〔海外ラボ紹介〕

Twenty years of narcolepsy research at Stanford University

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The Stanford Center for Narcolepsy was established in the 1980's by Dr. William C. Dement, one of the discoverer of Rapid Eye Movement (REM) sleep and a pioneer in the field of sleep disorders research and medicine. Since 1990, the Center for Narcolepsy is directed by Dr. Emmanuel Mignot, a medical doctor and a basic researcher. This laboratory is composed of scientists from all over the world dedicated to finding the cause of narcolepsy and developing new treatments for this disabling disorder.

Several hundred patients with the disorder are currently treated at the center or participate in various research protocols. Narcoleptic samples are collected from all over the world for genetic studies. The world's only colony of narcoleptic dogs -mostly Doberman pinschers and Labrador retrievers-is also bred and maintained to study narcolepsy at the genetic and neurochemical level.

Narcolepsy is a chronic neurological disorder characterized by a tetrad of symptoms: excessive daytime sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis. It is the only known neurological disorder with such a dramatic disorganization of sleep. Narcoleptic patients have the unusual tendency of falling asleep directly into REM sleep and experience cataplexy, a sudden episode of muscle weakness akin to REM sleep atonia, when emotionally stimulated. Although several histopathological studies have been performed in idiopathic human narcolepsy, no clear morphological abnormalities have been consistently reported. This suggests that the pathophysiology of narcolepsy involves a minute imbalance in the neurochemical mechanisms regulating sleep rather than a localized lesion or a large developmental abnormality.

Most cases of human narcolepsy are sporadic in nature. Human family and twin studies indicate environmental influences and multiple gene effects. In 1983, narcolepsy was shown to be closely associated with HLA-DR2, DQ1 by Drs. Yutaka Honda and Takeo Juji in Japanese patients. This association was quickly confirmed in Caucasians, with 95-98% of all Caucasian patients being DR2 positive. Further studies performed at the Stanford University Center for Narcolepsy have now established that across all ethnic groups, the association is tightest with a DQB1 allele, DQB1*0602 rather than with DR2, a subtype that is found in 8-35% of controls in various ethnic groups. This is especially important in the African-American population in which DR2 is a poor marker for narcolepsy. HLA-DQB1*0602 is an especially good marker for narcolepsy with cataplexy. More recent studies also suggest that, as for other HLA-DQ associated diseases, complex HLA allele interactions are involved in predisposing to narcolepsy.

Although most diseases with an HLA association are autoimmune in nature, however, all attempts to demonstrate that human narcolepsy is an autoimmune disease have failed. Studies using additional genetic markers in the HLA-DQ region have

shown that HLA-DQB1*0602 and another HLA gene allele located nearby, HLA-DQA1*0102, are the actual narcolepsy susceptibility genes located in the HLA region. The studies of multiple CA repeat markers have also demonstrated that only the genomic segments surrounding the coding region of DQA1 and DQB1 are conserved across all narcolepsy susceptibility haplotypes. Further, the HLA-DQ region has now been entirely sequenced in control subjects and no other candidate narcolepsy genes have been found in the region.

Canine narcolepsy is the only known animal model of the human disorder. Dogs affected with narcolepsy have been shown to display abnormal rapid eye movement sleep, cataplexy and excessive daytime sleepiness. As with human narcolepsy, most canine cases do not have a family history. In Dobermans and Labradors, however, the disorder is transmitted as a single autosomal recessive trait and breeding experiments were successful in establishing a colony of dogs affected with the disorder in 1991. A large number of backcrosses were generated in the following years in anticipation of a linkage analysis. In 1991, a linkage marker for the canine narcolepsy gene was finally identified. A large insert bacterial artificial chromosome (BAC) canine genomic library was built to initiate chromosome walking around the linkage marker. By applying homology mapping between the human and dog genomes, chromosome walking, microsatellite marker typing, and sequencing, we finally identified mutations in the gene encoding the receptor for a novel neuropeptide, the hypocretin (orexin) receptor 2, as the cause of canine narcolepsy (see CELL, August 6, 1999 issue). This peptide was first believed to be important for the control of appetite but this discovery indicates a major role for sleep regulation.

Figure 1: Two narcoleptic Dobermans in the midst of a cataplectic attack. Cataplexy is defined a sudden episode of muscle weakness triggered by emotions. In humans, cataplexy is most typically triggered by positive emotions such as humor, playing, excitement and laughing. In dogs, cataplexy is most frequently triggered by the excitement of a good meal or playing with other animals.

Excessive daytime sleepiness and cataplexy are the most disabling symptoms of narcolepsy. Although non-pharmacological treatments, such as taking regular naps, are crucial to the management of narcoleptic patients, over 90% of patients still have to use medications to alleviate their symptoms. Excessive daytime sleepiness is usually treated with amphetamine-like stimulants. Although these stimulants reduce sleepiness, they unfortunately have little effect on cataplexy, and a combination of both stimulants and anticataplectic medications (typically tricyclic antidepressants) is usually needed to treat all symptoms. These treatments are purely symptomatic and are often unsatisfactory due to unpleasant side effects and incomplete therapeutic efficacy. Furthermore, the modes of action of these compounds on narcoleptic symptoms are largely unknown. The canine model of narcolepsy has been used in a series of pharmacological studies which aim to dissect out the mode of action of currently used treatments for narcolepsy with the hope of developing better treatments for human narcolepsy

Previous pharmacological studies have demonstrated that the monoaminergic and cholinergic systems are critically involved in the control of cataplexy. Activation of cholinergic transmission and/or inactivation of catecholaminergic transmission aggravate cataplexy. Furthermore, specific receptor subtypes which mediate cholinergic and monoaminergic control of cataplexy, such as the muscarinic M2, adrenergic postsynaptic a-1b, presynaptic a-2 and dopaminergic D2/D3 receptors, have been identified. Additionally, polygraphic studies in canine narcolepsy have demonstrated that the dopaminergic uptake system is critically involved in the control of EEG arousal, and that increased dopaminergic neurotransmission mediates the wake-promoting effects of amphetamine-like stimulants. Narcoleptic dogs are very sensitive to the compounds acting on these systems,

Figure 2: Picture of the current narcolepsy staff, an international team devoted to the understanding of narcolepsy and sleep disorders. Emmanuel Mignot, Director of the Center For Narcolepsy, sitting down second on the left on the first row. Seiji Nishino, associate Director, standing up in the right end corner of the group. Lin Ling, currently Research Associate in the Center and author of this article, is standing up in the last row, 5th position from the left side of the picture.

and an altered receptor number has been reported in the specific brain regions in canine and human narcolepsy. Some of the acting sites of these compounds have also been identified by local drug administration in specific brain areas of freely moving narcoleptic animals. Cholinergic stimulation in the pons and the basal forebrain, and stimulation of dopamine D3 autoreceptors in the midbrain, significantly aggravates cataplexy. Basal forebrain cholinoceptive hypersensitivity in narcolepsy is particularly interesting because this area is anatomically connected with the limbic system, suggesting an important link with the emotional triggers of cataplexy.

In parallel with the cloning of the canine narcolepsy gene (a mutation of the hypocretin (orexin)-2 receptor), it has been demonstrated by Dr. Masashi Yanagisawa that knock-out mice with a deficient prepro-hypocretin gene also have a phenotype similar to human and canine narcolepsy (CELL, August 20, 1999 issue). This result independently confirms that the hypocretin system is critically involved in the pathophysiology of narcolepsy. Interestingly, human cases of symptomatic narcolepsy are often reported to be associated with hypothalamic or basal forebrain lesions, areas close to the sites where the hypocretin neurons are located. Further studies are now ongoing to study the potential role of hypocretins in HLA associated human narcolepsy. Other studies are also needed to establish how the hypocretin and the cholinergic/monoaminergic systems mentioned above interact anatomically and functionally to regulate normal and abnormal sleep. In time, we believe our increased understanding of the pathophysiology of narcolepsy will lead to novel treatments for narcoleptic patients and other sleep disorders.

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