Autoantibodies Activating Human $\beta_1$-Adrenergic Receptors Are Associated With Reduced Cardiac Function in Chronic Heart Failure

Roland Jahns, MD; Valérie Boivin, PhD; Christian Siegmund, MD; Gerhard Inselmann, MD; Martin J. Lohse, MD; Fritz Boege, MD

**Background**—Autoantibodies against synthetic peptides of $\beta$-adrenergic receptors have been observed in human cardiomyopathy. However, it has never been shown that such antibodies really interact with native human $\beta$-adrenergic receptors, nor has the clinical impact of such an interaction been investigated in larger groups of patients.

**Methods and Results**—We screened 104 patients with dilated or ischemic cardiomyopathy (NYHA functional classes II to IV) and 108 healthy subjects for IgG antibodies reacting with $\beta$-receptor peptides. Such IgGs were further analyzed for binding and functional interactions with native recombinant human $\beta$-adrenergic receptors. Antibodies reacting with synthetic receptor peptides were present in 51% of the patients. However, only a subgroup directed against the second extracellular receptor domain also recognized native human $\beta$-adrenergic receptors situated in a cell membrane. All antibodies of this subgroup impaired receptor ligand binding and enhanced receptor-mediated signaling, which could be blocked by 5 $\mu$mol/L bisoprolol in vitro. Their prevalence was 1% in healthy subjects and 10% in ischemic cardiomyopathy, whereas it amounted to 26% in dilated cardiomyopathy and was associated with a significantly poorer left ventricular function.

**Conclusions**—Our data show that activating autoantibodies against human $\beta$-adrenergic receptors exist in $\approx$25% of patients with dilated cardiomyopathy. Counteraction of such autoantibodies might contribute to the beneficial effects of $\beta$-adrenergic receptor blockade in chronic heart failure. (Circulation. 1999;99:649-654.)

**Key Words:** antibodies $\bullet$ receptors, adrenergic, beta $\bullet$ cardiomyopathy $\bullet$ immune system

Dilated cardiomyopathy (DCM) is defined by progressive dilation and loss of function of the left ventricle in the absence of known causes.1 Autoimmune responses against various myocardial antigens2,3 have been proposed to be involved in its pathogenesis. Some recent observations indicate that autoimmunity against $\beta$-adrenergic receptors might also play a role: autoantibodies reacting with synthetic peptides derived from $\beta$-adrenergic receptors4 or affecting $\beta$-receptor–mediated functions in animal cells5,6 have been reported, their prevalences varying from 30% to 95% in patients and from 0% to 16% in healthy subjects.6,7 However, it has never been shown that such antibodies really interact with native human $\beta$-adrenergic receptors, nor has the clinical impact of such a direct receptor-antibody interaction been demonstrated in larger groups of patients. Furthermore, it is unclear whether such autoantibodies activate4,6 or inhibit5,8 $\beta$-adrenergic receptors and whether they interfere with ligand binding to the receptor4 or not.9

Several technical developments of the past few years make it possible to address these questions in a more direct manner. These include the cloning of the human receptor cDNAs10,11 and their expression in various cell types, which allows a much more precise definition of autoantibodies than either synthetic peptides or animal cell lines used in earlier studies. Furthermore, the generation of specific and subtype-selective antibodies12 provides positive controls for such experiments. In the present study, we used these instruments in a large number of heart failure patients and healthy control subjects to address the question of autoantibodies against $\beta$-adrenergic receptors in cardiomyopathy.

**Methods**

**Patients and Samples**

One hundred four patients were recruited in the course of routine heart catheterization, all suffering from heart failure (NYHA functional classes II to IV), with a left ventricular diastolic volume $>110$ mL/m$^2$ and an ejection fraction $<55\%$ (by ventriculography). DCM (n=65) was diagnosed when coronary heart disease was excluded by angiography and exposure to cardiotoxic substances, myocarditis, or other systemic heart diseases were not evident from clinical history. In ventriculography, all patients of this subgroup exhibited a diffuse reduction in wall motion. ICM (n=39) was diagnosed when a significant stenosis ($>75\%$) of $\geq1$ of the main coronary arteries was

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From the Medizinische Poliklinik (R.J., G.I., F.B.) and the Institut für Pharmakologie (R.J., V.B., C.S., M.J.L.), University of Würzburg, Germany. Correspondence to Dr Fritz Boege, Medizinische Poliklinik der Universität Würzburg, Klinikstraße 6-8, D-97070 Würzburg, Germany. E-mail boege.medpoli@mail.uni-wuerzburg.de

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Table 1. Clinical and Hemodynamic Data of Patients and Healthy Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DCM+ (n=17)</th>
<th>DCM− (n=48)</th>
<th>ICM+ (n=4)</th>
<th>ICM− (n=35)</th>
<th>Healthy (n=108)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59±3</td>
<td>55±7</td>
<td>55±6</td>
<td>59±2</td>
<td>49±18</td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>1/16</td>
<td>7/41</td>
<td>1/3</td>
<td>7/28</td>
<td>38/70</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>3.3±0.4*a</td>
<td>2.8±0.3</td>
<td>3.0±0.8</td>
<td>2.9±0.4</td>
<td>ND</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>87±3*</td>
<td>77±3</td>
<td>81±3</td>
<td>90±6</td>
<td>69±14</td>
</tr>
<tr>
<td>PVR, dyne · s · cm⁻⁵</td>
<td>171±18</td>
<td>157±18</td>
<td>162±81</td>
<td>143±24</td>
<td>ND</td>
</tr>
<tr>
<td>SVR, dyne · s · cm⁻⁵</td>
<td>1464±96</td>
<td>1448±57</td>
<td>1667±233</td>
<td>1427±72</td>
<td>ND</td>
</tr>
<tr>
<td>EFangio, %</td>
<td>30.9±2.2*</td>
<td>38.5±1.8</td>
<td>27±9.3</td>
<td>38±2.1</td>
<td>ND</td>
</tr>
<tr>
<td>Cl, L · min⁻¹ · m⁻²</td>
<td>2.21±0.1†</td>
<td>2.81±0.1</td>
<td>2.15±0.3</td>
<td>2.71±0.2</td>
<td>ND</td>
</tr>
<tr>
<td>Cont., mm Hg · s⁻¹</td>
<td>787±48*</td>
<td>1044±52</td>
<td>902±234</td>
<td>1102±54</td>
<td>ND</td>
</tr>
<tr>
<td>Relax., mm Hg · s⁻¹</td>
<td>721±49*</td>
<td>912±47</td>
<td>720±197</td>
<td>892±46</td>
<td>ND</td>
</tr>
</tbody>
</table>

PVR indicates pulmonary vascular resistance; SVR, systemic vascular resistance; EFangio, left ventricular ejection fraction (ventriculography); Cl, cardiac index; Cont., contractility (peak dP/dt); Relax., relaxation (peak negative dP/dt); and ND, not done. Cont. and Relax. were derived from left ventricular high fidelity time/pressure curves. Values are mean±SEM. Patients with DCM or ICM who were positive (+) or negative (−) for β-receptor autoantibodies (see Figure 1c) were compared by Student’s t test for unpaired samples.

*aP<0.05; †P<0.001.

ascertained by angiography and/or myocardial infarction was apparent in the clinical history. Regional wall motion abnormalities and ECG signs of transmural infarction were evident in all patients of this subgroup. At the time of sample acquisition, all patients were stable under therapy with diuretics, ACE inhibitors, digitalis, and nitrates. None of them were treated with β-adrenergic receptor agonists or antagonists. Healthy control subjects (n=108) were matched for sex and age. Table 1 summarizes basic clinical and hemodynamic data.

Immunosassays With Synthetic Antigens

Peptides corresponding to the second extracellular domains or fusion proteins of aminotermini and carboxytermini of human β₁- or β₂-adrenergic receptors were coated onto ELISA plates (5 ng/well) or spotted onto activated nitrocellulose membranes. BSA and a nonreceptor peptide served as specificity controls. Antigens were probed with the IgG preparations (ELISA: 25 and 12.5 μg/mL, 12 hours, 4°C; dot blotting: 50 μg/mL, 2 hours, 37°C), and bound IgG was detected with biotinylated secondary antibodies, streptavidin-peroxidase, and o-phenylenediamine (ELISA) or horseradish peroxidase–conjugated secondary antibodies and enhanced chemiluminescence (dot blotting).

Immunosassays With Intact Recombinant Receptors

Human β₁- or β₂-adrenergic receptors expressed in baculovirus-infected Sf9 insect cells were used for Western blotting and indirect immunofluorescence microscopy. Receptor-specific antibodies raised in rabbits served as positive controls. Cells infected with wild-type baculovirus were used to define nonspecific staining. Cell lysates were subjected to native Western blotting and incubated with IgG preparations (50 μg/mL, 12 hours, 4°C), and immunoreactive bands were visualized by enhanced chemiluminescence. For indirect immunofluorescence microscopy, intact unfixed Sf9 cells were incubated with IgG preparations (167 and 83 μg/mL, 6 hours, 4°C), counterstained with CY3-labeled secondary antibodies (Dianova), and spotted onto glass slides. Red epifluorescence was photographed at 400-fold magnification with fixed exposure times.

Functional Assays

Antibody effects on binding of the radioligand [³²P]ATP and 100 μmol/L kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly, a peptide-based substrate) as substrate. Each series of experiments was repeated at least 3 times.

Results

Demonstration of β-Adrenergic Receptor Autoantibodies

Screening With Synthetic Analogues of Selected Receptor Domains

IgG preparations of heart failure patients and healthy control subjects were initially screened for antibodies capable of binding to synthetic domains of human β₁- or β₂-adrenergic receptors (aminoterminus, second extracellular domain, and carboxyterminus) by ELISA. An increased reactivity was assumed for signals above the upper limit of the respective 95% CIs (mean±2 SD) of the healthy subjects. By this criterion, 51% of the patients showed increased reactivity with ≥1 of the selected receptor domains (Figure 1a). Of the positive sera, 24% showed multiple reactivity with >1 receptor domain (Figure 1a, numbers in parentheses) and 59% revealed cross-reactions between β₁- and β₂-adrenergic receptors (Figure 1a, hatched bars).
nonspecific binding (compare numbers in parenthesis between Figure 1a and 1b). Only 1 patient in each group showed specific reactions with the aminoterminal and the second extracellular domain of the β₂-adrenergic receptor.

**Demonstration of IgG Binding to Native Human β-Adrenergic Receptors**

To analyze binding of human IgG preparations to β-adrenergic receptors presented in their native conformation in a cell membrane, we performed immunofluorescence experiments with unfixed S9 cells transiently overexpressing intact human β-adrenergic receptors. A representative result is shown in Figure 2c: in cells infected with recombinant baculovirus coding for human β₁- (top) or β₂-adrenergic receptors (middle), a pattern typical for membrane proteins could be visualized by receptor-specific antibodies raised in rabbits (Figure 2c, Rabbit). Cells devoid of β-receptors were not stained (bottom). A similar receptor-specific immunofluorescence pattern was obtained with 87% of those IgG preparations, which according to ELISA and dot blotting were specific for the second extracellular domains of β-adrenergic receptors (Figure 2c, Patients). For some of these antibodies, we could also demonstrate specific staining of β-adrenergic receptors in renatured Western blots of S9 cell lysates (Figure 2b). However, this was not possible for all antibodies exhibiting a receptor-specific staining pattern in microscopy, suggesting either a lower sensitivity of the Western blot or a requirement for a specific native conformation of the receptor, which cannot be completely restored by renaturation. None of the IgG preparations from healthy subjects that did not react with synthetic receptor domains in ELISA (Figure 2c, Healthy) or of the patient IgG preparations recognizing aminotermini or carboxytermini of β-receptors (not shown) stained native β-adrenergic receptors expressed in S9 cell membranes.

These observations show that a native epitope within the second extracellular domain of β-adrenergic receptors can be targeted by autoantibodies under physiological conditions. As summarized in Figure 1c, we detected such autoantibodies in 26% (17 of 65) of the patients with DCM, 10% (4 of 39) of the patients with ICM, and only 1% (1 of 108) of the healthy subjects. It can also be seen that all these antibodies recognized the β₁-subtype of the receptor (Figure 1c, open boxes), and only a small subgroup cross-reacted with the β₂-subtype (Figure 1c, hatched boxes).

**Antibody Effects on Receptor Function**

**Interference With Ligand Binding**

Binding of the radioligand [³H] CGP 12177 to human β-adrenergic receptors expressed in S9 cells was determined after the cells had been incubated with various concentrations of human IgG preparations. In repeated experiments, none of the antibodies directed against aminoterminal or carboxyterminal receptor domains affected ligand binding. In contrast, antibodies directed against the second extracellular domains and capable of immunostaining native β-adrenergic receptors (ie, the subgroup shown in Figure 1c) decreased radioligand binding in a concentration-dependent manner by decreasing [³H]CGP 12177 affinity by up to 3-fold (data not shown),
indicating some degree of competition between these auto-
antibodies and ligand binding. However, the maximum of
inhibition at IgG concentrations as high as 167 μg/mL was
≤19 ± 3.5% (mean ± SEM, single values ranging from 10% to
35% inhibition).

Stimulation of β₁-Adrenergic Receptor Activity
Antibody effects on cellular cAMP production mediated by
human β₁-adrenergic receptors are summarized in Figure 3.
Antibodies directed against the second extracellular domain
of the β₁-adrenergic receptor and capable of binding to native
human β₁-receptors (ie, again as in Figure 1c) (1) increased
basal cAMP levels (1.25 ± 0.27-fold, P < 0.05), (2) enhanced
isoproterenol stimulation of cellular cAMP production
(1.27 ± 0.11-fold, P < 0.0001), and (3) increased basal and
isoproterenol-stimulated activities of cAMP-dependent pro-
tein kinase by 1.55 ± 0.18- and 1.20 ± 0.04-fold, respectively
(P < 0.005, not shown). In patients with DCM, there was a
modest positive correlation (R = 0.59) between immunoreac-
tivity in ELISA and the increase in isoproterenol-stimulated
cAMP (Figure 3, inset). For the 4 autoantibody-positive
patients with ICM, such an analysis was not meaningful. The
data clearly show that these antibodies act as activators and
sensitizers of β₁-adrenergic receptors and that the degree of
receptors (β₁/β₂) or infected with wild-type vector (W) were
either probed with IgG preparations of cardiomyopathic patients
reacting only with β₁ (Patient 1) or with β₁- and β₂-adrenergic
receptors (Patient 2) or probed with subtype-specific polyclonal
rabbit antibodies against human β₁- or β₂-adrenergic receptors
(Rabbit) or with an IgG preparation of an autoantibody-
negative healthy individual (Healthy).

Figure 2. Detection of β-receptor autoantibodies by various
approaches. a, Dots of synthetic second extracellular receptor
domains (β₁/β₂), a nonreceptor control peptide (Ctr), or BSA
were probed with human IgG preparations either not reacting
with β-adrenergic receptors (lane 4) or reacting specifically with
β₁- (lane 2) or β₂-adrenergic receptors (lane 1) or both subtypes
(lane 3) or showing an increased nonspecific protein binding
(lane 5). b, Renatured Western blots of cell lysates or c, intact
unfixed SF9 cells expressing recombinant human β-adrenergic
receptors (β₁/β₂) or infected with wild-type vector (W) were
either probed with IgG preparations of cardiomyopathic patients
reacting only with β₁ (Patient 1) or with β₁- and β₂-adrenergic
receptors (Patient 2) or probed with subtype-specific polyclonal
rabbit antibodies against human β₁- or β₂-adrenergic receptors
(Rabbit) or with an IgG preparation of an autoantibody-
negative healthy individual (Healthy).
TABLE 2. Basal and Isoproterenol-Stimulated cAMP Levels of β1-CHW Cells

<table>
<thead>
<tr>
<th>Assay Conditions</th>
<th>cAMP, fmol per 10^4 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisoprolol 5 μmol/L</td>
<td>Basal</td>
</tr>
<tr>
<td>−−</td>
<td>53±4</td>
</tr>
<tr>
<td>++</td>
<td>67±6</td>
</tr>
<tr>
<td>++</td>
<td>50±3</td>
</tr>
<tr>
<td>++</td>
<td>54±6</td>
</tr>
</tbody>
</table>

CHW cells expressing human β1-adrenergic receptors (120 fmol/mg) were stimulated with 10 μmol/L (-)-isoproterenol or not (basal) in the presence (+) or absence (−) of 5 μmol/L bisoprolol, and in the presence (+) or absence (−) of sensitizing human β1-receptor autoantibodies. Cellular cAMP was measured by 125I-cAMP scintillation proximity assay. Values are mean±SEM. Data were compared by Student’s t test for unpaired samples. *P<0.05, †P<0.001.

stimulation attainable with isoproterenol in the presence of such antibodies exceeds the maximum effect of the full agonist alone. We detected such sensitizing antibodies not only in patients with DCM (Figure 3, open circles) but also in those with ICM (Figure 3, solid circles) and even in 1 of the healthy subjects (Figure 3, triangle). In contrast, IgG preparations from healthy subjects (Figure 3, hatched box) or patients (Figure 3, open box), which had no reactivity against receptor domains other than the second extracellular domain and/or did not stain native β-adrenergic receptors, had no effect on basal or stimulated cAMP production. A mediation of these activating effects via the β1-adrenergic receptor was further supported by 2 observations: (1) maximal cAMP levels after stimulation of G proteins with NaF were not altered by sensitizing antibodies (data not shown), excluding a postreceptor effect; and (2) antibody-mediated increases in basal and agonist-stimulated cAMP concentration were blocked by bisoprolol, a selective β1-receptor antagonist (Table 2); preincubation of β1-receptor-expressing CHW cells with 5 μmol/L bisoprolol abolished not only the stimulation of cellular cAMP production by 10 μmol/L isoproterenol alone but also the increases in basal and (maximally) isoproterenol-stimulated cAMP effected by receptor autoantibodies.

**β-Adrenergic Receptor Autoantibodies and Left Ventricular Function**

Table 1 compares left ventricular function between patients with and without β-receptor autoantibodies (as defined by binding to native β-adrenergic receptors and enhancement of cAMP responses). In DCM, autoantibody-positive patients clearly had a poorer left ventricular function than antibody-negative patients. Their peak systolic contraction force (derived from left ventricular time-pressure curves), their left ventricular ejection fraction, and most notably their cardiac index (Figure 4) were significantly lower than in the antibody-negative subgroup. Table 1 also shows that autoantibody-positive patients had a higher heart rate (P<0.05), which could result from a direct autoantibody-mediated stimulation of β1-adrenergic receptors but could also reflect an adaptation to the more severely impaired cardiac function in these patients. In contrast, the antibody-positive and -negative (DCM--) subgroups did not differ significantly with respect to duration of the disease, onset of clinical symptoms, ECG abnormalities (left bundle-branch block and/or atrial fibrillation), and/or the medication at the time of sample acquisition.

In ICM, hemodynamic differences between autoantibody-positive and -negative patients had a similar trend but were not significant, because only 4 patients in this group were positive for β-receptor autoantibodies.

**Discussion**

Using the molecular tools developed during recent years, we present here evidence for human autoantibodies, which bind to native β-adrenergic receptors under physiological conditions. Their overall prevalence was 20% in patients with chronic heart failure and 1% in healthy subjects, which is clearly less than in most previous reports. We believe that these differences are essentially due to the more stringent criteria for such autoantibodies, which have become available by recombinant techniques. In agreement with previous reports, we found a high prevalence (51%) of IgG antibodies directed against various synthetic β-receptor domains when a similar peptide-based ELISA approach was used (compare Figure 1a). However, most of these antibodies were nonspecific; they did not react with native β-adrenergic receptors and had no effect on receptor function. Only half of the antibodies detected by binding to receptor fragments also recognized the native membrane-bound β-adrenergic receptor. All of these antibodies were directed against the second extracellular domain, which is known to affect ligand binding and suggested to induce immune responses. All of them increased basal and agonist-stimulated cAMP production in a receptor-mediated fashion, probably by stabilizing an active conformation of the receptor, which is also favored by binding of agonists.

The possible relation of such sensitizing β-receptor autoantibodies to the clinical course of chronic heart failure remains open to speculation: some recent transgenic animal models overexpressing β-adrenergic receptors and/or constitutively active β-adrenergic receptor mutants suggest that amplification of receptor-mediated myocardial signaling in-
creases cardiac contractility and thus (transiently) improves left ventricular function. However, it is also possible that chronically enhanced β-receptor activity potentiates the vicious circle of adrenergic overdrive, thereby promoting the clinical manifestations of heart failure. Thus, antibodies that stabilize an active receptor state and sensitize the β-adrenergic system for catecholamines could cause adverse long-term effects in the failing heart, an argument supported by their relatively high prevalence in DCM and their association with a more severely reduced left ventricular function. However, these coincidences do not necessarily imply a cause-and-effect relation: immunologically3 or genetically determined muscle damage19 might be responsible for both the depression of cardiac function and the elaboration of receptor autoantibodies, although in our study, a familiar basis for sensitizing β-receptor autoantibodies was not evident. The clinical effects of β-receptor autoantibodies could also be triggered and/or enhanced by autoimmune responses against other myocardial antigens,2–3 which were not examined in this study.

We could block the stimulatory antibody effects by bisoprolol in vitro. This observation might provide a mechanistic explanation for the recent beneficial effects of β1-adrenergic receptor antagonists in the treatment of DCM.20,21 Although a preliminary reanalysis of some of the patients of the Metoprolol in Dilated Cardiomyopathy trial has not shown a clear correlation between β-blocker benefit and prevalence of β-receptor autoantibodies,22 this analysis may be difficult to interpret because the β-receptor autoantibodies were defined solely by their binding to synthetic receptor peptides, which, as shown here, results in a large fraction of false-positive sera. Future trials on the therapeutic effects of cardioselective β-receptor antagonists could take advantage of the newly available tools to define antibodies that recognize and activate native human β-adrenergic receptors.

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References


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