ImmunoTools *special* Award 2014

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Immunological modulation of synaptic plasticity

Synapses are complex cellular junctions specialized for communication between neurons. A hallmark of synaptic specializations is their dependence on highly organized complexes of proteins that interact with each other. Therefore the loss or modification of key synaptic proteins might directly affect the properties of such networks and, ultimately, synaptic function. SNAP-25 is a component of the SNARE complex, which is central to synaptic vesicle exocytosis. In the last years, it has been demonstrated that SNAP-25 regulates intracellular calcium dynamics by negatively modulating neuronal voltage-gated calcium channels. The *SNAP25* gene has been associated with different psychiatric diseases, including Attention Deficit Hyperactivity Disorder (ADHD) and schizophrenia. Consistently, mice expressing half of the protein show moderate hyperactivity, and impaired associative learning and memory.

Synaptic plasticity is the ability of synapses to change in response to external stimuli. This process is called long-term potentiation (LTP) and involves in particular the postsynapse, which enlarges and gather receptors to the membrane in order to potentiate the neurotransmission. Impairment in synaptic plasticity can alter the entire neuronal network, favouring the appearance of cognitive defects. In the last years, different evidences have indicated an unexpected postsynaptic role of SNAP25. The protein was indeed shown to control NMDA receptors trafficking, while acute SNAP25 down-regulation resulted in defective synaptic plasticity. These data open the possibility that besides a presynaptic impact, chronic reductions of SNAP25 levels may impair the structure and/or function of the postsynaptic compartment, which would provide a logical frame for the protein involvement in psychiatric diseases, such as epilepsy, which are known to be characterized by defects at the postsynaptic compartment. In particular, SNAP25 mutations are indeed present in patients with epilepsy.

Nowadays, the link between epilepsy and inflammation is well established. Experiments in rodents demonstrate that seizures induce high levels of inflammatory mediators in brain regions involved in the generation and propagation of epileptic activity. Moreover, in recent years growing evidence suggest that astrocytes, microglia, blood leukocytes and blood-brain

barrier breakdown are involved in the pathogenesis of epilepsy. Consistently, IL1b is rapidly increased after seizures in neurons and post-mortem studies from epileptic patients reveal high levels of TNFa.

With the help of ImmunoTools reagents I aim at investigating how the inflammatory status of the network influences synapse plasticity, and therefore its transmission properties in the mouse model heterozygous for SNAP25.

In particular, I would administrate mouse recombinant cytokines IL1beta, IL2, IL6, and TNFa to neuronal cultures from het mice for SNAP-25 to elicit an inflammatory state and then I would evaluate structural and functional changes at synaptic level.

It is demonstrated that an alteration in the permeability of the blood brain barrier favours neuronal hyperexcitability. Therefore, my second aim will be to evaluate possible infiltration of lymphocytes which I can detect using CD4, CD8, NK-cells markers (and relative isotype controls) in slices.

In spite of epilepsy is thought as a pathology of neurons, it is fundamental to take into account that neurons grow in a complex network composed of astrocytes and microglia, which modulate the inflammatory status in the brain. I aim therefore at studying the glia immunomodulatory contribute in epilepsy. I plan to do so identifying activated microglial cells with CD11b, and the release of microvesicles from astrocytes (CD81, CD9, CD29). Eventually, trough ELISA assays I could quantify the amount of inflammatory proteins released by the glia.

In order to do that I will need a substantial amount of cytokines making this box valuable. Results from these experiments will contribute to elucidate how inflammation affects neuronal function and to get us nearer to new therapeutic targets.

ImmunoTools *special* AWARD for **Giuliana Fossati** includes 22 reagents

FITC - conjugated anti-mouse CD4, CD9, CD11b, CD29, CD81, CD117, a/b TCR, isotype control IgG2b,

PE - conjugated anti-mouse CD117, NK-cells, /d TCR,

APC - conjugated anti-mouse CD8a, CD11b, isotype control IgG2b,

recombinant mouse cytokines: rm FGF-b / FGF-2,rm IL-1beta, rm IL-2, rm IL-4, rm IL-6, rm IL-10, rm MCP1 / CCL2, rm TNFa DETAILS more AWARDS