Imaging the Rejecting Heart

In Vivo Detection of Major Histocompatibility Complex Class II Antigen Induction

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Background. Mice with abdominal heterotopic heart transplants were studied to determine whether scintigraphic detection of an increase in major histocompatibility complex (MHC) class II antigen expression could be used as a noninvasive method for diagnosing early rejection.

Methods and Results. Allografts from C3H/He (H2b) donors were transplanted into BALB/c (H2d) recipients (n=18). Two of the 18 allografted mice were treated with cyclosporine (15 mg/kg/day), and two isografted mice served as controls. Each mouse was injected intravenously with 100 μCi of 111In-labeled anti-MHC class II monoclonal antibodies (10-2-16 and 14-4-4S) 24 hours before scintigraphy. After imaging, the mice were killed for tissue counting and histopathology. Radiotracer uptake in the grafts reflected the severity of rejection as determined by histopathological criteria. The percent injected dose per gram of tissue in excised grafts was 4.8±1.8 (mean±SD) for normal grafts (n=8), 11.1±9.7 for grafts with grade IA rejection (n=3, NS), 18.0±3.8 for grafts with grade IIIB rejection (n=4, p<0.001 versus normal), 18.7±3.2 for grafts with grade IIIA rejection (n=3, p<0.001 versus normal), and 22.6±5.4 for grafts with severe rejection (grade IV) (n=3, p<0.001 versus normal). Rejecting allografts with lymphocyte infiltration but without significant myocyte necrosis could be identified by this scintigraphic method. In the BALB/c donor--C57BL/6 (H2b, Ie') recipient combination, rejecting allografts were visualized by 14-4-4S (anti-Ie4b') antibody but not by 10-2-16 (anti-IA b2d' ) antigen. This difference shows that class II antigens induced on donor hearts are solely responsible for the antibody uptake in positive scintigrams of rejecting allografts.

Conclusions. We conclude that 111In-labeled anti-MHC class II antigen antibody imaging is a sensitive and noninvasive method for detecting cardiac allograft rejection. (Circulation 1992;85:738-746)

Although a variety of techniques for the detection and quantification of cardiac allograft transplant rejection have been explored, only histological inspection of myocardial specimens obtained through endomyocardial biopsy has gained widespread use.1 Endomyocardial biopsy is invasive, costly, and prone to sampling error, and biopsy interpretation is subjective and possibly arbitrary. We2-3 and other laboratories4-6 have shown that 111In-antimyosin scintigraphy is useful for detecting moderate and severe cardiac rejection in human patients and experimental animals. Myocyte necrosis is essential to the detection of rejection by antimyosin scintigraphy in a mouse ectopic heart transplantation model.3 Because myocyte necrosis is an indicator of relatively advanced rejection, a more sensitive, noninvasive method of detecting cardiac transplant rejection is desirable.

The distribution of major histocompatibility complex (MHC) antigens in various organs and tissues is well documented in animals and humans.7,8 Normal, nucleated, nonlymphoid cells such as cardiac myocytes express low levels of MHC class I antigens (MHC K and D in mice) and do not express detectable levels of MHC class II antigens (MHC IA and Ie in mice). Recent immunohistological investigations show that expression of class II antigens increases in rejecting organs,9-14 in tissues undergoing autoimmune injury,15-17 in viral disease,18-20 and in inflammatory states.21 Enhanced class II antigen expression correlates with clinical evidence of rejection in transplanted human heart tissue obtained by endomyocardial biopsy.22,23 Sell et al22 found that

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histological evidence of moderate rejection in sequential heart biopsies was always preceded by a marked increase in the index of MHC antigens measured by radioimmunoassay. They also showed that this enhancement of MHC class II antigen expression could be normalized by immunosuppressive therapy. However, because diagnosis and follow-up of rejection by this method would require endomyocardial biopsy, the usefulness of the approach is limited by the invasiveness and sampling error inherent to the biopsy procedure. Therefore, the purpose of the present study was to determine whether scintigraphic detection of an increase in class II antigen expression could be used as a noninvasive method for diagnosing early cardiac allograft rejection.

Methods

Animals

Male, inbred BALB/c (H2k) and C3H/He (H2s) mice (4–6 weeks old) were obtained from the Charles River Breeding Laboratory (Boston, Mass.). C57BL/6 (H2s, IE−) male mice were purchased from Jackson Laboratory (Bar Harbor, Me.). All animal experiments were approved by the Committee on Research Animal Care Protocol Review Group and carried out according to Massachusetts General Hospital guidelines and the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Scintigraphy

Hybridoma cell lines 10-2-16 (anti-mouse IAααα) and 14-4-4S (anti-mouse IEααα) were obtained from ATCC (Rockville, Md.). The specificity and affinity of the monoclonal antibodies have been described. Hybridoma cells were grown in Dulbecco’s minimum essential medium supplemented with 10% fetal calf serum and 0.1% gentamycin, and monoclonal immunoglobulins were purified by protein A affinity chromatography (Pharmacia, Piscataway, N.J.). Monoclonal antibodies 10-2-16 and 14-4-4S were labeled with 111In by use of a bifunctional chelating agent (diethylenetriamine pentaacetic acid [DTPA]). Approximately 100 μCi of 111In-DTPA antibody (10-2-16, 14-4-4S, or both) was injected into the tail vein of the recipient mouse 24 hours before scintigraphy. Scintigraphy was performed with a gamma camera (Ohio Nuclear 100) equipped with a 3-mm pinhole collimator as described. Both energy peaks (173 and 247 keV) of 111In were used for count acquisition. For each scintigram, the intensity of radioactivity in the graft was compared with that of the native heart after the cardiac area had been set by computer planimetry.

Tissue Analysis

Mice were killed after scintigraphy. Venous blood was withdrawn and the autologous heart, transplanted heart, liver, spleen, kidneys, and lungs were excised. Both hearts were washed thoroughly with saline. The biodistribution of radioactivity was determined as described. The ratio of percent injected dose per gram of grafted heart (%ID) to that of autologous heart was determined for each mouse.

Organ Grafting and Animal Groups

Heterotopic mouse heart transplantation was performed by the microvascular technique as described. In 18 mice, C3H/He hearts were transplanted into BALB/c recipients. Two of this group were treated with cyclosporine (15 mg/kg/day s.c. injection). Two control mice were isografted (BALB/c heart into BALB/c recipient and C3H heart into C3H recipient). Experimental and control mice were randomly chosen for scintigraphy 2–8 days after transplantation and were injected with radiolabeled 10-2-16 and 14-4-4S antibodies.

In our second set of experiments, we analyzed radiolabeled antibody uptake in rejecting allografts in various donor–recipient combinations to show the specificity of antibody uptake. 111In-labeled 14-4-4S antibody was tested in the BALB/c recipient–C3H/He donor combination, 111In-labeled 10-2-16 antibody was tested in the C3H/He recipient–BALB/c donor combination, and each antibody was tested in two mice in the C57BL/6 recipient–BALB/c donor combination. Cardiac graft contractility was assessed daily by direct palpation, and scintigraphy was performed right after confirmation of a significant decline in the graft beat.

Figure 1. Light microscopic sections of C3H/He ectopic cardiac allografts transplanted into BALB/c recipients. Panel A: Grade IA, focal interstitial infiltrate without necrosis. Panel B: Grade IIIA, multifocal infiltrates with myocyte necrosis. Panel C: Grade IIIB, diffuse cell infiltration and necrosis. Panel D: Grade IV, diffuse infiltrates with hemorrhage and necrosis.
Histological Examination

Grafted and autologous hearts were embedded in paraffin and stained with hematoxylin and eosin. The samples were submitted for blinded histopathological evaluation by a cardiac pathologist without experimental history and were classified according to the International Heart Transplantation Working Formulation.1

Statistical Analysis

A value of \( p > 0.05 \) was considered nonsignificant in comparisons between multiple groups of data.29 All data were expressed as mean±SD. Linear regression was computed by the least-squares method.

Results

Histopathology and Radiotracer Uptake

Histological findings ranged from nearly normal to severe rejection (Figure 1). Labeled antibody uptake and histopathological data are listed in Table 1. The ratios of radioactivity by tissue counting of the grafted versus native heart in the two isografted mice were 1.9 and 2.6. This slight increase in radioactivity in isografts was also observed after injection of radiolabeled mouse immunoglobulin of irrelevant specificity. We conclude that nonspecific inflammation caused by operative manipulation was responsible for the increase in radioactivity.

Regardless of the time after transplantation and the presence or absence of cyclosporine therapy, the level of radiotracer uptake reflected the histological severity of rejection. The %ID in excised grafts was 4.8±1.8 (mean±SD) for normal grafts (\( n = 8 \)), 11.1±9.7 for grafts with grade IA rejection (\( n = 3 \), NS), 18.0±3.8 for grafts with grade IIIA rejection (\( n = 4 \), \( p < 0.001 \) versus normal), 18.7±3.2 for grafts with grade IIIB rejection (\( n = 3 \), \( p < 0.001 \) versus normal), and 22.6±5.4 for grafts with severe rejection (grade IV) (\( n = 3 \), \( p < 0.001 \) versus normal) (Figure 2). The ratio of radioactivity in the graft versus native heart was similar. The ratio was 2.2±0.6 for normal grafts, 6.6±4.6 for grade IA grafts (\( p < 0.05 \) versus normal), 13.5±5.8 for grade IIIA grafts (\( p < 0.001 \) versus normal), 12.7±1.7 for grade IIIB grafts (\( p < 0.001 \) versus normal), and 17.2±4.0 for grade IV grafts (\( p < 0.001 \) versus normal) (Figure 3). The %ID in excised grafts increased progressively with time in isografts (\( \% \text{ID} = 2.9\times\text{days} - 1.0, \ r = 0.65, \ p < 0.01, \ n = 16 \) (Figure 4). The ratio of radioactivity in the graft versus native heart also increased progressively with time (G/N = 2.3×days - 4.4, \( r = 0.65, \ p < 0.05, \ n = 16 \)). Radiolabeled antibody uptake in cyclosporine-treated allografts, for which there was no histological evidence of rejection, did not increase relative to that in isografts even 7 days after transplantation.

Scintigraphy

A good linear correlation was observed between the ratio of graft to native heart radioactivity measured by tissue counting and that measured by computer planimetry from the scintigrams (\( P = 0.33\times\text{G/N} + 1.4, \ r = 0.95, \ n = 20, \ p < 0.001 \), where \( P = \text{ratio of radioactivity in the graft versus autologous heart measured by computer} \).
planimetry). Correlation between the intensity of radiotracer signal from the scintigram and the %ID in the graft was also good (P=0.25×%ID+0.96, r=0.93, n=20, p<0.001). Strong and unequivocal accumulation of labeled antibodies was apparent in scintigrams of rejecting allografts from day 4 of transplantation (Figure 3). In contrast with mice with rejecting allografts, isografted mice and cyclosporine-treated mice showed no specific accumulation of radiolabeled antibodies 6 and 7 days after transplantation.

In an allografted mouse imaged on day 4 of transplantation (No. 7, Table 1) and another imaged on day 6 (No. 10), the tissue counting and scintigram revealed significant uptake of radiotracer, whereas the histological studies showed cell infiltrates but no significant myocyte necrosis. In two mice (No. 3 and No. 16) with significant myocyte necrosis probably caused by perioperative ischemia but without evidence of active rejection, there was no specific accumulation of radiotracer.

Dependence of Antibody Binding on the Class II Antigen Expressed

In the C3H/He (H2b) donor–BALB/c (H2c) recipient combination, the rejecting graft was clearly visualized by radiolabeled 14-4-4S antibody (anti-IE<sup>2</sup>4<sup>a</sup>,<sup>b</sup>). In contrast, in the BALB/c donor–C3H/He recipient combination, there was no increase in radiolabeled 10-2-16 (anti-IA<sup>a</sup>,<sup>d</sup>) antibody uptake in the rejecting allograft relative to nonrejecting allografts, even though the graft showed histological evidence of severe rejection 10 days after transplantation. In the BALB/c donor–C57BL/6 (H2<sup>e</sup>, IE<sup>e</sup>) recipient combination, in which one mouse was injected with radiolabeled 10-2-16 at 8 days after transplantation and another with radiolabeled 14-4-4S at 7 days after transplantation, rejecting allografts with H2<sup>e</sup> antigens in mice with H2<sup>b</sup> antigens...
were visualized by an anti-IE\textsubscript{a,b,p} antibody but not by an anti-IA\textsubscript{x,a,f} antibody (Table 2 and Figure 6).

Discussion

The results of this study demonstrate for the first time that anti-MHC class II antibody scintigraphy can be used to detect cardiac rejection. Scintigraphy was sufficiently sensitive to detect early rejection before development of significant myocyte necrosis. The results also show that MHC class II antigens induced on donor cardiac myocytes are the unique source of antigen accounting for the positive scan in the rejecting heart.

Although a variety of noninvasive techniques for detection and quantification of cardiac rejection have been explored, none has gained widespread use. Antimyosin scintigraphy is a potentially useful method for diagnosing cardiac rejection in the clinical setting because myocyte necrosis is a direct result of rejection and is usually regarded as an indication for additional rejection-directed therapy. Myocyte necrosis is not an early manifestation of rejection, however; it occurs at a relatively advanced stage. Also, antimyosin scanning cannot differentiate perioperative necrosis from necrosis caused by rejection. A more sensitive and specific scintigraphic method for detecting cardiac rejection would be useful.

MHC class I and class II molecules, known as transplantation antigens, play an essential role in the pathogenesis of rejection.\textsuperscript{8,30} Although low levels of class I antigens are expressed in normal cardiac myocytes,\textsuperscript{31} class II antigens normally are not detected.\textsuperscript{7,9} However, induction of both class I and class II antigens has been reported in rejecting kidney,\textsuperscript{8,10,12} heart,\textsuperscript{11,13,14,22,23} liver,\textsuperscript{32} and pancreas\textsuperscript{33} allografts. Milton et al\textsuperscript{11,34} observed a massive induction of donor-type class II antigens in rejecting rat cardiac allografts. In the report by Sell et al\textsuperscript{22} of a series of six patients who underwent cardiac transplantation, an increase in the level of MHC class II antigen expres-
sion always preceded histological evidence of moderate rejection. In all six patients, the expression of class II antigens decreased when rejection abated. These results led us to conclude that donor-type class II antigens induced on allografted hearts should be useful imaging targets for detecting early cardiac rejection.

Induced MHC class II expression on graft cells is most likely a result of \( \gamma \)-interferon, a lymphokine generated by activated T lymphocytes and natural killer cells. The donor MHC class II antigens can augment both cellular sensitization in the recipient and susceptibility to MHC class I– and class II–specific effectors in the donor targets. Because the large-scale induction of class II antigens could greatly stimulate activation of T cell–derived \( \gamma \)-interferon, induction of class II antigen would be maintained in myocardial and endothelial cells. In this way, an expanding and self-regenerating cycle would be initiated. Induced class II antigens could also serve as new targets for class II–specific cytotoxic T cells. This enhancement in MHC antigen expression could significantly drive the rejection process.

Two MHC molecules (IA and IE) have been identified as class II antigens in mice. Therefore, we selected these antigens as targets for radioimmunomaging. In our experiments in the C3H/He donor–BALB/c recipient combination, we used antibodies against IA and IE antigens that react with both donor and recipient antigens. In contrast to

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<th>Table 2. Uptake of (^{111}\text{In}-\text{labeled Anti-MHC Class II Antibodies 10-2-16 (Anti-IA}^{\text{H2}\text{d}}\text{) and 14-4-4S (Anti-IE}^{\text{d}}\text{) in Rejecting Mouse Heart Allografts in Various Donor-Recipient Combinations}</th>
<th>Mouse No.</th>
<th>Donor</th>
<th>Recipient</th>
<th>Days after transplant</th>
<th>Histological degree of rejection</th>
<th>Antibody</th>
<th>Graft/native heart (%ID)*</th>
<th>Scintigram</th>
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<td>21</td>
<td>C3H (H2\text{d})</td>
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<td>6</td>
<td>IIIA</td>
<td>14-4-4S</td>
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<td>1.6</td>
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<tr>
<td>23</td>
<td>BALB/c</td>
<td>C57BL/6 (H2\text{d})</td>
<td>8</td>
<td>IIIA</td>
<td>10-2-16</td>
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<td>C57BL/6</td>
<td>7</td>
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*Percent injected dose per gram organ; +, positive; –, negative.
nonrejecting allografts and isografts, which were not visualized by the radiolabeled antibodies, allografts with histological evidence of rejection were clearly identified. Radiolabeled antibody uptake in rejecting allografts increased progressively with time and in proportion to the severity of rejection. Although all allografts were being at the various times at which the mice were killed, beating was obviously impaired in allografts in mice killed 7 and 9 days after transplantation. Significant loss of myocytes in these mice may account for the relative reduction in radiotracer uptake observed in the late stage of rejection. However, the reason for the nonlinear correlation between histological grade of rejection and class II antibody uptake remains to be determined.

It is of interest that anti-class II antibody uptake was dissociated from myocyte necrosis. In an allografted mouse imaged on day 4 after transplantation and another imaged on day 5, the scintigrams revealed strong uptake of radiotracer, whereas histological studies showed evidence of cell infiltrates but no significant myocyte necrosis. There was a significant increase in %ID in these grafts in relation to that in isografts and nonrejecting allografts. One allografted mouse killed 3 days after transplantation and another cyclosporine-treated mouse killed after 7 days showed significant ischemic myocyte necrosis (probably caused by perioperative ischemia) and no evidence of rejection. These mice also showed no increase in radiotracer activity in comparison with histologically normal grafts. Therefore, we conclude that myocyte necrosis does not affect anti-class II antibody uptake in allografted hearts.

In our series of 20 mice, eight allografts were deemed normal on histological examination and four grafts were given a rejection grade of IA (cell infiltrate without myocyte necrosis). The ratio of %ID for graft versus native hearts in the eight mice whose grafts were normal was always less than that in the mice whose grafts showed histological evidence of rejection. In two of the four allografts with grade IA rejection (in which there was no evidence of myocyte necrosis), radiotracer uptake increased significantly and was clearly visible in the scintigraphic image, and in one isograft with focal monocytic infiltrates consistent with grade IA rejection, there was no increase in tracer uptake in comparison with histologically normal grafts. All grafts with advanced rejection (grade III or IV) revealed statistically significant increases in tracer uptake and were clearly visible by scintigraphy. Because the transition from mild to moderate cardiac rejection is subtle in humans and difficult to detect by histological examination, the use of anti-class II antibody scintigraphy to detect allograft rejection before the development of myocyte necrosis may have clinical relevance. It should be noted, however, that in a study by Sell et al.,23 no strict correlation was observed between extent of leukocyte infiltration and level of MHC antigen expression determined by radioimmunoassay.

Cyclosporine inhibits lymphokine production by helper T cells in vitro and is widely used to alleviate tissue allograft rejection in vivo.34 Cyclosporine also suppresses induction of MHC class II antigens, despite evidence of substantial leukocyte infiltration shown in cyclosporine-treated grafts.35 This suppression of MHC antigen induction is almost certainly a consequence of suppression of γ-interferon release from the infiltrating leukocytes.36,37 Not surprisingly, radiolabeled antibody uptake in cyclosporine-treated allografts did not increase relative to that in isografts, reflecting the absence of active rejection.

As shown in Table 2, rejecting allografts were imaged only by anti-class II antibody against donor antigen. Normal splenocytes, especially B lymphocytes, express class II antigens.4 The strong uptake of antibody in the recipient's spleen is consistent with the reactivity of the antibody with the recipient's class II antigens. Regardless of the reactivity of the antibody with the recipient's antigens, the rejecting BALB/c (H2k) grafts were visualized by 14-4-4S (anti-IEkrsf) but not by 10-2-16 (anti-IAkrsf) antibody. These results also rule out the possibility of nonspecific accumulation of antibody (immunoglobulin) in sites of rejection.

One difference between rejection in mice compared with humans and other large mammals such as pigs requires consideration. Acute rejection in mice is provoked primarily by class I antigens. In the mouse vascularized graft model with class II mismatching, spontaneous long-term tolerance has been observed.40 Class II mismatched grafts are invariably rejected in the pig kidney transplant model.41 This discrepancy may relate to the expression of class II antigens on the vascular endothelium of large animals (including humans) but not that of mice.42 The distribution of MHC antigens, as well as its effect on induction of class II antigens associated with acute rejection, must be investigated because of its potential to affect the sensitivity of anti-class II antibody scintigraphy.

This study demonstrates that cardiac rejection can be detected by anti-class II antibody scintigraphy at the stage at which there is leukocyte infiltration but no significant myocyte necrosis. This method may be particularly useful for distinguishing the active cellular infiltrates that are the first sign of a rejection episode from the innocuous and self-limiting infiltrates frequently seen with cyclosporine and apparently seen in one of our isografted mice. Since the level of HLA-DR antigen on rejecting cardiac cells decreases after intensive immunosuppressive therapy,22 MHC class II antigen scintigraphy may simplify the follow-up of patients with episodes of rejection.

Enhancement of class II antigen expression in rejecting allografts occurs not only in heart but also in other organ transplants. Because of the low expression of class II antigens in normal kidneys8,10 and pancreas,33 MHC class II antigen scintigraphy may also be useful for detecting rejection of these organs. In the detection of kidney rejection, this
method would be particularly useful for distinguishing renal failure caused by rejection from that caused by cyclosporine toxicity.

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References


**KEY WORDS** • transplantation • scintigraphy • cyclosporine • MHC rejection
Imaging the rejecting heart. In vivo detection of major histocompatibility complex class II antigen induction.

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