Beneficial Effects of Metoprolol Treatment in Congestive Heart Failure

Reversal of Sympathetic-Induced Alterations of Immunologic Function

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**Background**

Little information is available to explain why β-blockers are beneficial in certain patients with congestive heart failure (CHF). Since catecholamines alter immune function, we asked whether β-blocker treatment leads to enhancement of immune function.

**Methods and Results**

Fifteen patients with New York Heart Association class III-IV CHF secondary to dilated cardiomyopathy were titrated to a minimum dose of metoprolol 25 mg BID on a background therapy of digoxin, diuretic, and angiotensin-converting enzyme inhibitors. Cardiac and immunologic studies were done before and 6 months to 1 year after treatment. While these patients served as their own controls, an additional population of patients with heart failure was followed for a similar time period on traditional medications. A panel of seven delayed hypersensitivity skin tests were placed at 6- to 12-month intervals on the patient’s forearm. Seventy percent of all CHF patients were anergic (unable to respond to more than 1 antigen). The 30% who could respond averaged 2.2 antigens. After treatment with metoprolol, only 20% remained anergic (P<.001). The 80% of responders averaged 4.2 antigens (P<.001). Additionally, patients treated with metoprolol had an increased percentage of T cells, natural killer cells, and increased interleukin-2 receptor density upon stimulation with concanavalin A. These changes correlated to increases in ejection fraction. Patients not treated with metoprolol remained anergic and had no beneficial immunologic changes.

**Conclusions**

It appears that patients with dilated cardiomyopathy who are treated with metoprolol have enhancement of cell-mediated immunity and improvement of T-cell function; these improvements are correlated to improvement in ejection fraction. *(Circulation. 1994;90:1774-1780.)*

**Key Words**

• lymphocytes
• congestive heart failure
• T-cell receptors, adrenergic, beta
• immunology

The rationale for the use of a β-blocker in treatment of patients with congestive heart failure (CHF) mainly has to do with its inhibition of the adverse effects associated with long-term activation of the sympathetic nervous system. These effects, including vasoconstriction, activation of the renin-angiotensin system, myocardial β-adrenergic receptor down-regulation, and direct cardiotoxicity, are all believed to contribute to the spiraling downhill course as the disease progresses.1-6

Although recent reports have proven β-blocker treatment to be efficacious in some patients with CHF,7-15 its exact mechanism of benefit is unknown, causing other investigators to examine the “catecholamine–heart failure hypothesis” for other links to disease pathophysiology.16

One of these potential links is the effect of the sympathetic nervous system on immune function. CHF and heightened sympathetic nervous activity share certain characteristics with respect to immune alterations. Both conditions are associated with mitigation of suppressor T-cell function, natural killer activity, diminished mitogen responsiveness, and interleukin (IL)-2 function.17-30 Additionally, both conditions are associated with increased autoantibody production.31-35 This leads us to consider whether the sympathetic overactivity leads to immune alterations that might contribute to progression of left ventricular dysfunction in the presence of heart failure.

The goal of the present study is to describe the changes in lymphocyte populations and cell-mediated immunity after treatment of CHF patients with metoprolol.

**Methods**

**Patients**

The following study protocol was approved by the University of California San Diego committee for investigations involving human subjects. Informed consent was obtained from each subject.

**Metoprolol Group**

We studied 15 patients from the San Diego Veterans Administration Medical Center (age range, 42 to 78 years; average age, 65±4) with a history of CHF of at least 5 years’ duration who were New York Heart Association (NYHA) class II-IV and taking conventional treatment: digitalis, diuretics, converting enzyme inhibitors (n=10), hydralazine (n=3), or nitrates (n=2). Ten patients were known to have occlusive coronary artery disease as shown by either coronary angiography or a well-documented myocardial infarction. Five patients were classified as having idiopathic cardiomyopathy. Left ventricular ejection fraction determined by equilibrium gated radionuclide ventriculography averaged 24±4%.

We wanted to maximize our changes of improving left ventricular dysfunction so that we could analyze changes in immune parameters more completely, therefore we biased our
selection whenever possible to those patients we suspected might have a better outcome with treatment, that is, those with higher heart rates at baseline (mean, 98±10 beats per minute).

After initial blood drawing and skin testing, patients were given a test dose of metoprolol 5 mg po BID. If the patient tolerated this dose, it was increased weekly by 5 to 10 mg to a maximum of 50 mg po BID. Patients who could not tolerate a minimum of 25 mg BID were excluded from further metoprolol treatment.

Control Group
While each patient given metoprolol served as their own control, we also studied a group of 15 patients with CHF with similar characteristics as the treated group with respect to age (age range, 44 to 77 years; average age, 67±4), ejection fraction (23±5%), and NYHA classification. These patients were all taking digitalis, diuretics, and vasodilators but had a contraindication to β-blockers (chronic obstructive pulmonary disease, claudication, and/or drug-induced bradycardia), were unable to tolerate metoprolol 25 mg BID, or refused to take the drug. The heart rates in this group were lower than patients treated with metoprolol (mean, 84±8 beats per minute).

Protocol
Subjects in each group had had tetanus boosters within the past 10 years, and no patient had any evidence of immune-compromising disease such as cancer, infection, or autoimmune disease. The following tests were recorded at the time of entry into the study and were repeated between 6 and 12 months into the study period.

Skin Tests
The skin testing procedure consisted of pressing a Multitest CMI (Merieux Institute Inc) disposable plastic applicator with eight sterile test heads into the skin of the forearm with sufficient pressure to puncture the skin. The applicator is preloaded with seven delayed hypersensitivity skin test antigens (tetanus toxoid, diphtheria toxoid, streptococcus, tuberculin, candida, trichophyton, and proteus) and a glycerin negative control. The diameter was measured for each test at 48 hours after placement. Induration of 2 mm or more constituted a positive response. Anergy was defined as fewer than two of seven positive responses.

Mononuclear Cell Isolation
Venous blood drawn from an antecubital vein while the patient was supine was placed in tubes containing heparin, and an equal volume of RPMI (Roswell Park Memorial Institute) medium was added. Mononuclear leukocytes were isolated over a Ficoll-Hypaque gradient and washed according to previous methods. More than 90% of these cells were viable as assessed by trypan blue exclusion. A separate tube of blood was used for automated white blood cell count and differential.

Flow Cytometric Analysis of Mononuclear Leukocytes
Lymphocyte subsets were detected by mouse monoclonal antibodies (Becton Dickinson) conjugated directly with either fluorescein or phycoerythrin (see Table 1). Twenty microliters of monoclonal antibody reagent was reacted with 50 μL of mononuclear cells (10^6 cells) in 12×75-mm tubes for 30 minutes in an ice-cold water bath. Cells were washed twice with phosphate-buffered saline (PBS) supplemented with 0.01% sodium azide and finally resuspended in 1 mL of PBS supplemented with 0.05% paraformaldehyde. Cells were stored at −20°C until analyzed.

Immunofluorescence was measured with a flow cytometer (FAC-scan, Becton Dickinson) equipped with a 15-mW argon ion laser and interfaced with a model 310 computer (Hewlett-Packard Co). Data were analyzed with the Consort 30 data management program (Hewlett-Packard Co) supplied by the manufacturer. Five thousand cells were analyzed per sample. Electronic gating of the lymphocyte population was performed based on forward and side-scatter parameters. The percentage of positive fluorescence of the gated population was determined relative to the fluorescence of the negative control cells. The relative proportion of each subset was obtained as a percentage of the total lymphocytes counted, and the absolute number in each subset was calculated by multiplying the percentage of each subset by the absolute lymphocyte count derived from the white blood cells and differential count.

Concanavalin A–Stimulated Proliferation
Sterile isolated mononuclear leukocytes were resuspended in RPMI medium supplemented with 25 μg/mL gentamicin, 2 mmol/L glutamine, and 20% fetal calf serum at a final density of 10^5 cells/mL. 150 μL of the cell suspension was transferred to each well of a 96-well, flat-bottomed plate. In some wells, concanavalin A (4.5, 9, 18, or 36 μg/mL) was present. Cells were incubated for 4 days in a humidified incubator with 95% air/5% carbon dioxide. During the last 16 hours of incubation, 2 μCi of 3H-thymidine dissolved in 20 μL of RPMI medium was added to each well. At the end of the incubation period, the cells were harvested over glass filters, size C (GF/C). The filters were washed with 10 μL of water, placed into vials, and 4.5 mL of Liquiscint (National Diagnostics, Inc) was added. The incorporated radioactivity was quantified in a liquid scintillation counter. Each point was assessed in quadruplicate in each experiment.

Interleukin-2 Receptor Expression
Sterile isolated mononuclear leukocytes were suspended in supplemented RPMI medium (see above) in the absence and presence of concanavalin A (18 μg/mL). After 48 hours, the cells were centrifuged and the expression of IL-2 receptors was determined fluorometrically by means of anti-CD 25 antibodies.

Catecholamines
Plasma catecholamine levels were measured radioenzymatically by the method of Durret and Ziegler.

Data Analysis
Data shown are mean±SEM for the given number of patients for the experimental observation. The significance of differences were assessed as change from baseline for metoprolol-treated groups as well as conservatively treated patients using two-tailed paired t tests and ANOVA with Bonferroni corrections when necessary. Additionally, between-group analysis was performed on several immunologic parameters.

Results
Skin Tests
The average dose of metoprolol was 38±5 mg BID. Before treatment with metoprolol, 11 of 15 patients (73%) were anergic (<2 positive skin tests) (Table 2).
TABLE 2. Skin Test Status Before and After Treatment

<table>
<thead>
<tr>
<th>Test Status</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-) Metoprolol</td>
<td>10/15 66%</td>
<td>11/15 73%</td>
</tr>
<tr>
<td>(+) Metoprolol</td>
<td>11/15 73%</td>
<td>3/15 20%</td>
</tr>
</tbody>
</table>

*p < .001

After treatment, only 3 of 15 (20%) remained anergic (P < .001). This is in marked contrast to patients receiving standard treatment, where there was no change in overall skin test status. Of the 10 who were anergic at the outset, only 1 had improved skin test results, whereas 2 patients not anergic at the outset became so after follow-up.

Fig 1 shows the number of positive skin tests in patients not anergic (n=12) after treatment with metoprolol. After treatment, patients had an average of 2.2±0.3 positive skin results. After treatment with metoprolol, responders had an average of 4.0±0.4 positive tests (P < .001 versus pretreatment as well as posttreatment in patients not receiving metoprolol).

Ejection Fraction and Skin Test Status

Fig 2 shows ejection fraction before and after the treatment period. Patients treated with metoprolol had a modest increase in ejection fraction (29±2% versus 24±2%, P < .005) at follow-up (10±0.8 months). Patients receiving standard treatment had no significant change in ejection fraction at a mean follow-up time of 9±0.8 months (25±1.5% versus 23±1.5%). Of the 5 patients treated with metoprolol who had a <5 percentage point increase in their ejection fraction, 2 were anergic at follow-up. Of the 10 patients treated with metoprolol who had a >5 percentage point increase in ejection fraction with treatment, only one was found to be anergic at follow-up. In this group, the number of reactive tests per patient was 4.8±0.5 versus 2.0±0.3 in the patients with a <5 percentage point increase in ejection fraction (P < .03). Using linear regression analysis, we found a direct correlation between the increase in ejection fraction after metoprolol and number of positive skin tests (r = .533, P < .05).

Changes in Lymphocyte Subsets

Table 3 shows the baseline immunologic and catecholamine data in patients treated with metoprolol versus standard treatment. Baseline immunologic data were pooled because both groups of patients had identical values. Although not reaching statistical significance, in the metoprolol-treated group there were trends toward a decreased white blood cell count, increased number and percentage of mononuclear leukocytes, and decreased norepinephrine levels after treatment compared with baseline.

Fig 3 reveals the changes in lymphocyte subsets before and after treatment with metoprolol as well as in those patients undergoing standard treatment. Patients who were treated with metoprolol had significant increases in total T cells, T-suppressor/cytotoxic cells, and natural killer cells over baseline values. Patients receiving standard treatment had no increases. Due to little change in T-helper cell population with increases in T-suppressor/cytotoxic cells, the T-helper–T-suppressor/cytotoxic cell ratio was markedly decreased after metoprolol (inset, Fig 4) (1.23±0.2 versus 2.5±0.2, P < .001). Between-group analysis also revealed significant differences (P < .001) for the above subtypes.

Mitogen Proliferation Assay

The dose-response curves for tritiated thymidine incorporation in lymphocytes exposed to concanavalin A are shown in Fig 4. There was a significant increase in the amount of tritiated thymidine incorporation in lymphocytes from patients treated with metoprolol as compared with baseline data (P < .05). No change was seen in groups receiving standard treatment. There was also a trend for increasing IL-2 receptor–positive cells after treatment with metoprolol (55±7% versus 70±7%, P = .1).

Discussion

Sympathetic Nervous System in CHF: Rationale for Use of β-Blockers

Numerous studies have suggested that long-term activation of the sympathetic nervous system in CHF may contribute to the progressive deterioration of cardiac function.1–3 Although the mechanism of this is unknown, various effects such as vasoconstriction, triggering of the renin-angiotensin system, as well as direct cardiotoxicity may play a role.4 Recently, desensitization of cardiac β1-receptor–G-protein complex has been deemed important as an explanation for the decline in myocardial contractility.1,5

A direct therapeutic strategy that would inhibit the effects of the sympathetic nervous system activity in CHF would be available in the form of β-receptor-blocking agents. β-Blockade as a treatment for heart failure was first described in 1975 by Waagstein et al.16 Their report was based on clinical experience in 7 patients with congestive cardiomyopathy who showed hemodynamic improvement and no adverse effects after being treated with alpenrol or practolol for 5 months.

Since then there have been many trials using β-blockers as adjunctive treatment in CHF, including newly designed drugs with either partial agonist or vasodilator activity.7–15 Although small in caliber and sometimes lacking appropriate controls, many of these studies have
nevertheless suggested benefits in certain patients with heart failure, including improvement in NYHA functional class and hemodynamic state, increase in ejection fraction, and a reduction of symptoms. Recently, the Metoprolol in Dilated Cardiomyopathy Trial was completed, which was a placebo-controlled study prospectively studying the effect of long-term β-blockade with metoprolol on total mortality and need for heart transplantation.38 There was an estimated reduction in the sum of death and need for heart transplantation by metoprolol of 34%.

While studies are beginning to show a variety of propitious effects, we remain puzzled by the mechanism for these beneficial effects. The results of the present study point to a plausible hypothesis that the sympathetic nervous system has a role in the progression of heart failure in part through an immune-mediated mechanism.

Sympathetic Nervous System
Regulation of Immunity

Growing evidence suggests that the function of the immune system is partly under the control of the sympathetic nervous system.24,39,40 Anatomic studies have revealed an extensive presence of noradrenergic fibers in both primary and secondary lymphoid organs, innervating both the vasculature and the parenchyma of the tissues.39 In these tissues, lymphocytes and sympathetic nerve endings form contacts at a distance even shorter than in a synapse.24,39 The presence of surface receptors for sympathetic neurotransmitters makes lymphocytes susceptible to sympathetic stimulation. These receptors are of the β2-adrenergic subtype and couple to adenylyl cyclase to form the second messenger, cyclic AMP.41-45 It is known that cyclic AMP can inhibit mitogen and antigen-induced T-cell proliferation, cytotoxic T-lymphocyte function, as well as leading to a decrease in natural killer cell activity.17,23,24,40,44-46

As products of activated cells of the immune system, cytokines function as an interactive communication network to coordinate the immune response in the development of inflammation and immunity.57,48 One of the most important cytokines responsible for proliferation of T lymphocytes is IL-2, which is produced by a population of activated lymphocytes presumably acting through the IL-2 receptor.49-51 This interaction appears to be greatly influenced by sympathetic activity, as illustrated by Feldman et al,52 who demonstrated that β-agonists could block the expression of IL-2 in activated cells. We recently demonstrated that the sympathetic drive from exercise likewise blunted IL-2 receptor expression.36

Other cytokines, such as tissue necrosing factor, interleukin I, and γ-interferon, have proven to be elevated in states of adrenergic overactivity and may be under the control of the sympathetic nervous system.53-56 Not only are these cytokines important in immune surveillance, but they are capable of a dramatic inhibition of contractile function.54

Immunologic Abnormalities in Heart Failure

Several investigative groups have identified abnormalities in both humoral and cellular immunity in patients with dilated cardiomyopathy. These findings have included decreased T-suppressor/cytotoxic function, decreased natural killer cell activity, and a higher than expected incidence of heterophil antibodies against constituents of heart.30,28-31,57 In favor of an autoimmune pathogenesis of dilated cardiomyopathy is the linkage to the human leukocyte antigen class II family. A recent report including a meta-analysis of five studies shows a significant increase in HLA-DR4 antigen in patients with idiopathic dilated cardiomyopathy. Recently, several groups have detected autoantibodies against cardiac β-adrenergic receptors in patients with

Table 3. Baseline Immunologic and Catecholamine Data in the Study Group

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After (-) Metoprolol</th>
<th>After (+) Metoprolol</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells/µL</td>
<td>7508±680</td>
<td>7920±620</td>
<td>6406±600</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes/µL</td>
<td>1560±160</td>
<td>1530±140</td>
<td>1770±140</td>
<td>.2</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>21±2</td>
<td>19±2</td>
<td>28±3</td>
<td>.08</td>
</tr>
<tr>
<td>Norepinephrine, pg/mL</td>
<td>550±90</td>
<td>585±100</td>
<td>370±70</td>
<td>.14</td>
</tr>
<tr>
<td>Epinephrine, pg/mL</td>
<td>60±12</td>
<td>68±7</td>
<td>56±8</td>
<td>.8</td>
</tr>
</tbody>
</table>

*Metoprolol-treated patient before and after treatment paired t test.
idiopathic dilated cardiomyopathy, a potential explanation for the downregulation of cardiac β-receptors found in patients with end-stage heart failure.

We recently found a marked reduction in the number of circulating lymphocytes as well as a decrease in natural killer and T-suppressor/cytotoxic cells and an increase in the T-helper−T-suppressor/cytotoxic ratio in a large group of patients with heart failure. Upon further characterization of these patients, we found that alterations in the circulating lymphocytes of patients with CHF were not related to the cause of the disease but rather to its severity and could in fact be correlated with plasma norepinephrine levels. Moreover, the alterations in the patients with CHF were identical with those observed after treatment of healthy subjects with the β2-agonist terbutaline.

Results of the Present Study

In the present study, treatment with β-blockers was associated with an improvement of T-cell function and cell-mediated immunity and an alteration in the cellular milieu that may inhibit autoantibody production. While we did not perform natural killer cell activity, we and others have determined that an increase in natural killer cells is associated with increased 51Cr killing. It is difficult to know whether the immune changes were a consequence of treatment, either directly or indirectly through improvement in cardiac function. There is some evidence that a direct effect exists. First, we found a clear relation between improvement in cell-mediated immunity, T-cell function, and the extent of increase in ejection fraction after treatment with metoprolol. Patients who were not treated with metoprolol had no changes in their immune parameters. Most of these patients remained anergic. Second, virtually all patients in both groups were taking converting enzyme inhibitors at the study outset. While this class of drugs is known to improve left ventricular function and survival in patients with heart failure, these patients still had marked immune abnormalities, including a 73% incidence of anergy to a panel of skin tests. Yet, after β-blocker treatment, we found further improvement in ejection fraction along with normalization of T cells and cell-mediated immunity. This suggests the possibility of a direct interaction of the sympathetic nervous system on the immune system that may then lead to further progression of heart failure.

Our data show a reversal of sympathetic-induced alterations in natural killer cells, T-suppressor/cytotoxic cells, mitogen proliferation, and IL-2-receptor expression when β-blocker treatment was given late in the course of the therapeutic regimen of patients with CHF. These reversals were associated with improvement of left ventricular function additional to that seen with converting enzyme inhibitor treatment, suggesting that the underlying pathophysiology is improved. Since metoprolol is a β1-receptor blocker, other actions may account for its beneficial effect besides the β2-mediated release of immune cells from the spleen. These might include regulation of splenic blood flow and alterations of cellular adhesion molecules (unpublished data).

Limitations of the Present Study

This was an unblinded study in which patients treated with metoprolol served as their own controls with regard to cardiac and immune function. We specifically recruited patients most likely to benefit by β-blocker treatment, that is, those with an elevated heart rate (unpublished observations and Reference 60). Thus, our metoprolol patients cannot be compared directly with patients given standard treatment. There were, however, patients in the standard treatment group who had heart rates > 100 but who could not take metoprolol for other reasons (chronic obstructive pulmonary disease, claudication) and remained anergic despite standard treatment.

Although our data support the concept that sympathetic overactivity led to immune abnormalities, which led to progression of heart failure, we cannot prove a direct cause-and-effect relation. It is possible that the improvement in skin test status, T-cell function, and IL-2 receptors may occur independent of the improvement in ejection fraction. It is possible that with the improvement in ventricular function, patients became less "ill," which in and of itself might have affected...
outcome of some parameters of immune function. Nonetheless, the association with change in ejection fraction to immune status and the lack of such change in patients not treated with β-blockers suggests more than a causal relation. We believe that it is not just the improvement of left ventricular function that changes immune parameters. We recently performed a study in which we treated a group of normal patients with propranolol.61 We found that even with normal catecholamines, there was some enhancement of circulating T cells as well as an increase in concanavalin A–stimulated lymphocyte proliferation and IL-2 formation.

Conclusions

Our study shows that treatment of patients with CHF of either ischemic or idiopathic etiology with metoprolol resulted in improved parameters of cell-mediated immunity, especially the response to skin testing, and correlated with changes in ejection fraction. Additionally, there were increases in natural killer cell population, increase in T-suppressor/cytotoxic cells, and a trend toward increased mitogen proliferation and IL-2 receptor number. We believe that clinical improvement seen in patients taking β-blockers may in part be due to a blockade of sympathetic-induced immune abnormalities. If this is the case, then further investigation into the role of immunologically mediated progression of left ventricular dysfunction is indicated.

References

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