論文の内容の要旨

論文題目 Molecular mechanism underlying association of HLA-DQ with narcolepsy
- Interaction of hypocretin-derived peptides with narcolepsy-associated HLA-DQ molecules -
(ヒト白血球抗原 HLA-DQ とナルコレプシー関連の分子機序に関する研究
−ハイポクレチンペプチドとナルコレプシー関連 HLA-DQ 分子の結合性−

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The highly polymorphic HLA gene products are responsible for the presentation of peptide fragments as antigens to the immune system, and many autoimmune diseases are associated with HLA genes. A strong association of narcolepsy with HLA class II has been identified in previous studies, in which narcoleptic individuals of diverse ethnic backgrounds were found to carry a specific haplotype, DQA1*01:02-DQB1*06:02, suggesting that narcolepsy is an autoimmune disorder. Although the mechanism underlying autoimmunity in narcolepsy is not clear, hypocretin levels were found to be significantly decreased in narcoleptic patients, and hypocretin-producing cells were disrupted in the patients' brains. In addition, hypocretin deficiency has been detected in sporadic HLA-DQB1*06:02-positive adolescent-onset narcolepsy cases. Moreover, the DQB1*06:01 and DQB1*06:03 haplotypes are negatively associated with human narcolepsy, and DQB1*06:04 is neutral for narcolepsy. No definitive evidence linking HLA to hypocretin deficiency has been found, and the underlying immunological mechanism remains elusive.

It has been hypothesized that autoimmune-triggered hypocretin loss is the major pathogenic factor for narcolepsy. The susceptible HLA-DQA1*01:02-DQB1*06:02 haplotype product might recognize hypocretin, and therefore, mediate the autoimmune response. This hypothesis might explain why hypocretin-producing cells are destroyed in narcoleptic brain cells.

The crystal structure of hypocretin has been elucidated. However, the nature of the interactions between narcolepsy-associated HLA-DQ proteins and hypocretin-derived peptides has not been investigated. In this study, I constructed a DQA1*01:02-DQB1*06:02 cell line that expresses narcolepsy-associated HLA-DQ molecules and evaluated the binding of these molecules to hypocretin-derived peptides in order to elucidate the autoimmune mechanism of narcolepsy. Subsequently, I determined the interactions between HLA combinations and variant hypocretin peptides by cell-based and plate-based methods. Cell lysates of HLA-DQA1*01:02-DQB1*06:02 (susceptible), DQA1*01:03-DQB1*06:01 (resistant), DQA1*01:03-DQB1*06:03 (resistant), and DQA1*01:02-DQB1*06:04 (neutral) were incubated with synthetic prepro-hypocretin-derived peptides,

and their interactions were analyzed. Interactions between eight prepro-hypocretin-derived peptides and three different haplotype proteins related to narcolepsy were identified in this study. The binding affinities of different risk- and protective-HLA-DQ proteins with different hypocretin-derived peptides reported here provide insights into the role of hypocretin peptides in narcolepsy.

To the best of my knowledge, this is the first study to utilize a systematic binding assay to study the interactions between prepro-hypocretin-derived peptides and risk- and protective-narcolepsy haplotype HLA-DQ proteins. The plate-based peptide assay system is more efficient, sensitive, and specific than the cell-based assay. Therefore, the plate-based peptide assay is an ideal tool for screening autoantigens against the cell expression system in narcolepsy. My findings indicate that hypocretin peptides and the immune system act as specific triggers of narcolepsy. However, from the binding study alone, it is not possible to conclude whether narcolepsy is an autoimmune disease. The results suggest that characterization of hypocretin peptides may help understand the epitopes involved in narcolepsy and provide insights into the pathogenic mechanism of narcolepsy.