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Neurotransmitter receptors in health and disease

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Immunomotherapy of Myasthenia Gravis

We have demonstrated that mucosal administration of recombinant fragments corresponding to the extracellular domain of the human acetylcholine receptor (AChR) α-subunit protects rats against the induction of experimental autoimmune myasthenia gravis (EAMG) and immunosuppresses an exisiting disease. However, in severely affected rats, this antigen-specific approach may need to be supported by direct modulation of key cytokines and costimulatory factors known to be involved in the pathogenesis of EAMG. We employed antibodies either to the proinflammatory cytokine IL-18 or to the costimulatory factor CD40L, to immunomodulate EAMG. Both treatments impaired AChR-specific Th1 cell differentiation with no effect on Th2-type responses. The most significant suppressive effect of both treatments was observed 2-3 weeks after initiation of treatment and was later diminished. We suggest that antagonists to key cytokines and/or costimulatory factors be used in conjunction with the antigen-specific treatment by induction of mucosal tolerance with AChR recombinant fragments.

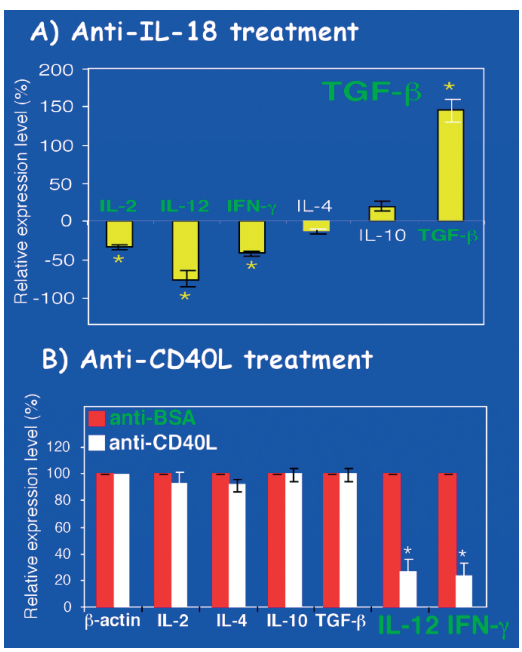

Fig. 1 *Effect of antibody treatment on cytokine profile*

The binding site of acetylcholine receptor (in collaboration with E. Katchalski-Katzir)

Neurotoxins, such α-bungarotoxin (α-BTX), bind specifically and with high affinity to AChR and are instrumental in the analysis of the ligand binding site of AChR. By using a combinatorial phage display-epitope library we have previously identified a 13-mer peptide mimotope that interacts with α-BTX and has similar structural motifs to the the binding region of the AChR α-subunit. Additional peptides derived from this library-lead peptide were designed and characterized. Four peptides, designated high affinity peptides (HAPs), homologous to the binding region of AChR, inhibited the binding of α-BTX to AChR with IC_{50} of 2 nM. The solution and crystal structures of complexes of α-BTX with HAP, were solved (in collaboration with T. Scerf and J. Sussman, respectively), demonstrating that the HAP fits snugly to α-BTX and adopts a beta-hairpin conformation. Superposition of the X-ray structures of the bound HAP and the homologous loop of the acetylcholine binding protein (AChBP), results in a model indicating that α-BTX wraps around the receptor binding-site loop, and in addition, binds tightly at the interface of two of the receptor subunits. Our proposed model explains the strong antagonistic activity of α-BTX, and accommodates many of the biochemical data on the mode of interaction of α-BTX with AChR.

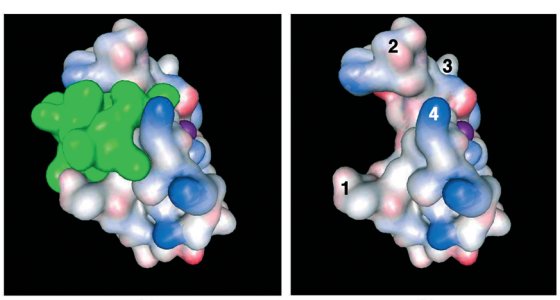

Fig. 2 *3D structure drawing of the α-BTX-HAP complex (a; HAP is shown in green) and of α-BTX with HAP removed (b)*

Dopamine receptors: Signal transduction in the D2 receptor family

Our interest is to elucidate the molecular and functional basis of dopamine receptor diversity. The G protein coupling mechanisms of D2, D3 and D4 receptors appear to be distinct. We demonstrated that the D2 dopamine receptor couples to G_i and to G_z to the same degree, whereas the D4 receptor couples more efficiently to G_z than to G_i. By contrast, D3 receptor can couple to G_z, and, albeit poorly, to G_i, but unlike D2 and D4, it also couples to the stimulatory G_s. To further investigate the molecular basis for the differences between D2 and D3 dopamine receptors in their second messenger coupling, we studied four chimeras each composed of different segments of the original D2 and D3 receptors. We demonstrated that chimeras with a third cytoplasmic loop of the D2 receptor, couples to G_i protein, like D2 receptor. On the other hand chimeras containing a third cytoplasmic loop of the D3 receptor have coupling characteristics of the D3 receptor and couples also to G_s protein. Thus, the third loop determines and accounts for the coupling of D2 and D3 dopamine receptors to G proteins.

Peripheral markers for schizophrenia

We are interested in the possible association of dopamine receptors and of acetylcholine receptors with schizophrenia. We attempt to develop reliable markers for schizophrenia by analyzing the correlation between schizophrenia and levels of these receptors in peripheral blood lymphocytes (PBLs). We have reported recently on a significant elevation of 2-6-fold in the levels of mRNA that encodes for the D3 receptor, in PBLs of schizophrenic patients, when compared with healthy controls.

In view of recent studies on the possible involvement of neuronal α-7 nicotinic AChR (α-7 AChR) in the pathogenesis of schizophrenia, we investigate the levels of mRNA for α-7 AChR in PBLs, as another potential biological marker for schizophrenia. In a preliminary study we observed a significant decrease (20-95%) of α-7 mRNA levels in PBLs of schizophrenic patients, compared with controls. The availability of two different biological markers (D3 and α-7) that can be tested in PBLs, makes the evaluation of schizophrenic patients by peripheral and objective tests, rather promising.

Selected Publications

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Acknowledgements

Sara Fuchs holds the Ernst B. Chain Professorial Chair of Neuroimmunology. Work in the lab is supported by grants from the Muscular Dystrophy Association of America and the Association Française contre les Myopathies.