Cardiac Dendritic Cells and Acute Myocarditis in the Human Heart

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Cardiac dendritic cells are considered to play an important role in the immunoresponse of the heart. However, it is unclear whether these cells occur in human myocarditis and whether they function in similar ways to those in rats. Cardiac samples were obtained from 22 autopsied patients with myocarditis, and compared with 20 age- and sex-matched controls. Formalin-fixed hearts were immunostained by the LSAB method. Cardiac dendritic cells were detectable even in the control hearts (1.5 cells/high power field (HPF)). In the acute phase of myocarditis, the number of cardiac dendritic cells increased up to 12.6 cells/HPF (p<0.001). In the subacute phase of myocarditis, T cells (36.6 cells/HPF) and HLA-DR+ cells (10.2 cells/HPF) continued to infiltrate the periphery of the inflammatory lesions, but they had no expression without inflammation. In this study, cardiac dendritic cells were reactive for HLA-DR, but negative for CD68, and were characteristically large monocytes with long, slender, dendritic processes. Accordingly, they were clearly distinguishable from macrophages. In the human heart, cardiac dendritic cells may be recruited in the acute phase of myocarditis, and seem to play an important role in the succeeding immunoresponse. (Jpn Circ J 2000; 64: 57–64)

Key Words: Cardiac dendritic cells; Cardiac myosin; Myocarditis; Major histocompatibility complex (MHC) class II

Acute myocarditis in humans is mostly caused by viral infection and many viruses have been proposed as candidates for the pathogenic organism. Immunological studies done to date have shown that infectious immunity, especially cellular immunity, is mainly involved in the cardiac damage of this disease! Furthermore, it is already known that, after the acute phase of myocarditis, an autoimmune mechanism must be secondarily provoked, which persists and develops the myocarditis! From this, Neu et al established a functional model of myocarditis in mice by immunization with cardiac myosin! Subsequently Smith et al succeeded in inducing myocarditis by T cell transfer into SCID mice using the same animal model! In 1990, Kodama et al proposed a novel rat model of lethal myocarditis, which was also provoked by cardiac myosin, and they showed that this model was transferable to healthy rats by activated T cells! The histology of the experimental autoimmune myocarditis is very striking, being characterized by the massive occurrence of cardiac dendritic cells, which is quite different from viral myocarditis.

In autoimmune myocarditis, the cardiac dendritic cells play an important role in initiating and provoking the inflammation. However, autoimmune myocarditis is not yet fully understood, especially as regards the human heart. It is still unclear whether cardiac dendritic cells, which are very important in the immunoresponse of the rat heart, are actually present and function in the human heart. Thus, the aim of the present study was to clarify whether cardiac dendritic cells are present in the human myocarditic heart.

Methods

Subjects

Histological samples were obtained from 42 cases that were autopsied at either Kitasato University or Niigata University; 22 hearts were from patients with acute myocarditis (17 males, 5 females; mean age, 43.0±23.8 years), and 20 hearts were from patients who died of non-cardiac diseases, such as trauma or cerebral bleeding, as the controls (12 males, 8 females; mean age, 42.2±19.2 years). There was not significant difference in the age of the patients with myocarditis (Table 1).

The diagnosis of myocarditis was confirmed according to clinical and histological criteria for the disease. Of the 22 cases of myocarditis, 14 had died of pump failure, and 8 cases were sudden death.

Section Preparation

Four to 5 tissue blocks were excised from the anterior wall, lateral wall, and posterior wall of the left ventricle, the ventricular septum, and the right ventricle. Tissue blocks were also prepared from lymph nodes, tonsil and skin. Each block was fixed using 10% formaldehyde. After dehydration and paraffin embedding according to conventional methods, serial sections of 4-μm thickness were prepared from each block. After the sections were arranged on Silane-coated slides and partially dried on a stretch board, they were dried for 12h in an incubator at 37°C, to prevent destructive changes during the staining process. Frozen sections (in liquid nitrogen) were used as the controls to compare with the formalin-fixed samples.

Several transverse sections were cut from paraffin-embedded samples and stained with hematoxylin and eosin for conventional pathology.

(Received August 4, 1999; revised manuscript received October 13, 1999; accepted October 15, 1999)

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Japanese Circulation Journal Vol.64, January 2000
Immunostaining

Protocol 1 (Identification of the Cardiac Dendritic Cells)

The following mouse monoclonal antibodies were used as the primary antibody to identify the cardiac dendritic cells: HLA-DR (Dako, Kyoto, Japan), CD1a (Langerhans cells; MBL, Nagoya, Japan), CD21 (interdigitating cells; Dako), CD23 (interdigitating cells, Dako), CD35 (follicular dendritic cells; Dako), S100 (Dako) and CD68 (macrophages; Dako). Cardiac tissue sampled from representative cases with active myocarditis, was used to compare with the lymph nodes, tonsil and skin of those patients, and to investigate how to differentiate cardiac dendritic cells by immunostaining from ordinary dendritic cells in other organs.

Protocol 2 (Examination of Cell Infiltrates in Active Myocarditis) The following mouse monoclonal antibodies were used finally as the primary antibodies to examine the cell infiltrates in the heart: CD3 (pan T cells; Dako), CD79a (B cells; Dako), CD68 (macrophages; Dako), and HLA-DR (major histocompatibility complex (MHC) class II; Dako).

In both the first and second protocol, the antigen–antibody reaction was performed by the labeled streptavidin-biotin method. The immunostaining was expressed by color staining with 3,3'-diaminobenzidine tetrahydrochloride (DAB), after which the nuclei were post-stained with Mayer-hematoxylin.

Double Immunostaining

At the same time, each preparation underwent double immunostaining to distinctly differentiate the cardiac dendritic cells and macrophages. HLA-DR+ cells, namely MHC class II+ cells, were stained black using a Vector SG Substrate Kit (Vector, Tokyo, Japan), and CD68+ cells, namely macrophages, were stained red with Vector Red Alkaline Phosphatase (Vector) to differentiate them during color imaging.

Classification and Cell Counting

Myocarditis was classified into 2 subgroups according to the clinical course: an acute phase group and subacute phase group. The acute phase included those who had died of the disease within 2 weeks of its onset, and the subacute phase included those who had died more than 2 weeks later. To compare cell counting among the 3 groups, 70–80 randomly selected high-power fields (HPF) of each heart sample were chosen, and the cells were counted at high magnification (x400). The average number of each infiltrate was expressed as the representative value.

Statistical Analysis

Data are shown as mean±SD. Statistical analyses were performed using STATVIEW V.4.5 software. To compare the data among the 3 groups, Kruskal-Wallis analysis of ranks was used. Differences were considered to be statistically significant at a p value less than 0.05.

Results (Table 2)

As shown in Fig 1, CD1a+ cells, namely Langerhans cells, were clearly observed in the skin. The interdigitating cells, positive for CD21 and CD23, and the follicular dendritic cells, positive for CD35, were also obvious in the tonsil, skin and thymus. Those ordinary forms of dendritic cells were intensively reactive against both the HLA-DR and S100. In simple morphology, they belonged to the spindle-shaped group and had large mononuclear cells.

HLA-DR+ cells, which were seen in the human heart with active myocarditis, were quite different from the aforementioned dendritic cells. Most were characteristically large mononuclear cells having long, slender, dendritic processes similar in structure to the ordinary form of dendritic cells. However, these HLA-DR+ cells were not positive for CD1a, CD21, CD23 or CD35 at all. In addition to those negative findings, these large cardiac monocytes stained negatively with S100, although both neurofibers and fibroblasts were clearly positive (Fig 2).

In comparison with the HLA-DR+ cells, the macrophages, another kind of large mononuclear cell, were round and very reactive for CD68. They had abundant cytoplasm, including phagocytic granules. On close examination, using double staining, which stained the HLA-DR+ cells...
Fig 1. Comparison between ordinary dendritic cells and cardiac dendritic cells. (---; 50 μm). (A,B) HLA-DR; (C,D) S100; (E,F) CD1a. By immunostaining serial sections, Langerhans cells in the skin were confirmed to be positive for HLA-DR (A), S100 (C), and CD1a (E). However, cardiac dendritic cells were positive for HLA-DR (B), but not for S100 (D) or CD1a (F).

Fig 2. Immunohistochemical characteristics of cardiac dendritic cells in the acute phase. (---; 40 μm) (A) HLA-DR, (B) S100, (C) CD68. By immunostaining serial sections, cardiac dendritic cells were confirmed to be positive for HLA-DR (A), but negative for S100 (B) and CD68 (C).
black and the macrophages red, differentiation was easily achieved. Occasionally cells that were double stained with the antibodies against both HLA-DR and CD68 appeared and these double-stained cells belonged to the group that were spindle-shaped and had large mononuclear cells. These results did not differ between the frozen samples and the formalin preparations.

Protocol 2

Control Heart In the myocardial interstitial space, 2 kinds of large mononuclear cells were observed; one was positive for HLA-DR, and the other was positive for CD68. The HLA-DR cells were infrequent in the control myocardium (Fig 3). The number of HLA-DR+ cells was 1.2±1.3/HPF. The cells were present in the epicardium and also the endocardium. In the control heart, HLA-DR+ cells were spindle in shape, and had a large nucleus in the center of the cytoplasm. Each CD68+ cell also had a large mononuclear, but the cytoplasm was very rich and abundant in cell organelles. The CD68+ cells (1.9±1.5/HPF) were rarely seen in the vascular space or among the muscle fibers. The others, CD3+ (1.2±1.1/HPF), CD79a+ (1.0±1.0/HPF), and S100+ cells (1.6±1.2/HPF), were also poorly developed.

Acute Phase of Myocarditis There were 15 of the 22 hearts with acute myocarditis belonging to this subgroup. Each type of inflammatory cell was very productive. They were broadly scattered throughout the whole myocardium (Fig 4). In 12 hearts of this subgroup, T cells were dominant, mainly infiltrating the myocardium (Figs 4C, D). In the remaining 3 hearts, the proportion of infiltrating B and T cells was almost equal. There were also abundant neutrophils. Large mononuclear cells were occasionally seen in the inflammatory lesions. These monocytes were in close contact with the cardiac muscle cells and protruded into several dendritic processes. HLA-DR antigens reacted on the surface of the large mononuclear cells, which were characterized by protruding dendritic processes (Fig 4A). The number of HLA-DR+ cells in the acute phase was 12.6±7.1/HPF, which was significantly higher than that of

Fig. 3. Immunostaining of normal myocardium: (−; 50μm). HLA-DR+ cells were sporadically observed in the myocardial interstitium in all 20 control hearts. The HLA-DR+ cells in the epicardium and around the endocardium were large spindle-shaped monocytes.

Fig. 4. Immunostaining of the myocardium in the acute phase of myocarditis: (−; 50μm) (A) HLA-DR, (B) CD68, (C) CD3, (D) CD79a. There was an increased number of HLA-DR+ cells in the acute phase of myocarditis (A). These cells were in contact with myocytes, and had dendritic processes. T cell infiltration predominated in many cases (C, D). There was also abundant infiltration of neutrophils, and extensive, diffuse infiltration of CD68+ macrophages (B).
Fig 5. Comparison of the number of HLA-DR+ cells, including cardiac dendritic cells, in the acute phase, subacute phase and normal groups. The number of HLA-DR+ cells in both the acute phase and subacute phase groups was significantly higher than in the control group (p<0.001 for each). The number of HLA-DR+ cells in the acute phase and subacute phase did not differ.

Fig 6. Immunostaining of myocardium in the subacute phase of human myocarditis. (—; 50 μm) (A) HLA-DR, (B) CD3. The number of inflammatory cells in the cicatrized myocardium appeared less than in the acute phase. In active myocarditis, myocardial cytolysis and cell infiltrates adjacent to the cardiocytes remained around the necrotic lesions, and were HLA-DR+, and dendritic-forming mononuclear cells and T cells were detectable near by.
Fig 7. Section of the myocardium from the acute phase group that was double-stained with the antibodies against HLA-DR and CD68. (--: 50 μm). Using the double stain technique, the large dendritic-forming monocytes stained black and were easily differentiated from the macrophages stained red. There were a few sporadic cells that stained with both, and these cells were sensitive to both HLA-DR and CD68 antibodies. They appeared to have both surface antigens.

the control (p<0.001, Fig 5). The CD68+ cells (15.6±8.4/HPF) were infiltrating extensively, as shown in Fig 4B.

Subacute Phase of Myocarditis Seven hearts belonged to this subgroup. The number of HLA-DR+ cells infiltrating the heart was 10.2±5.3/HPF. Four cases showed predominantly T cell infiltration. In active myocarditis, myocardial cytolysis and cell infiltrates adjacent to the cardiocytes remained around the necrotic lesions and were HLA-DR-, and dendritic-forming mononuclear cells and T cells were detectable in the surrounding areas (Fig 6).

Double Staining With HLA-DR and CD68 The dendritic-forming mononuclear cells, which infiltrated in the acute phase of myocarditis, were reactive to antibody against HLA-DR. On the other hand, the macrophages were only very sensitive to the antibody against CD68. Noticeably, cells that double stained with the antibodies against both HLA-DR and CD68 appeared occasionally in the acute and/or subacute phases (Fig 7).

Discussion

Identification of Cardiac Dendritic Cells

It is extremely difficult to identify cardiac dendritic cells by the immunostaining technique that employs formalin-fixed material. This difficulty has been overcome by a recently developed highly sensitive staining system, which enables successful immunostaining of formalin-fixed specimens using labeled streptavidin biotin and catalyzed signal amplification (CSA). Even previously autopsied hearts have become available for immunological investigation through this new immuno-staining system.

Generally speaking, dendritic cells are released from the bone marrow to express MHC class II, and are antigen-presenting cells for helper T cells. Hart and Fabre first reported the presence of cardiac dendritic cells in 1981, and Spencer and Fabre discriminated immunohistologically between cardiac dendritic cells and macrophages in the normal rat heart. Our colleagues, Suzuki et al. also showed that at the onset of autoimmune myocarditis in rats, these cells proliferate quickly and are activated, and are sporadically present in the normal myocardium. However, the role that cardiac dendritic cells play in human myocarditis is unknown.

Cytoplogically, the cardiac dendritic cells seem to belong to a subtype of dendritic cells. They function as antigen-presenting cells in the cardiac immune response. The ordinary form of dendritic cells are intensively reactive for HLA-DR and S100, whereas, as found in the present study, the HLA-DR+ mononuclear cells occurring in the myocardium are not positive for S100 (Fig 1). Although the immunostained form of dendritic cell was quite different from the ordinary form of dendritic cell in the thymus, tonsil and skin, we identified them as cardiac dendritic cells, as described by Hart and Fabre and Zhang et al. who had already identified cardiac dendritic cells by using only the immuno-attitude for MHC class II and morphological characteristics. Structurally, the large HLA-DR+ mononuclear cardiac cells occurring in active myocarditis had long and slender dendritic processes, a structural similarity that enabled us to recognize these cells. Therefore, from the results of the present study, we devised criteria for identifying these cells: (1) positive for HLA-DR, (2) negative for CD68, (3) spindle shaped with a large mononuclear cell that has dendritic processes, and (4) including a cell double-stained with both HLA-DR and CD68. Accordingly, we gave less consideration to the immuno-attitude for the S100 in this definition. Most ordinary dendritic cells are positive for S100, but, in the brain, S100 stains glial and ependymal cells. Moreover, Schwann cells of the peripheral nervous system are also positive, as are cartilaginous tissue and fat tissue. Therefore, S100 is not a specific marker for the total number of dendritic cells. For example, Pavli et al. reported that in the colon dendritic cells poorly expressed S100 and Fano et al. and Zimmer et al. reported that S100 had various characterizations in each organ. Furthermore, in the present study, neurofibers were abundant in the myocardium, and the number of fibroblasts increased in the inflammatory stage. To avoid overstimulation of fibroblasts, we did not consider the immunostaining-attitude for S100 staining at all. With regard to this issue, it needs further investigation, especially concerning immuno-electronmicroscopic analysis.

The cardiac dendritic cells were negative for CD1a, CD21, CD23 and CD35. Such an immuno-attitude differed from that of ordinary dendritic cells. It is thought that cardiac dendritic cells may change their character either in the blood vessels and/or the heart during transport from their place of origin (ie, bone marrow).

Acute Phase of Myocarditis

The hearts of patients who had died during the acute phase of myocarditis contained a large number of cardiac dendritic cells that were positive for HLA-DR, and the number of cardiac dendritic cells in the acute phase group was significantly greater than that in the control group. This indicates that cardiac dendritic cells positive for HLA-DR proliferate in the acute phase of myocarditis. Most cardiac dendritic cells were in contact with myocytes and developed dendritic processes. This morphological finding suggested that cardiac dendritic cells might exert a destructive effect on the myocyte. Mainly T cells had infiltrated the heart of 12 of the 15 cases, which suggests early mobilization of cardiac dendritic cells in myocarditis. It is most likely that they also present altered cardiac myosin molecules released from injured cardiocytes to helper T lymphocytes as antigens.
Subacute Phase of Myocarditis

Of the patients who died of myocarditis during the subacute phase, the number of T cells, B cells, HLA-DR+ cells, and CD68+ cells in the cicatrizated myocardium appeared to be less than in the acute phase. In active myocarditis, myocardial cytolsys and cell infiltrates adjacent to the cardiocytes remained around the necrotic lesions, and were HLA-DR positive, with dendritic-forming mononuclear cells detectable very close by. This suggests that inflammatory cell infiltration may persist for several years in some cases. Polymorphonuclear giant cells and cardiac dendritic cells, and T cells in the active inflammatory lesions, were detectable in chronic patients. The polymorphonuclear giant cells were positive for CD68 and negative for HLA-DR and S100, and, therefore, seem to be derived from macrophages. Because giant cell myocarditis frequently responds to immunosuppressive therapy, the participation of autoimmunity in myocarditis has been considered. That polymorphonuclear giant cells were present in giant cell myocarditis would suggest the possibility of an autoimmune mechanism. The present findings in the subacute subgroup are quite similar to those from the experimental model of autoimmune myocarditis in rats. Woodruff et al. have emphasized in their murine experimental model using coxsackie virus infection and that, after acute inflammatory changes, autoreactive T cells would be provoked and that they played an important role in developing myocardial heart damage through a secondary immune response. Our results support their proposal; namely, an autoimmune response follows acute viral myocarditis, even in the human heart.

Double Staining Cells

Occasionally, cells that were double stained with the antibodies against both HLA-DR and CD68 appeared. These double stained cells were scarce in the acute (2.6±1.4/HPF) and/or subacute phases (2.5±1.2/HPF). They were characteristically large, round monocytes with abundant cytoplasm. It is suggested that those cells have a dual role. HLA-DR reactive with B cells, activated T cells, macrophages, and antigen-presenting cells. CD68 stains macrophages in a wide variety of human tissues, but antigen-presenting cells (eg, dendritic cells) are negative. In this study, the double-stained cells had the ability to present the antigen to T cells, but also performed phagocytosis.

Further Investigation

In spite of recent advances in the immunological analysis of several autoimmune diseases, the morphological aspects of human myocarditis are insufficiently understood. Most cases of human myocarditis are caused by a virus, but its etiology has not been fully elucidated. Cases of delayed active myocarditis have been reported. Immunosuppressive agents, such as corticosteroids, are effective in the treatment of delayed active myocarditis, and discontinuation of that agent induces a relapse; therefore, it has been suggested that an autoimmune mechanism is involved in active myocarditis. What is the autoimmune response in the myocardium? One answer to this question can be found in the experimental autoimmune myocarditis model established by Kodama et al. who demonstrated that injection of myocardial myosin-reactive T cells leads to the mobilization of many cardiac dendritic cells in the myocardium and the release of cytokines, thereby inducing lethal myocarditis. Cardiac dendritic cells play an essential role in the autoimmune response and are responsible for the immune response.

Cardiac dendritic cells have been reported in acute myocardial infarction, as well as the hypertensive heart, stroke and atherosclerotic vessels, although their specific role is unknown. Cardiac dendritic cells also play an important role in antigen presentation in the rejection response in heart transplantation.

The finding that cardiac dendritic cells are recruited in acute viral myocarditis suggests the involvement of a secondary immune response, which in turn suggests that the immune response is prolonged after the initial myocardial damage by the viral infection has subsided. Cardiac dendritic cells have been studied from the viewpoint of remodeling in the failed myocardium and the immune response in transplanted hearts. The present study disclosed another role of cardiac dendritic cells in human myocarditis and so contributes to the further progress in the diagnosis and treatment of myocarditis.

Conclusion

The present study demonstrated for the first time the active form of cardiac dendritic cells in human myocarditis. Cardiac dendritic cells were characterized as large monocytes with dendritic processes, immunohistochemically positive for HLA-DR and negative CD68. The number of cardiac dendritic cells dramatically increases during the acute phase of myocarditis and are thought to be involved in the autoimmune response following inflammation.

Acknowledgments

The authors wish to thank Professor M. Naito in the Second Department of Pathology, Niigata University School of Medicine, for providing valuable samples and also his encouragement throughout this study. We thank Drs N. Aoyama, T. Inoue, C. Matsuda, H. Takeshima for their assistance and valuable comments in this work. We also thank Ms Y. Yasui, Kitasato University School of Medicine, for her kind assistance. This study was supported by a grant for scientific research from the Ministry of Education, Science and Culture of Japan (No. 08771116) and by a grant from the Vehicles Foundation of Japan (1995–1997).

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