Immune Factors in Narcolepsy

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Summary: Most but not all subjects with the narcoleptic syndrome have the human leukocyte antigen (HLA) DR2 (and DQ1). The narcolepsy-DR2 association is the highest disease-HLA linkage known, and occurs in nonfamilial as well as familial cases of the narcoleptic syndrome. In other forms of daytime drowsiness, there is no relationship with a specific HLA, although some subjects considered to have "essential" hypersomnolence probably have the narcoleptic syndrome. The cause of the narcoleptic syndrome remains unknown, although in a few instances the condition follows infection. There is no evidence for a circulating sleep factor in the blood or in the cerebrospinal fluid of narcoleptic subjects, and no unequivocal marker of cellular immunity has yet been found. However, a few subjects with the narcoleptic syndrome have oligoclonal bands or raised immunoglobulin concentration in the cerebrospinal fluid. It is highly likely that the narcoleptic syndrome is an immune-mediated disorder, occurring in a genetically susceptible (DR2/DQ1-positive) subject. Key Words: Narcolepsy—HLA antigens—Sleep factors.

The cause of the narcoleptic syndrome is quite unknown. The recent finding (1–3) that most if not all narcoleptic patients are DR2/DQ1-positive has led to speculation that possession of these antigens is directly linked to the disease, or alternatively that narcolepsy is associated with an immune process determined by the human leukocyte antigen (HLA) (3). We examined three problems:

(a) Is the association between the narcoleptic syndrome and the HLA DR2/DQ1 absolute, or is another gene in close linkage disequilibrium with DR2 responsible for the disease?
(b) Is there any present evidence for an immune abnormality in the narcoleptic syndrome?
(c) Do nonimmune mechanisms or humoral factors cause the disease?

HLA ANTIGENS IN SLEEP DISORDERS

We selected a group of 62 consecutive excessive daytime sleepiness (EDS) patients attending the King's College Hospital Sleep Disorder Clinic (30 men and 32 women, 61 white and one black subjects; mean age 47 years). Forty-one subjects had the narcoleptic syndrome as determined by clinical presentation. Thirteen of these subjects reported a
first-degree relative with narcolepsy, cataplexy, or both. All subjects had narcolepsy-cataplexy, 19 had sleep paralysis, and 20 had hypnagogic hallucinations. Two subjects with monosymptomatic narcolepsy (short periods of daytime drowsiness), eight subjects with more prolonged day sleep periods (hypersomnolence), six subjects with obstructive sleep apnea, and five subjects with persistent daytime drowsiness following an encephalitic illness were investigated.

Venous blood samples were taken into sodium citrate for HLA A, B, and C typing or into EDTA for HLA DR and DQ typing. Typing for 60 HLAs was carried out with our own plates (Guy’s Hospital Tissue Typing laboratory) made up from workshop sera or from sera standardized against eighth or ninth workshop reagents. Microcytotoxicity testing with phase-contrast microscopy (HLA A, B, C) and trypan-blue exclusion (HLA DR and DQ) was used throughout to assess cell death.

HLA distribution in 62 subjects with different types of daytime drowsiness is shown in Table 1. Although the relationship between HLA DR2/DQ1 and the narcoleptic syndrome is very close, the linkage is not absolute, and possession of the DR2 antigen alone will not therefore account for the disease. The one black subject with severe typical narcolepsy-cataplexy and a family history of narcolepsy was not DR2-positive. In a sibship of three white sisters, one with narcolepsy-cataplexy and two with monosymptomatic narcolepsy, the sister with cataplexy (but not the two sisters with narcolepsy alone) was DR2-positive.

The following definitions were used as the basis for clinical diagnosis in relation to HLA tissue type. Both clinical and polysomnographic diagnoses are open to a number of errors. Subjects with the narcoleptic syndrome had at least one, but not necessarily two, REM sleep onset periods during multiple sleep latency tests, whereas subjects with hypersomnolence had NREM sleep onset (4). Perhaps genetic studies will eventually allow for a more accurate classification of sleep disorders.

**Narcoleptic syndrome**

Synonyms for the narcoleptic syndrome include narcolepsy, primary narcolepsy, and polysymptomatic narcolepsy. This syndrome consists of excessive daytime sleepiness with
cataplexy or sleep paralysis and/or a history of frequent definite pre-sleep dream recall. The great majority of such cases, once established, are life-long and show no evidence of structural brain disease (5,6). However, one DR2-positive patient had a 3-year complete symptomatic remission, another developed multiple sclerosis more than a decade after the onset of narcolepsy, and one developed severe narcolepsy during cerebral irradiation for the prophylaxis of lymphoma.

**Monosymptomatic narcolepsy**

Monosymptomatic narcolepsy consists of EDS, cataplexy, or sleep paralysis alone. For a discussion of this syndrome, see Roth (7). This is likely to be a subdivision of the narcoleptic syndrome, not a separate disorder. However, it is disturbing to find (Table 1) different HLA D-related antigens in different affected family members of a sibship in which different members have monosymptomatic and polysymptomatic narcolepsy.

**Familial and nonfamilial narcolepsy**

Many family studies, including investigation of a few monozygotic twin pairs with the narcoleptic syndrome, have established that ~30% of all narcoleptic subjects have an affected first-degree relative (8–10). It must be remembered, however, that a family history of sleep disorder obtained without personal interview of all family members is unreliable (10).

The clinical presentation of the narcoleptic syndrome, and polysomnographic findings, are usually identical in familial and nonfamilial narcolepsy, although in our experience the mean age of onset of narcolepsy is very slightly lower (by 1.5 years) in familial than nonfamilial cases (11).

**Essential or idiopathic hypersomnolence**

This syndrome has been defined by several authors, notably Roth (7). Subjects have an excess of sleep during 24 h, but without any of the accessory symptoms of narcolepsy. In practice, the term is sometimes applied to sleepy patients without a definite diagnosis. In other instances, the age of onset of daytime drowsiness, the type and persistence of sleep disturbance, and the presence of a family history all closely resemble the narcoleptic syndrome (12). Any large group of patients with idiopathic hypersomnolence almost certainly includes a few with the narcoleptic syndrome (12).

**IMMUNITY AND THE NARCOLEPTIC SYNDROME**

There is no present evidence that any immune or autoimmune process causes the narcoleptic syndrome. However, there are several indications of possible immune involvement in the disease.

(a) Lymphocytic infiltration or other definite pathological change has never been found in the brain of subjects with the narcoleptic syndrome. However, to our knowledge only a single detailed neuropathologic study has been reported to date (13). Here, no specific abnormality was found. In many so-called cases of secondary narcolepsy (i.e., EDS, almost always without cataplexy, and sometimes following encephalitis), periventricular-periaqueductal cellular infiltration is found (14). It must be stressed that in most of these examples, the clinical presentation of daytime drowsiness has little resemblance to that seen in the narcoleptic syndrome.

(b) No circulating antibody or evidence of cellular immunity has yet been demonstrated in the narcoleptic syndrome. However, there is a recent report of raised cerebrospinal fluid (CSF) immunoglobulin concentration in two subjects and of oligoclonal bands in one subject with typical narcoleptic syndrome (11).
(c) The narcoleptic syndrome is not clearly related to other HLA-associated autoimmune diseases such as type I diabetes mellitus or Graves' disease. However, there is a poorly defined relationship between the narcoleptic syndrome and one other DR2-related disorder, multiple sclerosis (15,16), although it is often assumed that when these two conditions coexist, brainstem demyelination is the cause of narcolepsy, and postmortem examination in one such case has demonstrated ponto-mesencephalic plaques (17). This interpretation may be incorrect. Two separate conditions, although with common genetic or environmental determinants, may be responsible.

There are no recorded examples of the narcoleptic syndrome in association with myasthenia gravis, but typical narcolepsy-cataplexy has been described in association with systemic lupus erythematosus, type II diabetes mellitus, encephalitis lethargica and pernicious anaemia (18-20; P. Behan, unpublished observations).

(d) The onset of narcolepsy is occasionally sudden, and some patients describe a preceding febrile illness or respiratory infection. This is found in <10% of all narcoletic subjects and is doubtless fortuitous in some instances, although it is difficult to escape the conclusion that, very occasionally, the narcoleptic syndrome can be “caught.”

**IS THE NARCOLEPTIC SYNDROME DUE TO A HUMORAL FACTOR?**

Sleep factors have been sought for 50 years without success. Piéron suggested in 1913 (21) that sleep might result from the accumulation of a metabolic factor, and others have suggested that sleep itself may produce such a substance or hypnotoxin. None of the old or new candidates, including muramyl peptides, delta-sleep-inducing peptide, or factor S of Pappenheimer (22,23), that have been proposed for this role have stood the test of time or have an established physiological role. Surprisingly, most of these experiments have involved animals, not humans, and the narcoleptic syndrome has not been investigated in this respect. However, if the narcoleptic syndrome is due to a sleep factor, this may be transferable to animals. Such a sleep factor could be either a normal body metabolite or an abnormal substance, the product, for example, of humoral immune mechanisms. It has recently shown that interleukin-1 (a factor released by macrophages and which activates T-lymphocytes) enhances slow wave activity in the sleep electroencephalogram of the rat (24). Also, infection may induce the expression of specific antigens on brain cells (25), in turn leading to an immune response. These mechanisms may be more applicable to postviral infection hypersomnolence than to narcoleptic syndrome itself.

We have examined the effect of plasma and CSF of patients with the narcoleptic syndrome on animal behavior. Although preliminary results were mainly negative, we give brief details of these experiments below.

Plasma and CSF from four subjects with severe narcolepsy-cataplexy were investigated. All stimulant drug and other treatment was stopped 7 days before samples were obtained. At the time of plasma-CSF sampling (2:00–3:00 p.m.) all subjects were drowsy. Control plasma and CSF samples, taken at the same time of day, were obtained from age- and sex-matched subjects with neurological illness but with no sleep disorder. High molecular weight (>65,000) CSF fraction concentrates (× 10 and × 50) were prepared to separate globulin and other protein components.

Benzodiazepine receptor binding activity was assessed in the CSF by one of the authors, (R. D.; Schering AG). Binding activity in the CSF of narcoleptics was not excessive.

The behavioral effects of plasma, CSF, and CSF concentrates from both narcoleptic and control subjects were studied in Wistar rats. Separate intraperitoneal (0.3–0.55 ml/animal)
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and intraventricular (0.025 ml/animal) injections were made. Intraventricular injection was done by cannula implanted into the left lateral ventricle 2 weeks prior to study. The effect of each injection was determined by three to 12 animals, and a total of 60 animals was studied.

Animal behavior was characterized and rated by two independent observers (N. L. and H. W.) who were unaware of whether narcoleptic or control material was used. Exploratory behavior, locomotor activity, rest-activity cycles, grooming, and other behavioral parameters were characterized and rated for a 2-h period following injection and after 24 h, after which the animals were killed.

No major difference between the effects of narcoleptic and control sample material was observed.

These animal behavioral experiments, done without simultaneous physiological controls, do not, of course, confirm or refute the hypothesis that a hypnotoxin accounts for narcolepsy, nor do they give any evidence for or against a humoral immune basis for the disease. A plasma or CSF sleep factor in narcoleptic subjects, if present, may require more sensitive or sophisticated techniques, using different animal species for demonstration. Alternatively, the effects of such a hypnotoxin may be confined to susceptible (DR2-positive) humans, and not be present in animals.

REFERENCES

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