The immune system, the brain and narcolepsy

"If narcolepsy is found to be a selective autoimmune brain disease, it raises the possibility that other such diseases remain to be discovered."

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The brain has long been considered immune-privileged and protected from peripheral immune insults by the BBB. Indeed, few autoimmune diseases are known to affect the brain parenchyma when compared with the impressive list of autoimmune conditions targeting virtually every organ of the body. These autoimmune diseases can typically be divided into organ-specific categories (e.g., Type I diabetes and thyroiditis), or are the result of a more diffuse response (e.g., systemic lupus erythematosus). Furthermore, most immune diseases known to affect the brain either do not target neurons or are not brain region-specific (e.g., multiple sclerosis, encephalitis, CNS manifestations of systemic lupus erythematosus or autoimmune vasculitis), with the possible exceptions of some autoimmune ataxias [1]. Interestingly, the brain also has its own specialized set of antigen-presenting cells, the microglia, and recent results have shown that immune molecules, such as MHC class I or complement proteins, also regulate brain development [2–4]. The relatively low frequency of brain region-specific autoimmune diseases is surprising considering the antigenic complexity of the brain.

Possible explanations for this paradox may come from the study of narcolepsy, where increasing evidence points toward an autoimmune destruction of approximately 70,000 human hypothalamic neurons containing the neuropeptide hypocretin/orexin as the culprit for most cases of this pathology [5,6]. Narcolepsy affects 0.02–0.05% of the population and is characterized by sleepiness, sudden transitions into REM sleep, cataplexy (muscle weakness associated with emotions such as laughing) and a disease onset around adolescence [7]. Indeed, a loss of hypocretin, or of one of its receptors, produces all the symptoms of narcolepsy in animals and the disorder is associated with a loss of hypocretin neurons and dramatically decreased levels of hypocretin-1 in the CSF, a highly diagnostic feature [8]. The first suggestion that narcolepsy involved the immune system came from the pioneering studies of Honda and Juji in 1984, when it was discovered that almost all cases of narcolepsy carry the specific human leukocyte antigen (HLA) subtype DR2 – an antigen only observed in 25% of the general population [9]. Studies in African–Americans subsequently found that DQB1*0602, rather than DR2, most likely in the presence of DQA1*0102 in the formation of the antigen-presenting DQα1/DQβ1 heterodimer, was the culprit behind this very tight association [10]. The association of narcolepsy/hypocretin deficiency with DQB1*0602 is among the highest single allele association reported for any HLA-linked disease. However, despite intensive investigations, no evidence has been found for autoimmunity in narcolepsy, with the exception of a few studies suggesting indirect effects through functional testing of potential autoantibodies [11,12], none of which have been independently replicated. Most notably, the search for autoantibodies directed against hypocretin and hypocretin-producing cells has only yielded negative data [13].

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The primary role of HLA is antigen presentation to T lymphocytes and subsequent coordination of humoral- and cellular-based adaptative immune responses. HLA proteins were first discovered as critical polymorphic proteins to match for successful graft transplantation. Later studies found that their extreme polymorphism/diversity in a population is critical in explaining interindividual immune responses to selected antigens. Indeed, more than 20 HLA genes are known, each encoding a large number of alleles – up to 150 different subtypes for some loci. Amino

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acid diversity primarily occurs at the level of the antigen-binding cleft of HLA molecules, an area where the antigen binds to activate the T-cell receptor (TCR). The diversity of HLA molecules in the population ensures a large amount of antigen presentation diversity across populations, thus better protecting the population against infections. Indeed, the course of various infections is well known to be HLA dependent, for example in the case of HIV infection, hepatitis C or leprosy [14–16].

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Interestingly, whereas in infectious diseases course or severity rather than incidence are typically modulated by HLA, the most tightly HLA-associated diseases are organ-specific autoimmune disorders. These include, to cite a few, HLA B27 and spondyloarthropathies, DQA1*05/DQB1*02 and celiac diseases and DR3/DR4 and Type I diabetes. How the various HLA alleles predispose to these diseases is unknown but it likely involves molecular mimicry with foreign antigens, such as those derived from external pathogens and/or from a failure of immune tolerance. Recent genome-wide association studies (GWAS) in various autoimmune diseases have shown extremely strong association within the HLA, as well as very minor effects at various immune-modulating loci, some of which are shared across various autoimmune disorders [17–19]. In most autoimmune diseases, similar to in narcolepsy, genetic susceptibility is mostly mediated through HLA and other genetic effects are minor, interacting with the environment to yield to disease.

With this background, two recent studies are now further suggesting an autoimmune component for narcolepsy [18, 20], although the absolute proof is still lacking; the identification of immune-related pathogenic cytotoxic T-cell clones or of pathogenic autoantibodies is still not available. In the first study, a GWAS study published in *Nature Genetics* [20], Hallmayer and colleagues studied 1930 narcolepsy patients in comparison with 2164 controls, identifying and replicating rs1154155 within the TCR α-locus as a marker for narcolepsy across multiple ethnic groups (Asians, Caucasians and African–Americans). Although rs1154155 only confers a small risk for narcolepsy in comparison with HLA-DQ, it is remarkable owing to its location within the J segment of the TCR α-chain. As the TCR is the receptor for HLA-peptide presentation, this result suggests autoimmunity. The result is nonetheless surprising as none of the other autoimmune diseases that have been subject to GWAS have shown association within the TCR receptor loci [17].

Second, searching for a trigger for the autoimmune process, we tested patients with narcolepsy and age-matched controls for markers of immune response to β-hemolytic streptococcus and *helicobacter pylori* – two infections known to trigger autoimmunity [18]. This search was also investigated based on early reports suggesting an increase in antistreptolysin O (ASO) titers in narcolepsy versus control sera – a result that was, however, not replicated in a further larger study [21–23]. It has indeed been our clinical experience that narcolepsy is increasingly recognized close to onset, whereas 10–20 years ago, the disorder was diagnosed more than 10 years after onset (median time). We reasoned that a possible infectious trigger would not be detectable long after the onset of narcolepsy, thus explaining variable results obtained in these first studies. If streptococcal infections were indeed a trigger for narcolepsy onset, it would be best detected in newly identified patients, many of which had had recent onset. Thus, these patients were the focus of this recent study.

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When compared with controls, we found that ASO and anti-DNAse B titers were highest close to narcolepsy onset and decreased with disease duration. For example, ASO of 200 or higher (anti-DNAse B ≥ 480) were found in 51% (45%) of 67 patients within 3 years of onset, compared with 19% (17%) of 67 age-matched controls ($p < 0.001$) or 20% (15%) of 69 patients with long-standing disease ($p < 0.001$). CRP and anti-*Helicobacter pylori* IgG did not differ with controls. As streptococcal infections are known to trigger other autoimmune manifestations (e.g., syndenham chorea,

rheumatic heart disease and possibly obsessive compulsive disorder), narcolepsy may also be a post-streptococcal autoimmune condition.

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These two discoveries have implications for our understanding of narcolepsy and for the study of autoimmune diseases in general – notably those affecting the brain. Interestingly, narcolepsy onset is not associated with a detectable inflammatory process, as exemplified by the measures of C-reactive protein or neuroimaging studies surrounding disease onset [18,24]. We thus suggest that in narcolepsy, the immune-mediated destruction of hypocretin cells is a selective self-limited process, without significant epitope spreading. The limited epitope spreading [25] may be a feature of neurons as an immune-protected target and could explain difficulties in detecting autoimmune abnormalities in narcolepsy and other neuron-related autoimmune diseases. The fact that HLA class II expression remains constantly repressed in neurons (unlike in glial cells), even in the face of local inflammation, a poorly studied phenomenon, would protect differentiated neurons (a cell type that cannot be easily replaced) against immune-related collateral damage. The specificity of this process may also explain the TCR genetic association – an association not found in other autoimmune diseases. In this model, Rs1154155 or a tightly linked marker within the TCR Jα region, could favor the occurrence of specific variable-joining TCRα pathogenic T-cell clone recombinants or mark a coding change within a J segment that alters TCRα–peptide binding. The fact that narcolepsy, unlike other autoimmune diseases that have been subjected to GWAS, is associated at the genetic level with the TCR locus could reflect oligoclonal/monoclonal selectivity of the T-cell-mediated immune process in the pathology. By contrast, most other known autoimmune pathologies involve complex polyclonal responses (and possibly multiallelic TCR associations that would be difficult to detect).

How would streptococcal infections be involved in this context? In the first model, destruction of hypocretin neurons could occur through a molecular mimicry of streptococcus-derived antigens with hypocretin cell bearing antigens, as suggested in rheumatic heart disease for cardiac valvular tissue [26]. In this case, if both TCR idiotype and peptide could be identified, narcolepsy could offer the unique possibility of modeling a specific trimolecular HLA–peptide–TCR complex that leads to autoimmunity. TCR–peptide–HLA interactions have indeed been established in specific instances of autoimmunity, such as in experimental models after peptide injections in mouse models, but, to our knowledge, have never been formally identified in any 'natural' human autoimmune disease.

Alternatively, interactions could be mediated through streptococcus superantigen binding, rather than peptide presentation. Superantigens are produced by various bacteria; notably *Streptococcus* and *Staphylococcus* are known to stimulate a large number of T cells (more often binding TCRβ rather than TCRα), and are less specific. However, the first model of TCR-peptide–HLA is more likely, considering the high-target specificity (hypocretin cells) of narcolepsy. Interestingly, superantigens are known to be involved in the mediation of toxic shock and DRB1*1501-DQB1*0602 is protective against *Streptococcus pyogenes* septic shock [27]. Finally, these infections could simply make it permissive for other, more specific, factors to trigger narcolepsy, for example, by increasing blood–brain permeability, or by reactivating a dormant pathogenic T-cell clone via superantigen activation.

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Although still speculative at this juncture, a model is emerging where narcolepsy is a selective autoimmune disease. The possibility that unique DQB1*0602–TCR narcolepsy-causing interactions would occur at the level of the brain independently of autoimmunity is still possible, as the TCRβ locus has been shown to be expressed in the brain [28]. However, in this case only joining-constant unrecombined mRNA products were found without corresponding identification of coding products (TCRα was not studied). Even if expressed, these truncated proteins would not be likely to be functional enough to interact with HLA. This, together with the fact that neurons are unable to express HLA class II molecules such as HLA-DQ even under extreme stimulation (in contrast to microglia and astrocytes, or Class I antigens), makes this model less likely.

If narcolepsy is found to be a selective autoimmune brain disease, it raises the possibility that other such diseases remain to be discovered. The fact that hypocretin lesions can produce a very easily clinically identifiable phenotype (narcolepsy–cataplexy) may have made these discoveries possible (similarly to autoimmune ataxias and loss of Purkinje cells [1]). Indeed, considering brain plasticity, specific phenotypes are rarely the result of discrete neurochemical lesions. This is due to the fact that specificity of function is ensured by both circuit organization and cellular/molecular diversity. In the sleep field, for example, almost all circuits are redundant and phenotypic recovery is typical after large brain lesions [29]. It is thus possible that other brain area-selective autoimmune diseases do occur, with selected neuronal cell loss, but have nonspecific clinical effects, for example, expressing as overlapping psychiatric manifestations. The recent finding that HLA is a susceptibility locus for schizophrenia and bipolar disorder in large-scale GWAS studies provide some support for this concept [30–33]. We hope that in time, narcolepsy will not only inform us about sleep, but also regarding other autoimmune diseases of the brain.

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