Myocarditogenic Epitopes and Autoimmune Myocarditis

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Abstract

Experimental autoimmune myocarditis is provoked by immunization with cardiac myosin. This animal model finally develops into dilated cardiomyopathy through repetitive myosin injections. To identify the myocarditogenic epitope, therefore, it is imperative not only to understand the mechanism of induction, but also to produce specific therapies, such as a blocking therapy to suppress the autoimmune process. Thus, we attempted to identify the myocarditogenic epitope using recombinant peptides. β -cardiac myosin heavy chain (CMHC) was amplified from rat mRNA by a reverse transcription polymerase chain reaction method. The PCR primers were designed to narrow the epitopic amino acid portion from each N-terminal to C-terminal site. These PCR products were cloned into an *E. coli* expression vector to produce fusion proteins consisting of a Histidine-tag and a myosin peptide. The segment of amplified CMHC including the epitopic amino acid sequence to provoke moderate myocarditis *in vivo* was reported previously. Each peptide solution was emulsified in an equal volume of complete Freund's adjuvant and given as an immunization to 7-week-old rats. On day 21 after immunization, the rats were sacrificed, and the fresh heart was observed pathologically. Through this immunization, we could restrict the myocarditogenic site. Lastly, this peptide was found to be located on residues from 1,124 to 1,153. Using ELISA, the antibodies against myocarditogenic peptides were easily identified. Whether or not the antibody productivity is linked to myocarditogenicity is discussed. (Internal Medicine 42: 3–6, 2003)

Key words: myosin heavy chain, autoreactive T cells, myocarditogenicity

Pathomechanism of Experimental Autoimmune Myocarditis

In 1989, we established a novel animal model of autoimmune myocarditis (1). Experimental autoimmune myocarditis,

namely EAM, was provoked in Lewis rats by immunization using cardiac myosin. The features were quite different from those of viral infection (2). The whole figure and pathomechanism of this model is very unique as mentioned previously (3, 4). The histology of EAM is remarkably characterized with enormous cell infiltrate including multinuclear giant cells and broad myocardial necrosis, which are dominantly detected in the epicardial site of every ventricular wall (Fig. 1). Through the transfer experiment, this myocarditis is mediated by T cell autoimmunity (5). The pathomechanism of this disease is explained as follows (4). Myosin fragments as causative epitopes, cardiac dendritic cells as antigen presenters, and myosin autoreactive T cells as effectors are the main elements in this process. The cardiac myosin activates the local lymph nodal myosin reactive Th1 T cells from Th0 T cells and the T cells move to the heart. And as they can encounter the same antigen in the myocardium, these T cells are greatly expanded through dendritic mediation. Consequently, they release interferon γ and IL-2. These cytokines then activate macrophages which allow them to release IL-1 β , TNF α and NO. The amount of NO discharge provokes serious inflammation. Seven weeks later, the T cells gradually shift from Th1 to Th2. This shift diminishes the inflammation. Interestingly, after several months have passed, such a cycle can be reproduced by repetitive immunization with the cardiac myosin (6). Accordingly, it has been proven that this model develops into autoimmune cardiomyopathy at the animal level.

Epitopes of Cardiac Myosin Heavy Chain

As a matter of course, one question has emerged: Which fragmental peptides of the cardiac myosin provoke the autoimmune myocarditis? As broadly known, cardiac myosin is a large peptide. It consists of 1,974 amino acids and the molecular weight reaches up to 210 kilo Dalton. To the date, through numerous experiments, only two representative epitopes have been nominated as primary candidates in Lewis rats. Inomata, my colleague, in 1995 discovered one (7). As shown in Fig. 2, this peptide is located in Segment 2 of the rod portion and consists of 96 amino acids. Another candidate was disclosed by Wegmann in 1994 (8). They ap-

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Figure 1. Features of experimental autoimmune myocarditis. The histology is remarkably characterized by infiltrate of enormous sized cells and broad myocardial necrosis, which are dominantly detected in the epicardial site of every ventricular wall.

proached it using the anchor protein theory. The peptide consists of 17 amino acids and is located in the light meromyosin. Concerning the former epitope, to determine the myocarditogenicity, three experiments had to be undertaken. The first one was done by digesting fragmental peptides from naive cardiac myosin. Through this method, we detected the 96 amino acids as mentioned above, Inomata's peptide. Next, artificially synthesized peptides were employed. In the case of Inomata's peptide, we failed to further identify exact site of the epitope through these artificial products, because there it occurred nothing in spite of immunization with synthesized peptides. But in Wegmann's peptide, we succeeded in provoking myocarditis using artificial ones. Thus, we started the third experiment in Inomata's peptide. The recombinant type of cardiac myosin was chosen to determine the myocarditogenicity (9). The peptide was induced in *E. coli*, to which a part of the myosin gene had been formerly ligated to a protein expression vector. So far, some distinguished investigators have emphasized that the cardiac myosin chain α is stronger than the β in myocarditogenicity (10–12). However, using recombinant myosin, we could demonstrate no difference in initiating and promoting myocarditis between the two (13). We attempted to determine the C-terminal in every amino acid using recombinant myosin immunization. We also approached the N-terminal. Finally, we could determine the exact amino sequence and its site. In fact, the total number of amino acids was 30 from 1,124 E to 1,153 E. Thus, the site is called Kohno's peptide. In comparison with the standard size of other T cell-mediated autoimmune diseases, the length of the epitope was much longer. Thereafter, we added several experiments; We studied the structure and function of the myocarditogenic epitope. The recombinant peptide could provoke the disease and circulate antibodies against the peptide as shown in Fig. 3. However, when the peptide was cleaved in the middle by Lys-endoproteinase, and the cleaved peptides were purified

Figure 2. Major epitope of the cardiac myosin heavy chain. To date, only two fragments have been nominated as myocarditogenic epitopes in the rat. A fragment located in segment 2 of the rod portion and consisting of 96 amino acids was discovered by Inomata et al (7), and another fragment was found by Wegmann et al (8). This peptide consists of 17 amino acids and is located in the light meromyosin. It is called the CM 2 peptide.

by RP-HPLC, then we acquired two major peptides; we called them peak-4 (from 1,111 E to 1,132 K) and peak-5 (from 1,140 L to 1,166 K). Although we immunized rats with these peptides, nothing happened. But, when we conjugated the former peptide, peak-4, to the latter one, peak-5, using glutaraldehyde, it began to function as an epitope. Interestingly, in the synthesized peptide study, only peak-5 showed high antigen productivity. To understand the phenomenon, we propose the hypothesis as shown in Fig. 4. Namely, peak-5 may activate B-cells. This activation probably enhances antigen-presenting activity. And, the stimuli may cause the epitope to spread including peak 4 and, finally the epitope may activate the autoreactive T cell and provoke autoimmune myocarditis. On the other hand, Wegmann's peptide is not so complicated and it is very illustrative to T cell immunity. The peptide may be digested and presented by antigen presenting cells. The T cell triad seemed to induce autoimmune myocarditis. This difference was reflected on the specificity of autoreactive T cell activation (Fig. 5). In the presence of dendritic cells, the T cells isolated from whole myosin immunized rats were tested. In the case of Wegmann's CM 2, the T cells were specifically activated by the peptide in comparison with the controls. On the other hand, in Kohno's peptide, the cells did not indicate specificity against the antigen stimulations. These findings support the possibility that our epitope requires a specific characteristic for antigen presenting.

Comments

The recombinant cardiac myosin, Kohno's peptide, which is composed of 30 amino acids, completely reproduced our EAM model. But even up to the present time, we can not

		Myocarditis	Antibodies
1. Recombinant products			
Ep- α	KKLKENQARIEEELEELFAERTARAKVEKLRSDDL TRELEE I SERLEEAGGATSVQIEMNK	⊙	(+++)
Ep- β	KKLKEQARIEEELEELFAERTARAKVEKLRSDDL SRELEF I SERLEEAGGATSVQIEMNK	⊙	(+++)
2. Cleavage by Lys-Endoproteinase			
Pk-4	ELQARIEEELEEELEAERTARAK	⊗	(-)
Pk-5	LRSDDL SRELEE I SERLEEAGGATSVQIEMNK	⊗	(+++)
3. Conjugation of HPLC Pk-4 + HPLC Pk-5			
Pk (4+5)	ELQARIEEELEELFAERTARAK + LRSDDL SRELEE I SERLEEAGGATSVQIEMNK	⊙	(+++)
4. Synthesized peptides			
SQLQKKLKEQARIEEELEEELEAE		⊗	(-)
ELQARIEEELEEELEAERTAR		⊗	(-)
EEEELEEAERTARAKVEKLRSDDL		⊗	(-)
RTARAKVEKLRSDDL SRELEF I SER		⊗	(+)
LRSDDL SRELEE I SERLEE		⊗	(+++)
SRELEE I SERLEEAGGATSVQIEM		⊗	(-)

Figure 3. Structure and function of the myocarditogenic epitope. The C-terminal of this epitope was determined in every amino acid by using recombinant myosin immunization. We also approached the N-terminal. Finally, we could determine the exact amino sequence and its site. The total number of amino acids was 30 from 1,124 E to 1,153 E. As shown here, the recombinant peptide could provoke the disease and circulating antibodies against the peptide. However, when the peptide was cleaved in the middle by Lys-endoproteinase, nothing happened. But, when we conjugated the former peptide, peak-4, to the latter one, peak-5, using glutaraldehyde, it began to function as an epitope again.

Figure 4. Function and structure of Kohno's peptide. Probably, peak-5 activates B-cells. This activation enhances antigen-presenting activity. And, the stimuli may cause the epitope to spread including peak 4 and, finally the epitope may activate autoreactive T cells and induce autoimmune myocarditis.

clearly understand why this myocarditogenic segment needs such long peptides.

In previous reports, some myocarditogenic peptides had been indicated in other rodent models of autoimmune myo-

carditis. Proposed epitopes were located in different sites of myosin heavy chains, and the severity of provoked myocarditis was also different in each EAM model. Accordingly, these findings support the possibility that several different mechanisms may be involved in EAM. For example, Wegmann's CM 2 consists of 17 amino acids, and the peptide can provoke myocarditis easily. Autoimmunity in this model seems to be according to classical T-cell mediation. On the other hand, using artificial synthetic peptides, Inomata's 96aa segment could not provoke myocarditis. Despite narrowing to 30aa in Kohno's peptide, the artificial peptide provoked nothing quite different from the recombinant one. Certainly different T cell mediation is involved in our autoimmune model in comparison with Wegmann's.

On function studies, the latter part of the Kohno's peptide was a determinant in activating B cells, and the other former part was related to the myosin autoreactive T cells by spreading of the epitope. Accordingly, we suspected our model is closely linked with not only T cell-mediated immunity but also B cell. Referring to antigen-specific T cell activity, this unique epitope has shown quite a different stance from Wegmann's. If we could detect specifically autoreactive T cells against Kohno's peptide not only *in vitro* but also *in vivo*, it would provide us with a significant aid to make the diagnosis of autoimmune myocarditis and autoimmune cardiomyo-

Figure 5. Specificity of autoreactive T cell activation. The difference between the two peptides was detected by on the specificity of autoreactive T cell activation. The autoreactive T cells were easily activated by Wegmann's peptide but not by Kohno's.

pathy in human beings easier and more accurate.

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