MODULATION OF IMMUNE RECEPTORS FUNCTION AS NOVEL THERAPEUTIC STRATEGY FOR ACUTE CNS DAMAGE

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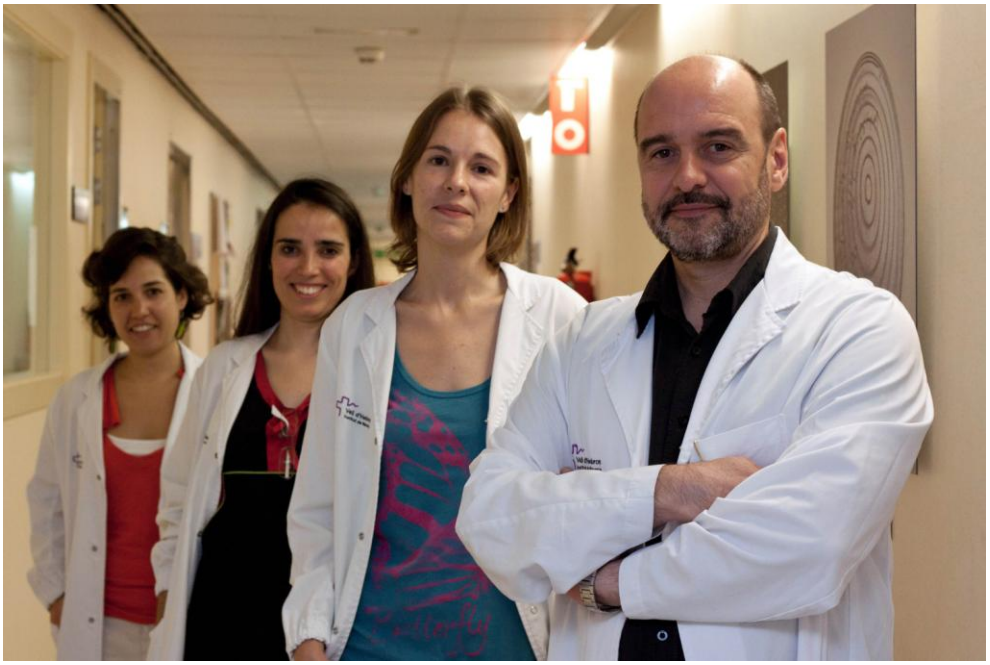

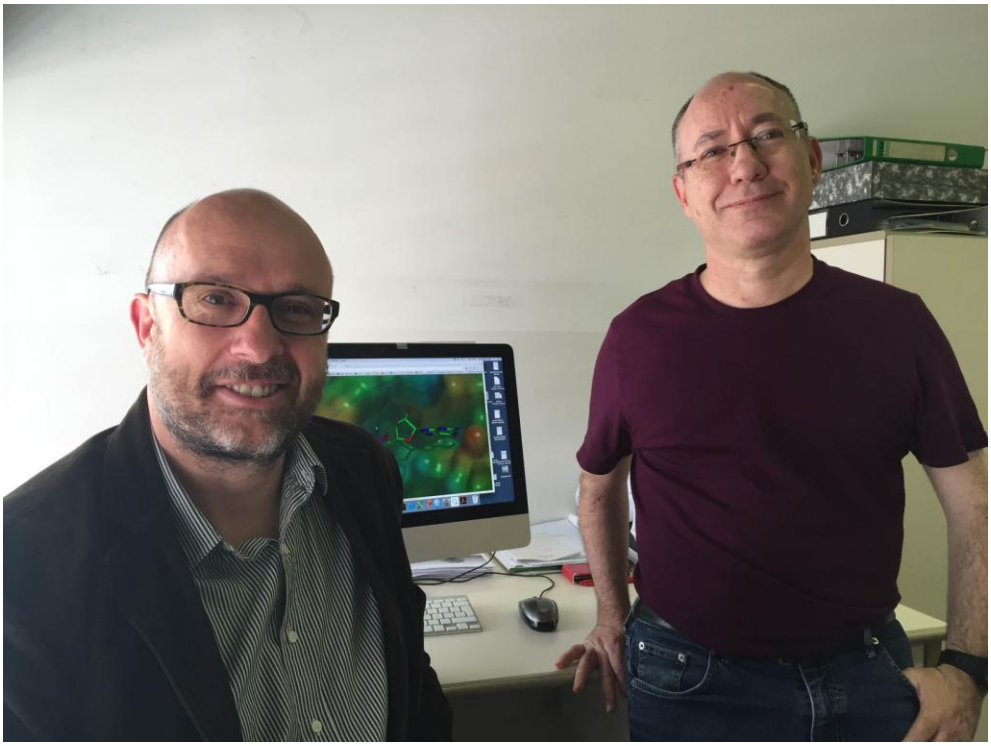

1. Abstract

Acute spinal cord and cerebral lesions result in central nervous system (CNS) damage usually associated to different degrees of functional loss. In this context, a neurodegenerative process is initiated, which is characterized by the presence of neuronal damage and an inflammatory response where glial cells (microglia and astrocytes), endothelial cells and cells of the peripheral immune system (macrophages, lymphocytes, dendritic cells) can be involved at different degrees according to the extension of neuronal damage and tissue injury induced.

Microglial cells are the main representatives of the CNS endogenous immune system. Under physiological conditions, these cells are constantly monitoring the CNS parenchyma and, thanks to a wide range of membrane receptors, they are able to recognise a healthy environment as well as detect alterations implying a threat for normal CNS function (pathogens, neuronal damage, exogenous neurotoxic agents, anomalous endogenous molecules resulting from pathologic processes). Following an acute lesion to the CNS, tissue homeostasis is altered and microglial cells become activated in order to neutralize negative changes. However, activated microglial cells can produce several pro-inflammatory molecules as well as oxygen and nitrogen species which are potentially neurotoxic. Consequently, microglial activation has to be tightly controlled in order to avoid secondary noxious effects.

A maintained inflammatory response is believed to contribute to the extent of the final injury in acute lesions of the CNS. Therefore, this inflammatory response is a potential target to be considered from the therapeutic point of view. Thus, the modulation of the regulatory mechanisms involved in the induction, maintenance and resolution of the microglial inflammatory response is a possible therapeutic strategy for a successful neurorestoration.

As previously mentioned, microglial cells act as sensors of the CNS activity, and neurons play an important role in the maintenance of microglia in a resting/quiescent/surveillant state under physiological conditions. A series of contact-dependent and contact-independent inhibitory signals participate in this control. The aim of this project was to analyse the therapeutic potential of some key microglial receptors involved in the regulation of the neuroinflammatory response (CD200R1,

TREM2 and the CD300 family of receptors) in different experimental in vitro and in vivo models in the context of acute spinal cord or cerebral injury. We believe that these receptors and their ligands are altered in these pathologies and that the modulation of the inflammatory response through an action on these receptors can have a neuroprotective role.

A multidisciplinary approach was considered to develop this proposal. Thus, the project was carried out by five teams from different institutions covering a wide range of expertise, including bioinformatics, molecular immunology, neurobiology, neuropathology, behavioral neuroscience and gene therapy. On the one hand, in vitro approaches were used to study both ligand-receptor interactions and the role of these receptors in the microglial inflammatory response and the neuroprotective potential of their modulation. On the other hand, in vivo approaches were used to study the involvement of these receptors and their modulation in experimental animal models of acute CNS damage (neonatal hypoxic/ischemic encephalopathy and traumatic CNS injuries). Functional alterations were evaluated by behavioral studies, and bioinformatics was used to study molecular interactions ligand-receptor in order to identify molecules in the existing data bases that could act as agonists.

2. Results

CD200-CD200R1

Using in vitro approaches, we detected alterations in the expression of CD200-CD200R1 in response to inflammatory stimuli. We observed that transcription factors involved in the control of the inflammatory response modulate CD200R1 expression in reactive microglia, and that CD200R1 modulation has an effect on the inflammatory pattern of microglial cells and their neurotoxic potential. Thus, the inhibition of CD200-CD200R1 interaction potentiated microglial pro-inflammatory response, and CD200R1 overexpression or stimulation resulted in the inhibition of the pro-inflammatory response and the associated neurotoxicity. In vivo studies using experimental models of acute spinal cord or cerebral injury showed alterations in CD200R1 expression. CD200R1 inhibition using different experimental strategies pointed out that this receptor is necessary to limit the extent of neuronal injury on these lesions. In silico studies of the structure of CD200-CD200R1 allowed the characterization of the energy

landscape of protein–protein-interaction regions and the identification of hotspots. This opened the door to the search of potential small modulator-molecules in databases of molecules known to be able to cross the blood-brain barrier. Unfortunately, the structural information is still not very conclusive to allow the finding of agonists for this system.

CD300

Regarding CD300 receptors, we have generated valuable information at different levels. Our work has mainly focused on CD300f receptor. In vitro studies using cell lines allowed us to identify sphingomyelin as one of the physiological ligands of this receptor in humans. Furthermore, we found that the presence of a protein presenting sphingomyelin to CD300f is required for a physiological binding. This fact is very important because this protein can become a therapeutic target due to its ability to regulate the signaling of this receptor. By modeling the ligand-receptor complex in silico, we identified a putative region of calcium- mediated interaction between the two molecules. In vitro studies also showed the expression CD300f receptor and its ligand in neurons and glial cells, and its pro- and anti-inflammatory dual role. In addition, we observed that blocking the interaction between CD300f and their ligands induced neuronal death in mixed neuron-glia cultures. In vivo studies using animal models of acute spinal cord or brain injury showed an altered pattern of expression of CD300f in these situations. Moreover, blocking CD300f interaction with their ligands in these models resulted in enhanced neuronal damage. From these data, we designed a therapeutic strategy based on CD300f overexpression after brain trauma. Whereas short-term expression of CD300f decreased volume lesion, long-term overexpression was toxic. These observations suggest that this therapeutic approach should be refined, for example overexpressing CD300f only in microglia/macrophages using a specific promoter.

TREM2

As occurs with CD300 receptors, the physiological ligand of TREM2 is unknown, making it difficult to study the ligand-receptor system. By using an in vitro system, we observed that microglial TREM2 expression is altered in an inflammatory context. In vivo studies allowed us to observe that the cerebral pattern of TREM2 expression is modified during postnatal development, and that TREM2 expression is associated with different microglial phenotypes. We also observed that the cerebral expression of

TREM2 is altered in an experimental model of brain injury induced by perinatal hypoxia/ischemia. These observations, coupled with the fact that decreased expression of TREM2 is observed in neurodegenerative disorders, suggest an additional interest in the study of this receptor in the context of hypoxia/ischemia.

In the experimental model of perinatal hypoxia/ischemia, we also characterized the temporal pattern of cerebral expression of pro- and anti-inflammatory cytokines, as well as the involvement of STAT-3 transcription factor in the regulation of the inflammatory response. In addition, in this experimental model we evaluated functional alterations using several mouse behavioral tests.

Altogether, the results obtained suggest that the inflammatory response occurring after acute spinal cord and cerebral injury can be modulated through an action on inhibitory immune receptors which regulate microglial function. The development of tools that allow the modulation of the function of these receptors, either by an action on their expression or an action on their activation, would be useful to inhibit neuronal damage resulting from spinal cord and cerebral lesions.

From a technical point of view, it is important to emphasize the utilization of bioinformatics tools that have contributed to the study of ligand-receptor interaction in the case of CD300 and CD200R1. For CD300, it has allowed the understanding of the molecular basis of the interaction with the identified ligand, while in the case of CD200-CD200R1 it has made it possible to establish the molecular basis for the search of molecules that could function as CD200R1 ligands.

A significant contribution of this project is the development of gene therapy strategies for traumatic brain injury. We have analyzed both integrative deficient lentiviral vectors and modular recombinant nanoparticles which display different but complementary profiles. We show that both vectors can rapidly induce a transgenic protein, do not exacerbate the endogenous neuroinflammation or the tissue damage, and produce biologically relevant transgenic protein levels. Interestingly, both vectors displayed a neuroprotective profile per se, as shown by decreased histopathology and enhanced neurological performance.

3. Relevance and possible implications

Although this is a basic research project, knowledge of the molecular and cellular mechanisms involved in the development of neuronal damage in acute lesions of the CNS is critical to be able to identify potential targets from the therapeutic point of view. Our working hypothesis is that the control of the microglial inflammatory response after acute CNS damage can result in neuroprotection.

On the one hand, the results obtained show that, through an action on certain inhibitory immune receptors present in microglial cells, we can inhibit the inflammatory response and the resulting secondary neuronal damage in acute lesions of the CNS. These results can contribute to the design of therapeutic strategies for processes of neuronal damage associated to neuroinflammation, and highlight the interest of developing therapeutic approaches considering an action on the expression or the activation of these receptors.

On the other hand, we have shown the feasibility of two gene therapy strategies for the treatment of brain and spinal cord contusion, including either lentiviral vectors or nanovectors. The use of gene therapy strategies for traumatic brain injury under preclinical conditions that include the delayed injection of the vectors (4 hours after the lesion) had not been undertaken until now. The rapid, non-toxic production of biologically relevant concentration of therapeutic protein of both approaches can be adapted to different neuroprotective transgenes, even to the over-expression of more than one at the same time to induce synergic effects. Moreover, the selection of the lentiviral or nanovectors should be coupled to the necessary levels and duration of the desired protein expression. Finally, the tropism of both types of vectors is overlapping but different, and thus this is another tunable property of the strategy used. These results should be confirmed in bigger animals, and in models that include other comorbidities observed in clinical practice such as polytrauma or hypotension.

In summary, the research project has contributed to the identification of potential therapeutic targets to inhibit neuroinflammation and the resulting secondary neuronal damage after acute CNS injury, as well as to the development of pharmacologic strategies classified as advanced therapies (in this case gene therapy).

4. Publications

Papers published

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Behavioral profile of C7BL6/C mice following neonatal hypoxia-ischemia.
Giménez-Llort L., Muntsant A, Shrivastava K, Gonzalez B, Acarin L, Shrivastava K.

Perinatal hypoxia-ischemia induces long-term sensorimotor deficits, anxiety-like behaviors and cognitive impairment in a gender-selective manner that can be reversed by early- postnatal handling.
Muntsant A, Shrivastava K, Gonzalez B, Acarin L, Giménez-Llort L.

CD300f immune receptor regulates microglial activation.
Lago N, Negro-Demontel ML, Alí D, López-Valez R, Sayós J, Peluffo H.

Modelling of Calcium mediated activation of CD300 by sphingomyelin. Agulló L, Sayós J, Rubio S, Villà-Freixa, J.

Computational characterisation of agonists for CD200R1 activation. Agulló L, Solà C, Villà-Freixa J.