Myocardial Uptake of Antimyosin Monoclonal Antibody in a Murine Model of Viral Myocarditis

Akira Matsumori, MD, Tomoko Ohkusa, MD, Yoshiki Matoba, MD, Ikutaro Okada, MD, Takehiko Yamada, MD, Chuichi Kawai, MD, Nagara Tamaki, MD, Yuji Watanabe, MD, Yoshiharu Yonekura, MD, Keigo Endo, MD, Junji Konishi, MD, Ban-An Khaw, PhD, and Edgar Haber, MD

The myocardial uptake of $^{125}\text{I}$- and $^{131}\text{I}$-antimyosin monoclonal antibody Fab in experimental myocarditis in BALB/c mice induced by encephalomyocarditis virus was studied. The biodistribution of $^{125}\text{I}$-antimyosin demonstrated that the highest ratio of radioactivity appears in the heart of infected mice on day 14 (the ratio of percent dose per gram for the organ to percent dose per milliliter for blood; $9.75\pm2.79$ vs. $1.27\pm0.78$ at 24 hours in inoculated mice vs. control mice). There was no statistically significant difference between the mean activity ratios of tissues other than the heart in control and inoculated mice. The uptake ratio for the heart increased significantly 3 days after virus inoculation and reached a maximum on day 14 when myocardial lesions were most extensive and prominent. The uptake ratio decreased significantly, but it still remained high compared with controls on day 28 when cellular infiltration had decreased and fibrosis was evident. The scintigraphic images obtained with $^{131}\text{I}$-antimyosin monoclonal antibody clearly demonstrated that visualization of the heart in experimental myocarditis was possible 24 hours after administration of radiotracer, and localized activity was still observed in the 48-hour image. We conclude that antimyosin monoclonal antibodies localize selectively in the heart from the acute to subacute stage of viral myocarditis. These findings indicate that antimyosin scintigraphy is a reliable noninvasive method for the evaluation of patients suspected of having myocarditis. (Circulation 1989;79:400–405)

Monoclonal antimyosin antibodies have been useful in the in vitro localization of myocardial necrosis.1–3 The mechanism of antimyosin localization in the damaged myocardium is probably related to increased access of macromolecules to intracellular sites after loss of cell membrane integrity.4 Because myocardial necrosis is an obligatory component of myocarditis,5 radiolabeled Fab of antimyosin monoclonal antibody was used for the diagnosis of myocarditis.6 However, sequential uptake of antimyosin antibody or its histopathologic correlation has not been studied. In this study, we used our experimental model of viral myocarditis7 to investigate sequential myocardial uptake of radiiodinated antimyosin monoclonal antibody Fab, the relation between the uptake and histopathology, and the reliability of scintigraphic imaging.

Methods

Experimental Viral Myocarditis

A murine model of encephalomyocarditis virus was used.7 The virus stock was prepared as previously described.7 Virus stock had a titer of $3\times10^6$ plaque forming units (PFU) determined in tissue cultures of FL (human amnion) cells. Virus and control fluids were stored at $-70^\circ$ C. Inbred male BALB/c mice were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan. This strain has been continuously

From The Third Division, Department of Internal Medicine, Department of Clinical Molecular Biology (T.O.), Department of Nuclear Medicine (N.T., Y.W., Y.Y., K.E., J.K.), Faculty of Medicine, Kyoto University, Kyoto, Japan; and Cardiac Unit (B.A.K., E.H.), Massachusetts General Hospital, Boston, Massachusetts.


Address for correspondence: Chuichi Kawai, MD, The Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, 54 Kawaracho Shogoin, Sakyo-ku, Kyoto 606, Japan.

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maintained by brother-sister matings. At 4 weeks of age, mice were inoculated intraperitoneally with 0.1 ml virus suspension containing 300 PFU/0.1 ml. BALB/c mice without inoculation served as the controls. The mice were killed \( n = 6 \), \( n = 6 \), \( n = 5 \), \( n = 9 \), and 28 days \( n = 5 \) after the inoculation.

**Preparation of Antimyosin Monoclonal Antibody**

Antimyosin monoclonal antibody R11D10 was obtained as previously described. Brieﬂy, BALB/c mice were immunized with human left ventricular cardiac myosin, and the spleen cells were fused with plasmacytoma cell line Sp2/0-Ag14. The supernatants were screened by solid-phase radioimmunoassay. After puriﬁcation of antimyosin antibody, Fab was prepared.

**Radioiodination of Antimyosin Monoclonal Antibody**

Radioiodination of antimyosin Fab was performed by the chloramine-T method. Brieﬂy, 50 μg antimyosin Fab was incubated with 0.3 mCi Na\(^{125}\)I and 2.5 μg chloramine-T for 5 minutes. Free \(^{125}\)I was separated by Sephadex G-50 column chromatography. The speciﬁc activity of radioiodinated antimyosin was about 4.5 mCi/mg protein. For imaging studies, radioiodination with \(^{131}\)I was carried out by a similar method.

**Distribution Studies of \(^{125}\)I-Antimyosin Monoclonal Antibody**

Mice were treated with water containing 0.1% potassium iodine during the initial 2 days to inhibit the thyroidal uptake of radiotracer. Mice were killed, and organs were excised 24 hours after injecting 1.5 μCi \(^{125}\)I-antimyosin in a tail vein. Heart, liver, kidney, intestine, stomach, spleen, lung, skeletal muscle, skull, brain, and pancreas were weighed, and the uptake of the tracer was determined by a well-type auto-gamma counter. Tracer uptake was expressed as a percentage of the injected dose per gram of tissue and as a ratio of percent dose per gram for the organ to percent dose per milliliter for blood. The weight of the mice was normalized to 20 g. For biodistribution study on day 14, nine infected and six control mice were used.

**\(^{131}\)I-Antimyosin Monoclonal Antibody Imaging**

Mice were treated similarly with 0.1% potassium iodine. For myocardial imaging, four control mice and four mice on day 7 after virus inoculation were injected with 200 μCi \(^{131}\)I-antimyosin in a tail vein. The mice were anesthetized by pentobarbital (0.5 mg/body i.p.), and 24 hours after administering radiotracer, whole-body images were obtained in the posterior view with a gamma camera (Photon- Gamma LFOV, Searle, Chicago, Illinois) equipped with a pinhole collimator. Fifty thousand counts were accumulated for whole-body images at the energy peak of 364 keV with a 20% window. All scintigrams were interpreted independently by two observers who were unaware of the experimental conditions.

**Histopathologic Studies**

After the studies of biodistribution and imaging, the hearts were ﬁxed in 10% formalin solution, sectioned transversely at the midportion of the ventricle, embedded in parafﬁn, and stained with hematoxylin and eosin. Myocardial cell necrosis, cellular inﬁltration, and calciﬁcation were scored blindly by severity on a scale of 1 to 4+. A 1+ score indicated a limited focal distribution of myocardial lesions. A 4+ score indicated the presence of multiple lesions over the entire heart, whereas scores of 2+ and 3+ indicated intermediate severity.

**Statistical Analysis**

The statistical method used was analysis of variance. Results were expressed as the mean ± SD.

**Results**

**Histopathology**

On day 3, histologic abnormality was not evident (Figure 1A). Histopathologic changes were ﬁrst noted in the myocardium 5 days after inoculation with encephalomyocarditis virus. These changes consisted of focal myocytolysis and a few mononuclear cell inﬁltrations (Figure 1B). On day 7, myocardial necrosis and cellular inﬁltration were more prominent (Figure 1C). Extensive myocardial necrosis with calcification was evident on day 14 (Figure 1D). On day 28, cellular inﬁltration decreased, but myocardial calcification persisted. Myocardial ﬁbrosis was evident at this stage (Figure 1E). Scores of myocardial necrosis and cellular inﬁltration on day 5 (\( n = 6 \)) were 1.0 ± 0.0 and 1.2 ± 0.4, and these signiﬁcantly increased on day 7 (\( n = 5 \)) to 2.8 ± 0.4 and 2.4 ± 0.5, respectively (\( p < 0.001 \), Figure 2). Myocardial calcification was prominent on day 14 (1.1 ± 1.5, \( n = 9 \)). Myocardial necrosis and cellular inﬁltration were less severe on day 28 (\( n = 5 \)). Mild spontaneous pericardial calcification, which was limited to the right ventricle, was often seen in controls and infected mice and was not included in grading.

**Distribution Studies**

Biodistribution of \(^{125}\)I-antimyosin in infected mice on day 14 and age-matched control mice 24 hours after administering radiotracer is shown in Figure 3 as percent dose per gram for the organ and the ratio of percent dose per gram for the organ to percent dose per milliliter for blood. Only the heart shows a signiﬁcantly increased uptake of tracer when compared with either blood or corresponding organs in the control animals. Thus, these ﬁndings indicate that localization of antimyosin monoclonal antibody in damaged myocardium primarily reﬂects speciﬁc antigen-antibody interaction.
Sequential Study of Myocardial Uptake

The antimyosin Fab uptake ratio of percent dose per gram for the heart to percent dose per milliliter for blood increased significantly in mice 3 days after inoculation with encephalomyocarditis virus (2.37 ± 2.38, p<0.05, compared with 0.65±0.15 of 5-week-old control mice) (Figure 4). The uptake ratio increased gradually (from 4.12±1.90 on day 5 to 4.87±1.86 on day 7, and it reached a maximum on day 14 of 9.75±2.79), and the ratio decreased significantly on day 28 (5.35±2.63) but was still elevated when compared with the controls (p<0.05).

\(^{131}\)I-Antimyosin Imaging

The initial images (at 3 hours) showed blood pool activity, primarily in the viscera. In addition, the thyroid gland and bladder were visualized (not shown). Figure 5 shows a whole-body image in a representative mouse with myocarditis on day 7. The images of the normal mouse, obtained 24 hours after administration of antibody, are shown on the left. Radioactivity is redistributed to the region of the heart, but no \(^{131}\)I activity is seen in the cardiac region of the control animals. Intra-abdominal activity seemed to be due mainly to activity in the stomach. This localized activity in mice with myocarditis persisted in the image at 48 hours.

Discussion

This study demonstrates that measurement of \(^{125}\)I-antimyosin uptake of the heart is a sensitive
Necrosis
Infiltration
Calcification

* p<0.001 vs day 5 or day 28

FIGURE 2. Bar graph of histologic scores of myocardial lesions. Myocardial necrosis and cellular infiltration was more severe on day 7, calcification was prominent on day 14, but myocardial necrosis and cellular infiltration was less severe on day 28.

FIGURE 3. Bar graphs of biodistribution of $^{125}$I-antimyosin monoclonal antibody Fab in infected mice on day 14 after virus inoculation (n=9) and age-matched control mice (n=6) at 24 hours after injecting radiotracer. Panel A: Percent dose per gram for the organ; Panel B: ratio of percent dose per gram for the organ to percent dose per milliliter for blood. Only the heart shows a significantly increased uptake of tracer when compared to either blood or corresponding organs in the control animals.
technique for detecting acute viral myocarditis in the experimental animal. The $^{125}$I-antimyosin uptake ratio of the heart began to increase 3 days after virus inoculation when histologic abnormality had not yet appeared. On day 14, the uptake ratio of the heart reached a maximum, and histologically, myocardial lesions were most extensive and prominent at this stage. On day 28, when cellular infiltration had decreased and myocardial fibrosis was evident, the uptake ratio remained elevated. Thus, the increase in $^{125}$I-antimyosin uptake in the early stage of viral myocarditis correlated well with histopathologic changes and persisted long after virus inoculation. These findings suggest that antimyosin scintigraphy is useful for diagnosing viral myocarditis in its subacute to chronic stage and in the acute stage.

A number of laboratory tests can be done to diagnose viral myocarditis, but none is specific. An increased activity of such serum enzymes as creatine kinase, glutamic oxaloacetic transaminase, and lactate dehydrogenase is also common but is seen only during the early stages of the disease.

Radionuclide scintigraphic methods for viral myocarditis include $^{99m}$Tc-pyrophosphate imaging and $^{67}$Ga-citrate imaging. $^{99m}$Tc-pyrophosphate imaging is useful in the diagnosis of myocardial infarction, and although not specific for myocarditis, sequential study with this technique may help in the diagnosis of viral myocarditis. Although the mechanism by which $^{99m}$Tc-pyrophosphate accumulates in the damaged myocardium is unknown, it is clear that this agent concentrates selectively in acutely necrotic myocardium irrespective of the cause of the necrosis. $^{99m}$Tc-pyrophosphate has also been shown to localize in the myocardium in cases of perimyocarditis in experimental animals and humans.

In our experimental model of coxsackievirus myocarditis, cardiac uptake of $^{99m}$Tc-pyrophosphate reached a maximum on day 7 after virus infection but decreased significantly on day 14 when myocardial lesions were most prominent. Thus, $^{99m}$Tc-pyrophosphate may be useful in diagnosis only in the acute stage of viral myocarditis.

$^{67}$Ga-citrate gave positive results in several noncardiac chronic inflammatory diseases characterized by mononuclear cell infiltration. These include lymphoproliferative diseases involving the heart, myocardial abscesses, and bacterial endocarditis. Myocardial $^{67}$Ga imaging, though possibly helpful, is not specific for the diagnosis of myocarditis, and scintigrams made

![Figure 5. Color scintigram showing posterior whole-body images of inoculated (right) and control (left) mice at 24-hours after infusion of 200 μCi $^{131}$I-antimyosin Fab. Selective uptake is seen in the cardiac region (arrow) of the mouse with encephalomyocarditis inoculation but not in the control mouse. Intra-abdominal activity seemed due mainly to activity in the stomach.](image-url)
with this radionuclide have been positive in patients with pericarditis\textsuperscript{16} and dilated cardiomyopathy.\textsuperscript{17} Thus, O’Connell et al\textsuperscript{17} found myocardial gallium uptake in 19 of 39 patients with dilated cardiomyopathy in whom clinical, hemodynamic, and electrocardiographic characteristics failed to discriminate those who were gallium positive from those who were not. Also, \textsuperscript{67}Ga has given positive results in experimental myocarditis in rabbits.\textsuperscript{18}

Scintigraphic examination with antimyosin monoclonal antibodies have been used to localize and quantify regions of myocardial necrosis in myocardial infarction.\textsuperscript{1,2} In recent years, these antibodies have been applied in diagnosing myocarditis in humans.\textsuperscript{6} At a microscopic level, the antibody is bound only by necrotic myocytes in which cytoplasmic and nuclear details are lost.\textsuperscript{4} The mechanism of antimyosin antibody localization in the necrotic myocardium is probably related to increased access of macromolecules to intracellular sites after loss of cell membrane integrity.\textsuperscript{4} It is conceivable that disruption of membrane integrity may lead to antimyosin antibody uptake in cardiomyopathies or other inflammatory diseases of the myocardium similar to what occurs in myocarditis and that antimyosin uptake may not identify the cause of myocardial damage. However, significant higher antimyosin uptake in the chronic stage of viral myocarditis long after virus clearance suggests that antimyosin antibody uptake study is helpful in detecting myocardial damage in early and especially late stages of myocarditis. The mechanism by which antimyosin antibody uptake persisted to the chronic stage could not be identified in this study, but it may be due to persistent damage of cell membrane integrity of myocytes.

In this study, we demonstrated that there is specific and selective localization of antimyosin monoclonal antibody in an experimental model with viral myocarditis. The scintigraphic images obtained with \textsuperscript{131}I-antimyosin, though the number of animals was small and though the study was preliminary, clearly demonstrate that visualization of the area of damaged myocytes is possible 24 hours after intravenous administration and that this localized activity persists in the 48-hour image. Our data establish that scans with \textsuperscript{131}I-antimyosin should be performed 24–48 hours after radiotracer administration. If imaging is performed earlier than 24 hours, the blood pool radioactivity tends to mask specific cardiac uptake. At 72 hours, cardiac localization is no longer seen in this model.

In conclusion, antimyosin monoclonal antibodies were localized selectively and specifically in damaged myocytes of an experimental model with viral myocarditis. These findings indicate that antimyosin scintigraphy is a reliable noninvasive method for the evaluation of patients suspected of having myocarditis.

References


Key words: imaging, myosin, encephalomyocarditis, virus, BALB/c mice
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