Immunoglobulin Isotype Determines Pathogenicity in Antibody-Mediated Myocarditis in Naïve Mice

Anita P. Kuan, Lionel Zuckier, Li Liao, Stephen M. Factor, Betty Diamond

Abstract—Antimyosin reactivity is associated with cardiac damage in autoimmune myocarditis, an inflammatory heart disease characterized by a cellular infiltrate in the myocardium and myocyte necrosis. We are interested in the pathogenicity of antimyosin antibodies and their ability to cause autoimmune myocarditis. We have shown that antimyosin antibodies of the IgG isotype will induce disease in the DBA/2 mouse. In the present study, we show that IgM antimyosin antibodies do not induce myocarditis; however, these same antibodies become pathogenic when converted to the IgG isotype. Although IgM antibodies can penetrate the myocardium during cardiac inflammation, they are usually less able to leave the vascular compartment and penetrate cardiac tissue, thus accounting for their lack of pathogenicity. Thus, antimyosin B cells may be potentially pathogenic only after antigen activation and heavy chain class switching or under conditions that alter vascular permeability in the heart. (Circ Res. 2000;86:281-285.)

Key Words: immunoglobulin isotype — myocarditis — pathogenicity

A utoimmune myocarditis, characterized by a cellular infiltrate in the myocardium and myocyte necrosis,1 can be studied in mouse models. Infection with Coxsackie B3 (CB3) virus will induce antimyosin reactivity and autoimmune myocarditis in many mouse strains, and immunization with cardiac myosin will induce disease in the same susceptible strains.2,3 DBA/2 mice are susceptible to disease induction by myosin immunization, and antibody-mediated tissue injury contributes to pathogenesis in this strain.2,4 We have previously demonstrated that DBA/2 mice will also develop autoimmune myocarditis when IgG anticardiac myosin antibodies are passively administered to naïve mice.4

IgG autoantibodies are associated with many autoimmune diseases, although IgM autoantibodies are not without pathogenic potential. There has been some controversy regarding the reasons for the increased pathogenicity of IgG antibodies and uncertainty why some IgM autoantibodies are pathogenic whereas other autoantibodies must be of the IgG or IgA isotype to mediate clinical disease. It is possible that heavy chain class switching is associated with affinity maturation and that the altered affinity or fine specificity of IgG antibodies endows them with greater pathogenic potential. Alternatively, the different effector functions of the μ and γ heavy chain constant regions may account for the differential pathogenicity of IgM and IgG antibodies.

We chose to explore this question with an analysis of the pathogenicity of antibodies to cardiac myosin. In the present study, IgM and IgG anticardiac myosin antibodies with identical antigen binding domains were tested for pathogenicity in the DBA/2 mouse strain. IgM antibodies were found not to cause disease; however, IgG antibodies possessing the identical antigen binding domains were pathogenic. Studies of immunoglobulin distribution in vivo suggest that IgM antibodies fail to exit the vasculature and do not accumulate in cardiac tissue, except under conditions of cardiac inflammation. Thus, immunoglobulin isotype determines the distribution of autoantibody and its potential pathogenicity.

Materials and Methods

Hybridoma Production

All mice were obtained from Jackson Laboratories (Bar Harbor, Maine). The care and use of all animals used in the present study were in accordance with institutional guidelines. B-cell hybridomas were obtained by standard fusion technology from cardiac myosin immunized mice. Two IgM antimyosin-producing cell lines were isolated, one from a BALB/c mouse (12F7G3), the other (8G10H2) from an A.CA mouse. Spontaneously arising γ1 class switch variants were isolated by the sib selection technique of Spira et al5 and cloned on soft agar and antimyosin ELISAs performed as previously described.4

Antibody Production and Purification

Immunoglobulin was isolated from ascites fluid by ammonium sulfate precipitation and affinity chromatography on a γ-bind protein G-Sepharose column (Pharmacia) for IgG antibodies or an anti–μ column for IgM antibodies (Sigma). Purity of the immunoglobulin was checked by SDS-PAGE and specificity by antimyosin ELISA as above. Antibodies were quantitated by ELISA using isotype-matched purified Ig to generate a standard curve (Southern Biotechnology).
Pathogenicity of Antimyosin Antibodies

Purified monoclonal antimyosin antibody (100 μg in 200 μL saline) was injected intraperitoneally into naïve DBA/2 mice 5 days a week for 2 weeks. Mice were sacrificed 3 weeks later. Hearts were excised and immediately immersed in 10% formaldehyde/PBS, embedded in paraffin, and cut in 5-μm sections at five levels. Sections were deparaffinized before staining with hematoxylin and eosin. Blinded analysis was performed by a cardiac pathologist (S.M.F.).

Administration of Iodinated Antibody

Purified monoclonal antibodies were labeled with either 125I or 131I (specific activity between 1.0 μCi/μg and 1.5 μCi/μg) using the Iodogen technique (Pierce). Labeled antibody was separated from free iodine using a size exclusion column (PD-10, Sephadex G-25, Pharmacia).

To block thyroid uptake of iodinated antibody in vivo, mice were given nonradioactive iodine in their drinking water for at least 5 days before antibody injection. Each animal was injected with either 5 μg of 125I-IgM and 5 μg of 131I-IgM in 200 μL PBS or 5 μg of 131I-IgM and 5 μg of 125I-IgM in 200 μL PBS in IgM versus IgG experiments.

Infarction Induction

Myocardial infarction was induced in 6-week-old DBA/2 mice by coronary artery ligation as previously described in the literature.6

Results

Characterization of Antibodies and Induction of Antibody-Mediated Myocarditis

Previous studies have shown that antineural myosin antibodies of various IgG subclasses can induce disease in the DBA/2 mouse strain.4 To determine whether isotype affects pathogenicity, we elected to administer paired IgM and IgG antimyosin monoclonal antibodies into mice susceptible to IgG antimyosin-induced myocarditis. Two hybridoma lines producing IgM antimyosin antibodies were isolated, 8G10H2 and 12F7G3. Variants that had undergone spontaneous class switching to the IgG isotype, IgG1 subclass, were isolated from each IgM cell line. IgG antibody from each cell line was purified and shown to retain myosin binding (Figure 1), despite the loss of avidity that occurs when IgM antibodies undergo heavy chain class switching to IgG.

IgG and IgM antibodies were injected intraperitoneally into naïve DBA/2 mice to determine whether both isotypes would cause myocarditis, using an identical protocol of antibody administration. Although the 8G10H2 and the 12F7G3 IgG1 class switch variants were both found to induce disease (Table, Figure 2), no disease was detectable in mice receiving IgM antibodies (Table, Figure 3). IgG-treated animals exhibited myocyte necrosis in proximity to inflammatory cells, and several mice displayed valvulitis as well as myocardial inflammation (Figure 2C).

Entry Into the Vascular Compartment After Intraperitoneal Administration

In the above experiments, antibodies were administered to mice by intraperitoneal injection; thus, it is possible that IgM antibodies do not transit from the peritoneal cavity to the vascular bed as well as IgG. We, therefore, injected mice intraperitoneally with radiolabeled antibodies to determine whether both IgG and IgM antibodies entered the circulation. An equivalent percentage of each antibody entered the blood, with little to no difference between the percentage of IgG and IgM detected in the blood shortly after injection (data not shown). As expected, the IgM antibodies had a much shorter half-life in the circulation than the IgG antibodies (Figure 4).

Pathogenicity of Antimyosin Antibodies in DBA/2 Mice

<table>
<thead>
<tr>
<th>Antimyosin Antibody</th>
<th>8G10H2 (5)</th>
<th>12F7G3 (5)</th>
</tr>
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<tbody>
<tr>
<td>IgM</td>
<td>0 (5)</td>
<td>0 (5)</td>
</tr>
<tr>
<td>IgG</td>
<td>5 (6)</td>
<td>5 (5)</td>
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Naïve DBA/2 mice were injected with antimyosin antibodies 8G10H2-IgM and 12F7G3-IgM and their corresponding IgG antibodies. The number of mice developing myocarditis is listed. Numbers in parentheses represent the total number of mice injected.
Entry Into the Myocardium

When we analyzed antibody present in the myocardium of perfused hearts of antibody-injected mice, we found that only IgG antibody was sequestered in the myocardium of the injected animals (Figure 5), despite the previous presence of both IgG and IgM in the blood. Thus, IgM antibody is able to leave the peritoneal cavity and enter the circulation but appears to be unable to transit out of the vascular bed and accumulate in the myocardium. The lower amount of 8G10H2 IgG compared with 12F7G3 IgG in the heart is probably due to a lower affinity for myosin as seen by ELISA (Figure 1).

To see whether IgM can enter the myocardium under the conditions of cardiac inflammation, myocardial infarction was induced in DBA/2 mice by left coronary artery ligation. Mice were injected intraperitoneally with 125I-IgM on days 1, 7, and 14 after infarction and sacrificed 5 days after antibody injection. Mice injected on day 1 after infarction showed the greatest amount of IgM sequestered in the heart (Figure 5C), comparable to the amount of IgG deposited in the normal DBA/2 heart (Figures 5A and 5B). Decreasing sequestration occurred at later time points.

Discussion

It has previously been shown that monoclonal IgG antimyosin antibodies can induce disease in the susceptible DBA/2 mouse strain. Because the Fc portion of IgG antibodies determines effector functions of the antibody, the pathogenicity of these antimyosin antibodies may depend on their isotype. We found that antimyosin IgM antibodies did not induce disease in the DBA/2 mouse strain whereas IgG class switch variants with identical variable regions did. The differential pathogenicity of IgM and IgG antibodies, therefore, appears to be a function of the heavy chain constant region alone.

In this model of passive antibody administration, an equivalent percentage of the injected IgM and IgG leaves the peritoneal cavity and enters the circulation, yet only IgG antibodies penetrate the heart and cause disease. The neonatal Fc receptor (FcRn) is a major histocompatibility complex class I-related receptor first identified as the protein that mediates transfer of maternal IgGs across the neonatal intestine. The FcRn is expressed by endothelial cells and protects IgG from degradation. The endothelium of small arterioles and capillaries internalizes IgG into endosomes, where the acidic pH allows binding to FcRn, preventing...
degradation and instead recycling the antibody back to the vasculature.\textsuperscript{7,12,13} Hence, the FcRn is responsible for the greater serum half-life of IgG antibodies compared with IgM.\textsuperscript{12,14} It is conceivable that the FcRn may also release IgG into tissues; thus, the FcRn would also be instrumental in transporting IgG antibodies from the blood into tissue. Beta-2-microglobulin (\(\beta_{2}M\)) is a subunit of the FcRn. Preliminary studies show that antimyosin antibodies injected into mice with a targeted disruption of the \(\beta_{2}M\) gene do not enter the heart. Without the ability to bind FcRn, IgM antibodies may have no mechanism to leave the vasculature, transit through endothelial cells, and enter interstitial fluid in the heart. It is also possible that size alone prevents the large pentameric IgM antibody from entry into the heart.

In the presence of cardiac inflammation, however, IgM antibodies are able to enter the myocardium. During cardiac inflammation, the vascular barrier is damaged and becomes leaky, allowing the pentameric IgM antibodies to leave the vasculature and enter the myocardium. We are presently testing the pathogenicity of IgM antibodies under these conditions, but we anticipate that IgM antibodies, deposited in the heart, will mediate tissue damage.

Figure 4. Antibody clearance from the blood. Concentration of labeled antibody in the vasculature is expressed as a percentage of the initial value. For each time point, the mean value and standard deviation are displayed. Note that standard deviations are small and hence not clearly visible on many of the points. A, 8G10H2 IgM and IgG (\(n=4\)). B, 12F7G3 IgM and IgG (\(n=5\)).

Figure 5. Antibody in cardiac tissue. Perfused hearts were analyzed for presence of injected antibody. Values are expressed as percentage of injected dose (ID). A, IgG versus IgM in hearts of animals injected with 8G10H2 antibodies (\(n=4\)). B, IgG versus IgM in hearts of animals injected with 12F7G3 antibodies (\(n=5\)). C, IgM levels in hearts of animals subjected to myocardial infarction (MI) by coronary artery ligation and injected with IgM antibody 1 (\(n=4\)), 7 (\(n=4\)), and 14 (\(n=3\)) days after infarction (shaded bars) compared with control hearts injected on days 1 (\(n=3\)), 7 (\(n=2\)), and 14 (\(n=2\)) (striped bars).
It is interesting to speculate that autoantibodies of the IgM isotype, which are often nonpathogenic, may lack pathogenicity because they fail to enter the interstitial fluid of tissue. Many B cells make antibodies that are broadly cross-reactive, binding both autoantigen and microbial antigens. Including these cross-reactive B cells in the peripheral pool enlarges the repertoire of B-cell specificity in the periphery and permits reactivity with the broadest spectrum of microbial antigens. Cross-reactive B cells that leave the bone marrow without undergoing some form of tolerance induction would not be dangerous to the organism so long as they secrete only IgM antibodies. Only after undergoing heavy chain class switching to IgG would the antibody become potentially pathogenic. Somatic mutation and heavy chain class switching occur concurrently in activated B cells. A necessary consequence of somatic mutation of activated B cells, therefore, is not merely to increase affinity for foreign antigen but also to eliminate cross-reactivity with self-antigen. B cells producing cross-reactive IgG antibodies would need to be made tolerant. This is consistent with studies from this laboratory on the antibody response to phosphorylcholine (PC).\textsuperscript{15} We have shown that activated IgG-producing B cells may be more susceptible to deletion and anergy induction in the periphery. Cross-reactive anti-PC, anti-DNA IgM antibodies that are present in the spleen of PC-immunized mice during the primary response are not found in the secondary IgG response. IgG-producing B cells are susceptible to stricter regulation to prevent autoreactivity.

We suggest that antimyosin IgM antibodies cross-reactive with microbial antigens, such as group A streptococci M protein or Coxsackie B virus, are not pathogenic so long as there is no disruption of the integrity of the vascular endothelium. Cross-reactive IgG antibodies, in contrast, are pathogenic requiring more stringent regulation or generating a risk of autoimmune disease.

Acknowledgments

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References

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