Antibody-independent effects of B cells in multiple sclerosis (MS)

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Research Question and background

B cells are clearly implicated in the pathogenesis of multiple sclerosis (MS). Most research on B cells has focused on (auto)antibody production, but recent success of B cell depletion in MS treatment has renewed interest in B cell antigen presentation capacity. However, the antigen presentation function of B cells in MS is not well understood as studies are mostly limited to experimental autoimmune encephalomyelitis (EAE), the animal model of MS. This project aims to further elucidate antigen presentation by B cells in MS and its role in the activation of autoreactive T cells.

Methods and tissues used

The capacity of peripheral B cells from MS patients to activate autologous T cells by presentation of myelin and other autoantigens is examined. As a part of this study, we will measure the expression of costimulatory and major histocompatibility complex (MHC) molecules on B cells from the peripheral blood, cerebrospinal fluid and brain lesions of MS patients and healthy individuals. Immunohistochemistry is therefore performed on frozen or paraffin embedded brain slices of MS patients and controls, both using DAB and fluorescent staining.

Results and conclusion

Up to now, the immunohistochemical staining for CD20 (B cell marker), HLA-DR/DP/DQ (MHC class II) and CD80 (costimulatory molecule) has been optimized on brain sections of MS patients with many inflammatory infiltrates. First, DAB staining of the individual markers was performed. Later, double stainings for CD20/HLA-DRDPDQ and CD20/CD80 were optimized. B cells expressing MHC class II molecules and CD80 were found in the brain of MS patients. More patients have to be included, as well as controls. These experiments will provide better insights into the aetiology of MS, which is instrumental for the identification of novel therapeutic targets.

The pathogenic role of CD4+CD28- T cells in multiple sclerosis

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Research question and background

Premature immunosenescence has been linked to many autoimmune diseases, such as multiple sclerosis (MS) and rheumatoid arthritis (RA). In particular, $CD4^+$ T cells gain aberrant, possibly cytotoxic functions after repeated antigenic stimulation or homeostatic proliferation. Until now, the absence of CD28 has been used as a marker for these senescent $CD4^+$ T cells. However, a marker which is present on the surface of these cells can greatly benefit the isolation and further characterization of this subset of T cells. Flow cytometric characterization of these cells showed that CX_3CR1, the fractalkine receptor, was present on the vast majority of $CD4^+CD28^-$ T cells and mostly absent on $CD4^+CD28^+$ T cells. Therefore, this molecule can be used as a surface marker of senescent $CD4^+$ T cells to identify them in target tissues. Furthermore, interleukin (IL)-15 will also be studied for its pathogenicity-enhancing effects on these cells.

Methods and tissues used

Identification of $CD4^+CX_3CR1^+$ T cells was performed on brain tissue of 16 MS patients (both plaque tissue and normal appearing white matter) and 1 non-demented control by fluorescence double stainings. In addition, IL-15 expressing cells were identified in these tissues.

Results and conclusion

These fluorescence stainings revealed that $CD4^+CX_3CR1^+$ cells were present in 6 out of 16 tested MS patients. Also, double stainings for CX_3CR1 and other brain cell markers revealed that microglia, neurons and oligodendrocytes also express CX_3CR1. Moreover, an apoptotic caspase-3^+ oligodendrocyte was found in close proximity to a $CD4^+CX_3CR1^+$ cell. This might be an indication that $CD4^+$CX3CR1$^+$ cells are able to kill target cells in vivo. Additional in vitro experiments revealed that these cells have indeed cytotoxic capacities. These experiments also showed that IL-15 enhances their cytotoxic profile. In MS lesions, we found that astrocytes and microglia are a major source of IL-15. Therefore, we hypothesize that $CD4^+CD28^-$CX3CR1$^+$ T cells contribute to the inflammatory processes in a subgroup of patients with MS and RA.

A novel candidate in macrophage targeting for the treatment of autoimmune disorders

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Research question and background

Recently, a novel receptor, exclusively expressed on specific subsets of macrophages (mϕ), has been identified. Because of the important role of these mϕ in the immune system, targeted manipulation of these cells via this receptor might be promising in steering immunity. Therefore, we are developing a technology that can either provoke, modify or eradicate an immune response.
To evaluate if the technology can be used for treatment of multiple sclerosis (MS), we initially aimed to study expression levels of this receptor on mϕ in MS patients.

Methods and tissues used

Therefore, an immunofluorescent double staining for this receptor and mϕ has been performed on brain material of 19 MS patients, compared to 9 non-demented controls. Afterwards, the percentage of mϕ expressing the receptor has been calculated in 20 randomly chosen brain areas. Finally, also the location of these mϕ has been determined according to the lesion type, visualized by proteolipid protein (PLP).

Results and conclusion

Results illustrate that the percentage of mϕ expressing this receptor is significantly increased in the brain of MS patients (25%) compared to the controls (<10%). According to the PLP staining in combination with mϕ appearance, all MS cases were subdivided into an active and a chronic phenotype. An active lesion is characterized by a high number of PLP positive mϕ inside the demyelinated area. A chronic phenotype is differentiated by only a few PLP positive mϕ inside the demyelinated lesion but a strongly pronounced mϕ rim. Our results indicate that in the active phenotype, mϕ expressing the receptor are highly present inside the lesion while in the chronic patients; most of these mϕ have migrated towards the lesion rim. These findings can have some implications for therapy since cells inside an active lesion have been proposed to be responsible for the damage in MS brains while it is not yet clear whether cells in the rim are rather protective or harmful. This will be investigated in the near future. According to these data, it can be concluded that this receptor seems to be involved in pathological processes in MS.

List of publications 2011-2012 where NBB material is used

Broux B, Pannemans K, Zhang X, Markovic-Plese S, Broekmans T, Eijnde BO, Van Wijmeersch B, Somers V, Geusens P, van der Pol S, van Horssen J, Stinissen P, Hellings N. CX(3)CR1 drives cytotoxic CD4(+)CD28(-) T cells into the brain of multiple sclerosis patients. J Autoimmun. 2012 Feb;38(1):10-9. doi: 10.1016/j.jaut.2011.11.006. Epub 2011 Nov 26