Toward immune intervention in MS

Abstract in English
Towards immune intervention in MS

The aim of the studies described in this thesis was to modulate the immune responses that are believed to be involved in MS. This is a disease that is characterized by inflammation of the CNS resulting in neuronal dysfunction. Local inflammatory lesions contain T cells, macrophages and monocytes and are accompanied by the production of cytokines and chemokines.

In our research group, we hypothesize that the myelin-associated protein αB-crystallin plays a key role in the CNS inflammatory disease MS. This notion is based on studies that have shown that αB-crystallin is the dominant antigen in MS-affected myelin for human T cells. In both patients and healthy subjects a strong peripheral αB-crystallin reactive memory T-cell response exists qualifying it as a potential autoimmune target in MS. Also, as shown in this thesis, readily detectable antibody levels exist in human serum that are selectively directed at αB-crystallin.

These results indicate that there is not an unusual peripheral immune response in MS but rather a local CNS problem causing local inflammation. Thus, our present view of the immune responses that take place in MS is that they consist of a combination of a pre-existing peripheral T-cell response to αB-crystallin together with increased expression and presentation of αB-crystallin due to local CNS inflammation. Although αB-crystallin-reactive-T cells as such are not considered as the primary cause of CNS inflammation, they are believed to aggravate such inflammation to clinically overt levels. This would be consistent with the apparent lack of differences between the T-cell and antibody repertoires of MS patients on the one hand and of healthy controls on the other.

An animal model that combines a pro-inflammatory T-cell response against αB-crystallin and local CNS inflammation is still lacking. Therefore, commencing our study of immune modulatory methods, we first developed an animal model that combines both these immunological events. This combination is in line with our present views as to the relevant immune response in MS. CNS inflammation in animal models may be induced by immunization with myelin proteins, generating myelin-specific T cells. The presence of these T cells results in CNS damage in genetically susceptible mice. Despite extensive efforts, very few clinical or histological signs of CNS inflammation have been detected in rodents after immunization with αB-crystallin. This is because these rodents are fully tolerant to αB-crystallin in contrast to humans. Therefore, we used αB-crystallin−/− mice to generate αB-crystallin-specific T cells. We first showed that transfer of αB-crystallin reactive T cells obtained from αB-crystallin−/− mice into MHC-compatible wildtype mice in the absence of any CNS
Summary

stress, did not induce any form of CNS inflammation. Hence these T cells are not encephalitogenic when present in the periphery even in large numbers. Yet, when recipient mice were infected with the neurotropic Semliki Forest virus followed by a transfer of αB-crystallin-reactive T cells, these mice developed mild yet clear signs of acute EAE. In this model we confirmed that αB-crystallin-reactive T cells alone are not encephalitogenic but become so when an expedient pro-inflammatory environment in the CNS promotes the influx of T cells and local functional presentation of αB-crystallin to these T cells.

Mice deficient in αB-crystallin themselves also turned out to be very useful in studying immune modulation of αB-crystallin-specific immune responses. These mice developed a T-cell response against αB-crystallin together with an antibody response against this protein. Such an antibody response is also detected in humans. In this thesis, antibody levels against αB-crystallin of MS patients with and without uveitis were compared to healthy controls. The results showed that the established antibody levels are similar in MS patients and in healthy controls, meaning that they are a normal aspect of the adult human immune repertoire. Thus, both the antibody response and the T-cell response against αB-crystallin that can be induced in αB-crystallin−/− mice are representative for the human situation, making these mice very useful to test αB-crystallin-specific immune modulation.

We further focused on different methods for modulating the immune response. First, various flavonoids were studied for their anti-inflammatory capacities in vitro. These studies provide new evidence for an important role of certain flavonoids as anti-inflammatory compounds in reducing T-cell proliferation and the production of inflammatory cytokines. The T cells used were obtained from mice and from humans. Mouse-derived T cells were reactive to the peptide 139-151 of the myelin protein PLP. Human-derived T cells were reactive to αB-crystallin. We found markedly reduced activation of antigen-specific T cells in vitro. The structurally related flavonoids luteolin and apigenin were especially potent inhibitors of both proliferation and IFN-γ production by antigen-specific T cells. This was observed with both mouse and human antigen-specific T cells. This inhibition by flavonoids was very promising and we therefore subsequently examined whether flavonoids may also reduce a pro-inflammatory T-cell response in vivo using the model of EAE in SJL mice. Upon immunization with PLP139-151, these mice develop both acute and subsequent chronic signs of EAE, which is known to be primarily mediated by T cells. Reduction of the T-cell response in vivo would therefore be expected to lessen clinical signs. Indeed, when mice were fed with luteolin, apigenin or morin, the proliferative T-cell reactivity was reduced. Yet, to our surprise, recovery from inflammation in actively induced EAE was reduced by two
out of four flavonoids tested. Curcumin, the only food component used that was not a flavonoid, had mild beneficial effects in reducing the inflammation. Reduced recovery from EAE was even more pronounced when PLP_{139-151}-specific T cells, isolated from flavonoid-fed mice, were transferred into untreated recipient mice. These mice developed clinical signs of EAE and showed also a clearly reduced recovery from the acute inflammation.

Another way to influence the immune response is by using probiotics. Probiotics are live microbial food supplements that, in contrast to most other bacteria, exert health benefits for the host in having anti-inflammatory properties not only in the gastrointestinal tract but also in the periphery. Probiotics most likely exert their immune-modulating effects by altering DC function. The effects of probiotics on DC were studied, primarily in order to find markers that can predict whether the selected probiotics species may be beneficial for use in vivo. As a first step, the expression levels of Toll-like receptors (TLR) on DC under influence of probiotics were explored. TLR shape the adaptive immune response that is mediated by DC, by direct recognition of pathogens. Also, surface markers and cytokine production by DC were monitored after probiotic stimulation. Probiotic-stimulated DC reduced the expression of TLRs 1, 2, 4 and 6 and increased the expression of TLRs 7-9, as compared to unstimulated DC. Also, DC displayed increased expression of the antigen-presenting molecule HLA-DR and the costimulatory molecules CD40, CD80 and CD86. Therefore, the strains used are indeed capable of activating the DC which will be of relevance to direct and polarize the T-cell response.

A more specific approach in immune modulation is targeting the antigen-specific response. As discussed, αB-crystallin reactive T cells are not primarily responsible for the pathogenesis of MS but they are considered a major driving force that aggravates and maintains local inflammation in the CNS. Abrogation of the peripheral αB-crystallin-specific T-cell response is therefore expected to reduce inflammation in MS. We studied tolerance induction specific for αB-crystallin in αB-crystallin^{-/-} mice. This study showed that a strong established T-cell response against αB-crystallin can be reduced by at least 80% after intravenous administration of αB-crystallin. The established tolerance was antigen specific, rapidly induced and the reduced response remained present for a period of at least 18 weeks. Tolerance induction as developed in mice proved highly effective and can be potentially very useful as a therapeutic approach in MS.