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Immunogenetics of Narcolepsy

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Introduction

The important role of genetic factors in the pathogenesis of narcolepsy was realized very early in the course of research on this disease. One of the first recognized instances of narcolepsy, reported by Westphal in 1877 (1), was a familial case, as the mother of his patient suffered from the same disease. More than 100 years later, in 1984, the groups of Juji and Honda in Tokyo (2), and of Langdon and Parkes in London (3), found what could be the biochemical basis of hereditary factors in narcolepsy. Almost simultaneously they reported that all of their narcoleptic patients carry the HLA type DR2, while this antigen is present in only 25–33% of the general population.

Since then, immunogenetic methods have played a crucial role in narcolepsy research. As this field may not be familiar to those working in traditional areas of sleep research, this presentation will first give a short introduction, and then summarize some recent immunogenetic data which further outline the role of the HLA-system in the pathogenesis of narcolepsy.

Basic Aspects of the HLA-System

The Human Leukocyte Antigens (HLAs) are proteins which can be found on the surface of almost all human cells which contain a nucleus. Usually they are identified on leukocytes by serological methods, using specific antibodies. They first gained recognition in connection with transplantations, because they play an important role in the detection and rejection of foreign tissue by the immune system. HLA-identical transplants have a better graft survival than HLA-incompatible ones. HLA-antigens are, as we know now, also involved in the mediation of many other immunological processes in the body.

The structure of the HLA-antigens is genetically encoded on the short arm of chromosome 6 in an area called the «major histocompatibility complex» or «HLA-region» (Fig. 1). In the HLA-region a large number of genes has been identified already, but there are still major sections with unknown functions. Besides the various HLA-genes there are also genes which encode complement factors and enzymes for cortisol synthesis. The gene products of the HLA-region are divided into three «classes» according to their structure and function (Fig. 2): class I contains the HLA-groups A, B, and C, which are involved in the cell mediated immune response. They are target structures for self-recognition by cytotoxic T-cells. Class III, which is located between classes I and II, comprises genes for complement factors and for cortisol synthesis. The class II proteins are concerned with the immune response against soluble antigens. One of them is the DR antigen; neighboring ones are DQ and DP. The DR-region consists of several individual genes. They encode the two separate protein chains which together form the DR-molecule. The DRA is the gene for the alpha-chain, and shows little polymorphism. The DRB gene, which is present in several additional, usually inactive copies

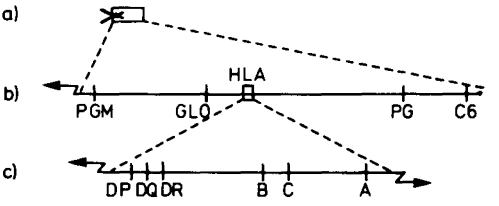

Fig. 1: Location of the genes for HLA-antigens on the short arm of chromosome 6, in schematic magnification. a) Chromosome 6 during mitosis. b) Relative location of some important genes on the short arm of chromosome 6. HLA = HLA-region («major histocompatibility complex»); PGM = phosphoglucomutase 3; GLO = glyoxalase I; PG = pepsinogen; C6 = complement component 6. c) HLA-region with gene-loci for HLA-antigens A, B, C, DQ, DP, DR.

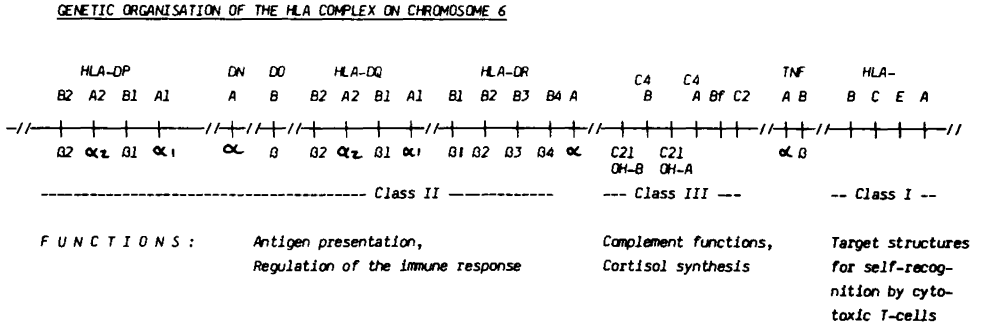

Fig. 2: Localization and function of genes in the HLA-region on chromosome 6: HLA A, B, C = Class I histocompatibility genes; HLA DN, DO, DP, DQ, DR = Class II histocompatibility genes. HLA-antigens usually consists of two separate protein chains, which are encoded by different genes; «A» denotes the gene for the alpha-chain, «B» the gene for the beta-chain (e.g., HLA-DRA = gene for alpha-chain of DR-antigen). «B1», «B2» etc.: multiple copies of the respective gene. C4 = complement component 4; C21-OH A = C21-hydroxylase A; C21-OH B = C21-hydroxylase B; Bf = Properdine-factor (B-factor); C2 = complement component 2; TNF = tumor necrosis factor.

(DRB2, DRB3, etc.), encodes the beta-chain of the DR-molecule. This gene exists in multiple allelic versions which are responsible for the variability of the DR-molecules.

As it may well be that it is the DR2-molecule itself, and not any neighboring gene, which is involved in the pathogenic process of narcolepsy, the function of this protein will be described in more detail. The DR-molecules, like other class II molecules, are primarily located on the surface of «accessory cells», which are mainly a type of macrophage. As mentioned above, the DR-molecule consists of two separate protein chains, alpha and beta, which are linked by noncovalent binding. Both chains are fixed in the cell membrane at one end (Fig. 3). On the far end of the molecule the proteins form a binding site for antigens. By X-ray cristallography the three-dimensional structure of this binding site has been analyzed; it has the form of a groove. The walls of this «groove» are formed by two protein alpha helices, with the bottom being made up of several parallel strings of the protein chains, looking somewhat like a gridiron. The variations between the HLA-DR types involve specific parts of the beta-chain in the section of the «walls», which results in a slightly different shape of the «groove». The function of the class II molecules is schematically shown in Fig. 4. In the process of recognition and binding of antigens, the class II molecules closely interact with the T-cell-receptor (TCR) in a stepwise reaction. The TCR is an immunoglobulin-like receptor on the surface of a T-lymphocyte, which

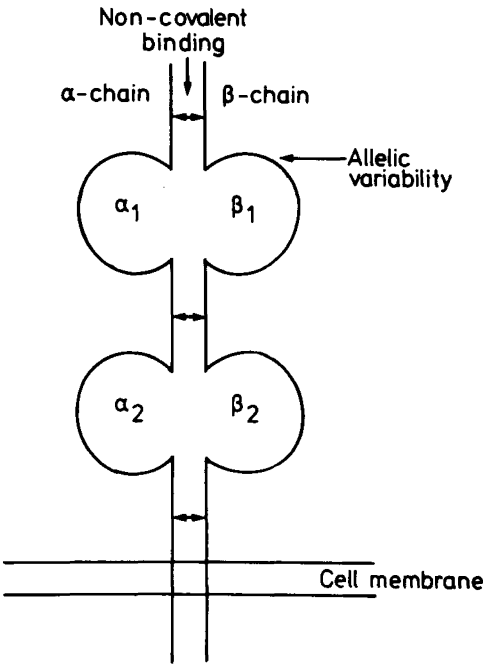

Fig. 3: Structure of class II molecule (e.g., HLA-DR molecule). Two protein chains are fixed in the cell membrane of an accessory cell. Alpha and beta chain are linked by noncovalent binding. Allelic variability of HLA-DR types is due to variations of the protein sequence in the beta-1 loop.

is highly specific for a certain antigen. The first step of this reaction is a non-specific binding of an antigen by the class II molecule. The binding strength to a certain antigen is determined by the form of the «groove», it varies according to the specific properties of a certain HLA-DR type. This binding is called «presenting» of the antigen. The «presenting» is a prerequisite for the next step, which is the binding of the TCR to its specific antigen. After this, the T-lymphocyte reacts in its specific way by stimulating further steps of the immune response.

For the DR-locus, now 16 allelic types have been identified, for other HLA-loci even more variants are known. They are expressed codominantly, so that the serologically defined HLA phenotype of an individual is determined by the genes on both chromosomes 6.

A statistical association with certain HLA types is known for a number of diseases. Most of them «run in families», like narcolepsy, but they do not show a clear Mendelian pattern of

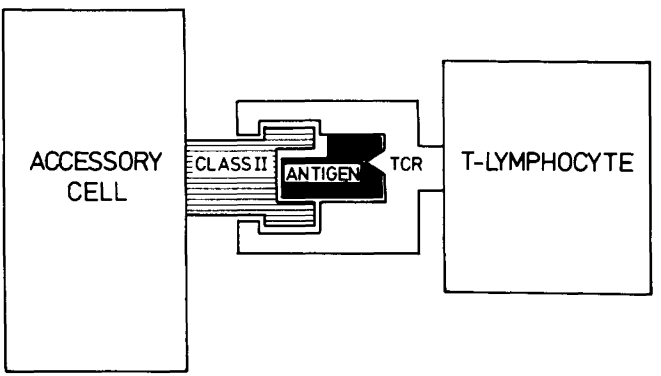

Fig. 4: Interaction of a class II molecule and a T-cell-receptor (TCR) in the process of antigen binding by a T-lymphocyte. The TCR can only bind to its specific antigen when it is presented by a class II molecule like HLA-DR or DQ.

transmission, such as, dominant or recessive. The strongest association next to narcolepsy is known for spondylitis ankylosans, Bechterew's disease. About 90% of these patients carry the HLA B27, compared to 8–10% in the general population. Other examples are, in the order of decreasing strength of association, coelilac disease (DR3 + DR7), juvenile type of diabetes mellitus (DR3 + DR4), and multiple sclerosis, which is associated with DR2.

HLA-Antigens and Narcolepsy

Since 1984, a number of follow-up studies have been performed on the association between HLA DR2 and narcolepsy in different populations around the world, and there appear to be interesting differences depending on the racial background of the patients. In Japanese narcoleptics, representing the Oriental race, not a single DR2-negative case has been found among 190 patients (4). In the Caucasian race DR2-negative patients are very rare, but they are found at a rate of 1–2% in various countries (5–7, see also 8). There is only one study which refers to a Negroid population. In 18 narcoleptic patients Neely et al. (9) found DR2 only in two thirds of the patients. Other authors also reported single cases of DR2-negative narcolepsy in Negroes (5, 10).

In a study which was performed in cooperation with the Neurological Clinic of the Charles University in Prague (11), we examined a total of 131 patients who suffer from narcolepsy, according to the criteria proposed by Honda (12). 129 of these patients carry the HLA DR2, and the other two do not. The results were confirmed by duplicate serological testing and by restriction fragment length polymorphisms (RFLP) analysis. The complete HLA types of the DR2-negative patients were A11/-, B55/-, DR4/5, DRw52/53, DQw3/-, and A2/-, B60/61, DR1/5, DRw52/-, DQw1/3. Clinically, it was not possible to find any difference between the two DR2-negative patients and the others. In 6 of the patients, narcolepsy can be considered secondary to trauma or infection. All of them were DR2-positive, a finding which corresponds to the results of Matsuki et al. (4) who also found all of their patients with symptomatic narcolepsy to be DR2-positive.

The standard method of HLA-typing is based on the reaction of living lymphocytes with defined antibodies. An alternative method, which in certain cases permits the detection of subtypes of serologically defined HLA types, is the analysis of RFLP. This technique shows genetic variations directly on the level of the DNA. In principle, the RFLP analysis is performed as follows: the DNA is extracted from the cell nuclei and digested by «restriction enzymes» which «cut» the DNA chain wherever a specific sequence of nucleotides occurs. The resulting «restriction fragments» are separated by electrophoresis and subsequently hybridized with a radioactively labeled probe which specifically binds to the gene under study. Finally, the fragments of the gene to which the probe was bound, are made visible by autoradiography.

With this method, two subtypes (splits) of HLA DR2 can be distinguished, using the restriction enzyme Taq 1. These splits are now officially listed as DRw15 and DRw16. Similarly for HLA DQw1, which is strongly associated with DR1, DR2 and DRw6, the splits DQw5 and DQw6 were detected. DQw5 associates with DR1 and DRw16, DQw6 goes with DRw6 and DRw15. The DRw15 is much more frequent in the German population than DRw16. In 118 random controls, DRw15 was present in 14%, DRw16 in 0.9%. From our 129 DR2-positive narcoleptic patients, 128 were DRw15/DQw6, one was DRw16/DQw5. This is a very recent result and it will have to be followed up, especially regarding the single DRw16 patient. The DR2-negative patient who was positive for DQw1 carries the DQw5 split of this antigen. This result does not confirm the notion of some authors that the primary association of narcolepsy might not be with DR2 but rather with DQw1. In this case one would expect that the DR2-negative narcoleptics carry DQw6, like the DR2-positive ones.

Another interesting result of our study refers to the DR type on the second chromosome 6 of

our patients. From all published studies, it is obvious that the effect of DR2 is dominant, i.e., the frequency of DR2 on the second haplotype is not higher than statistically expected. The same result was found in our 131 patients, but there is another important difference in the distribution of the DR types compared to the controls. Only 7 out of 131 narcoleptic patients carry DRw6, corresponding to a gene frequency of 2.7%, while in the 118 controls the gene frequency of DRw6 was 18%. These values were confirmed by RFLP analysis. The difference is significant even after correction for the increased rate of DR2 in the patients. When reviewing the literature on this point, we found that many other authors had reported similar results in Caucasians, without further discussing this topic (4, 10, 13–15, see Tab. 1). This rare occurrence of DRw6 in narcoleptic patients could indicate a certain protective effect of this gene against the hypothetical pathogenic agent which sets off narcolepsy.

Tab. 1: Phenotype frequency of HLA-DRw6.

			Narcolepsy		Controls	
Author	Country	Year	n	f (%)	n	f/2 (%)
Matsuki et al. (4)	Japan	1988	190	7.9**	310	9.6**
Billiard et al. (13)	France	1988	35	5.7	110	9.1*
Lock et al. (10)	U.K.	1988	60	8.3	60	11.7*
Meier-Ewert et al. (15)	W- Germany	1988	59	3.4	418	12.3*
Montplaisir and Poirier (14)	Canada	1988	48	2.1	150	11.7*
Andreas-Zietz and Albert (unpublished results)	W-Germany and Czechoslovakia	1988	131	2.7	118	9.0*

Phenotype frequency of HLA-DRw6 in narcoleptic patients and controls. In the controls, the frequency value was divided by the factor 2 in order to correct for the almost 100% frequency of DR2 on the first haplotype of the narcoleptic patients, which lowers the a priori probability for other HLA-phenotypes.
* significant difference between patients and controls ($p < 0.01$).
** combined values for DRw13 and DRw14.

Summarizing the results, the following points evolve:

1. Narcolepsy is very highly associated with HLA DR2, but this association is, at least in Caucasian and Negroid populations, not equal to 100%. A common effort will be necessary to determine if the DR2-negative narcoleptic patients share certain features which distinguish them from the DR2-positive ones.
2. With the available data, it cannot be decided whether the susceptibility gene for narcolepsy is the DR2 gene (DRB1) itself or a gene very closely linked to it.
3. The very low frequency of HLA DRw6 in narcoleptic patients may be a hint that the second haplotype is also involved in the pathogenesis of narcolepsy.

After all these immunogenetic considerations, we should not forget, however, that in the pathogenesis of narcolepsy non-genetic factors must be involved too. This is emphasized by recent reports on monozygotic twins who are clearly discordant for narcolepsy (16, 17).

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