Apoptosis in Acute and Chronic Myocarditis

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SUMMARY

Programmed cell death or apoptosis plays a major role in the modification of morphologic and functional maturity in various normal organ systems. However, it is also related to certain diseases. We conducted a pathological study of the apoptosis of cardiomyocytes in six cases with myocarditis (three of acute myocarditis and three of chronic or persistent myocarditis) using histochemical methods. In normal hearts obtained from autopsy cases, apoptosis was seen in endocardial cells. There was no apoptosis in myocardial cells, except for a few in myocytes with two nuclei. In myocarditis, although the myocytes of all cases with acute myocarditis did not show apoptosis, one of the three cases with chronic or persistent myocarditis showed many apoptotic myocytes. Apoptosis may be one of the mechanisms causing myocyte damage in myocarditis. (Jpn Heart J 35: 745-750, 1994)

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In myocarditis, the myocardium is involved in an inflammatory process under the influence of several factors, such as direct myocardial invasion by the pathogen, immunologically mediated myocardial damage and myocardial toxins. Therefore, the clinical course and prognosis may vary.

Diagnosis of myocarditis by endomyocardial biopsy is difficult because of sampling error and the focal nature of the lesion. There is poor clinicopathological correlation in the diagnosis of myocarditis. Moreover, we cannot determine the prognosis of individual cases with myocarditis. One of the reasons may be that abnormalities of the myocytes in myocarditis cannot be detected by previous methods alone.

Since Kerr et al described programmed cell death or apoptosis, which was different from necrosis, there have been many studies of apoptosis and disease, especially immunological disease. However, there has been no report of the...
relation between heart disease and apoptosis.

More recently, apoptosis, or programmed cell death, has been detected by the pathological technique named the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling) method. To investigate whether or not apoptosis plays a role in myocarditis, we conducted a pathological investigation of the apoptosis of myocytes in myocarditis.

**MATERIALS AND METHODS**

Myocardial tissues obtained from three patients with acute myocarditis (two males and one female, aged 30–52 years) and from three patients with chronic myocarditis (three males, aged 23–29 years) pathologically diagnosed according to the Dallas criteria of endomyocardial biopsy, were used for this study. Acute myocarditis was clinically diagnosed by symptoms, such as high fever, ECG change and acute heart failure associated with the common cold. One of the three cases with acute myocarditis showed a distinct increase in virus (Coxsackie A16) neutralizing titer. The patient with chronic or persistent myocarditis did not have a significant increase in neutralizing titers of any viruses. As the normal control, we studied three autopsied hearts without cardiovascular disease obtained within two hours of death (three males aged 50–68 years, heart weight: 250–320 g).

We used the modified TUNEL method to investigate the tissues. These specimens were frozen in liquid nitrogen and the blocks were cut into sections about 3–4 μ thick by cryostat. After fixing with acetone, these slides were incubated with 3% H₂O₂ in methanol. The sections were rinsed with double distilled water, and immersed in terminal deoxynucleotidyl transferase (TDT) buffer (30 mM Trizma base (pH 7.2), 140 mM sodium cacodylate, 1 mM cobalt chloride). TDT and biotinylated dUTP in TDT buffer were then added to cover the section, and incubated in a moist chamber at 37°C for 60 min. After rinsing in phosphate buffer saline, the sections were covered with horseradish-peroxidase-conjugated streptavidine for 30 min at room temperature. The sections were soaked in DAB substrate for about 5 min, then counterstained with methyl green.

We used human lymph node sections incubated with 130 mg/ml DNase at 37°C for 90 min as a positive control. To demonstrate the distribution of apoptotic cells, some of these frozen sections were stained with hematoxylin-eosin.

**RESULTS**

Many lymphocytes in the lymph node pretreated with DNase were positively stained by the TUNEL method (Figure 1).
Figure 1. Human lymph node pretreated with DNase used as a positive control (TUNEL stain × 40). Many lymphocytes are stained positively by the TUNEL method.

Figure 2. Apoptosis in a normal heart (TUNEL stain × 20). L = lumen of the heart; E = endocardium. Endocardial cells show apoptosis.

In normal control hearts, apoptotic cells were revealed mainly in the endocardium by the TUNEL method (Figure 2). Although almost none of the myocytes were stained by this method, some with two nuclei showed apoptosis.

The patients with acute myocarditis showed pathological myocardial cell damage and cell infiltration in the biopsy specimen (Figure 3A). However, no specimen from the patients with acute myocarditis showed apoptotic myocytes (Figure 3B). Chronic or persistent myocarditis demonstrated interstitial fibrosis and slight cell infiltration (Figure 4A). One of the three patients with chronic myocarditis showed many myocytes nuclei that were positive by the TUNEL method (Figure 4B).
Figure 3. Apoptosis in acute myocarditis. A: Myocardial cell damage and cell infiltration can be seen (H-E × 20). B: There are no apoptotic cells in myocardium with acute myocarditis (TUNEL stain × 20).

Figure 4. Apoptosis in chronic myocarditis. A: Interstitial fibrosis and infiltration by a few cells can be seen (H-E × 20). B: Many nuclei of myocytes were stained positively (brownish) for apoptosis. (TUNEL × 20).
DISCUSSION

Apoptosis seems to be involved in cell turnover in many healthy adult tissues and is responsible for focal elimination of cells during normal embryonic development.\(^3\) Apoptosis has been reported in certain organs, including the liver, intestine, epidermal tissue and lymphoid tissue.\(^7,9\) In this study apoptosis was seen mainly in the endocardium of the normal heart. The endocardium is damaged by blood flow and may be repaired naturally. Therefore, apoptosis may play some role in remodeling of the endocardium in the normal heart. Myocardial cells did not show apoptosis except for a small number of cells with two nuclei.

We studied acute and chronic myocarditis diagnosed by endomyocardial biopsy and observed many apoptotic myocytes in one case of chronic myocarditis, but not in the cases of acute myocarditis. The findings of this study suggested that apoptosis may be one of the mechanisms causing myocyte damage in myocarditis.

Apoptosis is induced by several factors, such as glucocorticoids,\(^10\) radiation,\(^11\) tumor necrosis factor,\(^12\) cytotoxic T cells,\(^13,14\) nerve growth factor,\(^15\) and viruses.\(^5,16\) Although we could not find what induced apoptosis of myocytes in myocarditis, viral infection was clinically suspected in the cases studied.

As apoptosis was seen in chronic myocarditis in this study, it may be related to the stage of myocarditis. Although myocarditis is generally a self-limited disease, some patients with viral myocarditis may have a subacute, recurrent, or chronic course.\(^17\) The causes of chronic myocarditis are not known. In the mouse, B cell-mediated and humoral autoimmune mechanisms have been suggested as the major pathogenetic factors in chronic viral myocarditis.\(^18,19\) This may be related to differences in individual susceptibility to the virus.\(^20,21\) Moreover, viral persistence may be the pathogenetic factor underlying chronic myocarditis.\(^22\) Genetic susceptibility to a virus may provoke abnormal programmed cell death or a persistent virus may induce apoptosis of myocytes.

Some patients with myocarditis develop dilated cardiomyopathy.\(^23\) Apoptosis might be related to the change from myocarditis to dilated cardiomyopathy. Further study is needed to determine the mechanism of apoptosis in chronic myocarditis.

REFERENCES