDM supplemented with TGFb (10ng/ml) and IL-2 (100U/ml) under stimulation with anti-CD3 (5ug/ml) and anti-CD28 (10ul/ml) in the presence of anti-IL-4(10ug/ml) and anti-IFNg (10ug/ml). In order to obtain Th1 fraction, naïve $CD4^+CD62L^+$ T cells will be cultured in IMDM supplemented with IL-12 (10ng/ml) in the presence of anti-IL-4 (10ug/mL) and anti-IFN-gamma (10ug/mL) under stimulation with anti-CD3 (5ug/ml) and anti-CD28

(10ul/ml). To gain Th2 cells, $CD4^{+}62L^{+}$ T cells will be cultured in IMDM supplemented with IL-4 (40ng/mL) in the presence of anti-IFN-gamma (10ug/mL) under stimulation with anti-CD3 (5ug/ml) and anti-CD28 (10ul/ml). Such polarized Th1, Th2, Th17 and Treg cells will be used for RNA extraction and subsequent microRNA profiling. In addition the role of accessory cytokines, like IL-10, IL-25/IL-17E, IL-27, IL-31 or IL-33 on Th differentiation will be assayed via analysis of the spectrum of microRNA changes.

The ImmunoTools IT-Box-Cy55M will be a great benefit to me as it would enable me to investigate the effect of cytokines such as IL-27, IL-31, IL-33, IL-10 in the process of differentiation and their impact on polarization towards anticipated T cells fractions.

ImmunoTools IT-Box-Cy55M for Maria Cichalewska

includes 55 recombinant mouse cytokines

rm EGF, rm Eotaxin / CCL11, rm FGF-a / FGF-1, rm FGF-b / FGF-2, rm FGF-8, rm Flt3L / CD135, rm G-CSF, rm GM-CSF, rm GRO-a / CXCL1, rm GRO-b / CXCL2, rm IFNgamma, rm IL-1alpha, rm IL-1beta, rm IL-2, rmIL-3, rm IL-4, rm IL-5, rm IL-6, rm IL-7, rm IL-9, rm IL-10, rm IL-11, rm IL-13, rm IL-15, rm IL-16, rm IL-17A, rm IL-17C, rm IL-17F, rm IL-19, rm IL-20, rm IL-21, rm IL-22, rm IL-25 / IL-17E, rm IL-27, rm IL-31, rm IL-33, rm IP-10 / CXCL10, rm LIF, rm MCP1 / CCL2, rm M-CSF, rm MIP-1α/ CCL3, rm MIP-1β / CCL4, rm MIP3α / CCL20, rm MIP3β / CCL19, rm NGF-beta, rm PDGF-AA, rm PDGF-BB, rm RANTES / CCL5, rm sCD40L / CD154, rm SCF, rm SDF-1α / CXCL12a, rm SDF-1β / CXCL12b, rm TNFα, rm TPO, rm VEGF DETAILS