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β-adrenoceptor control of immune function in congestive heart failure

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1 We have determined the number of β-adrenoceptors and the isoprenaline-stimulated cAMP generation in lymphocyte subsets. The *in vitro* β-adrenergic sensitivity was greatest in $T_{suppressor/cytotoxic}$- and natural killer cells and smallest in T_{helper}- and B-cells. B lymphocytes appear to have a poor receptor coupling to adenylate cyclase as they have many β-adrenoceptors but generate only little cAMP in response to isoprenaline.
2 A 7 day treatment of healthy volunteers with terbutaline decreased the number of circulating cells in those lymphocyte subsets with a high *in vitro* sensitivity to β-adrenoceptor stimulation (i.e. $T_{suppressor/cytotoxic}$- and natural killer cells) but not in those with a poor *in vitro* sensitivity (i.e. T_{helper}- and B-lymphocytes).
3 Similar alterations of circulating lymphocyte subsets were found in patients with congestive heart failure (CHF). These alterations were not related to the aetiology of CHF but to its severity and could be correlated with plasma catecholamine levels.
4 We conclude that prolonged exposure to β-adrenoceptor agonists or enhanced sympathetic activity can decrease the number of circulating lymphocytes with an increase in the T_{helper}-/$T_{suppressor/cytotoxic}$-cell ratio.

Keywords heart failure β-adrenoceptors immune system lymphocytes

Introduction

Growing evidence suggests that the function of the immune system is (at least partly) under the control of the sympathetic nervous system. Lymphoid tissues are densely innervated with sympathetic nerve fibres (Felten *et al.*, 1987), and lymphocytes have membrane receptors for catecholamines which are of the β_2-adrenergic subtype (Brodde *et al.*, 1981). As the major sympathetic neurotransmitter noradrenaline is β_1-selective (Lands *et al.*, 1967), noradrenaline is unlikely to act on the β_2-adrenoceptors of circulating lymphocytes. Within the lymphoid tissues, however, the sympathetic nerve fibres end in close proximity to the lymphocytes (Felten *et al.*, 1987), so that very high local concentrations of noradrenaline might be achieved which could be sufficient to act on lymphocyte β_2-adrenoceptors.

Congestive heart failure is a disease state of increased sympathetic activity (Francis, 1988; Packer, 1988). Although enhanced adrenergic drive helps to maintain perfusion pressure in the face of a decreased effective plasma volume, prolonged sympathetic activation may ultimately contribute to the further deterioration of cardiac function. Thus, an inverse relationship between

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plasma noradrenaline concentrations and survival has been demonstrated in patients with CHF (Cohn & Rector, 1988). Alterations of the immune system have also been implicated in the pathogenesis of CHF (Fowles, 1987; Huber, 1987). Therefore, the present studies were performed to elucidate whether an alteration of the immune system may be caused by enhanced sympathetic activity. They consist of three parts: determination of the adrenergic sensitivity of lymphocyte subsets *in vitro* (Maisel *et al.*, 1989a), assessment of the effects of a 7 day treatment with the β-adrenoceptor agonist terbutaline on the number and subset composition of circulating lymphocytes (Maisel *et al.*, 1989b), and measurements of number and subset composition of circulating lymphocytes in patients with CHF (Knowlton *et al.*, 1988; Maisel *et al.*, 1989b).

β-adrenoceptor sensitivity of lymphocyte subsets *in vitro*

Twenty-eight subjects (age 24–43 years) of either sex (6 women, 22 men) participated in our study. All were drug-free, and had no history of hypertension, heart disease, diabetes, or thyroid disease. Informed consent was obtained from each subject, and the study protocol was approved by the University of California Committee for Investigations Involving Human Subjects. Blood (120 ml) was withdrawn from each subject between 08.00 h and 10.00 h after a 20 min resting period in the sitting position. Blood was anticoagulated with sodium citrate (0.38% final), and mononuclear leucocytes (MNL) were isolated and washed at room temperature according to previously published techniques (DeBlasi *et al.*, 1986).

MNL subsets were isolated by sequential positive selection using the following specific monoclonal antibodies (Becton Dickinson, Mountain View, CA): T_{helper}-cells with anti-Leu 3a, $T_{suppressor/cytotoxic}$-cells with anti-Leu 2a, B-cells with anti-Leu 12, natural killer cells with anti-Leu 7, anti-Leu 11, or anti-Leu 19, and monocytes with anti-Leu M3. The sequential subset isolation was performed over 2 days, with selection of T_{helper}-, $T_{suppressor/cytotoxic}$-, and natural killer cells on day 1, and B-cells on day 2. Depletion of the appropriate MNL subsets was monitored by flow-cytometric analysis of the remaining MNL. Our separation methods yielded MNL subsets of good viability (> 95% as assessed by trypan blue exclusion) and high purity. The Leu $3a^+$ T_{helper}-cell fraction contained < 1% Leu $2a^+$ cells, and the Leu $2a^+$ positive $T_{suppressor/cytolytic}$-cell fraction had < 2% Leu $3a^+$ cells. As CD 8 antigen detected by anti-Leu 2a is expressed on some Leu 7^+ cells, isolation of Leu $2a^+$ cells partially depleted the Leu 7^+ natural killer cell populations. Thus, the majority of natural killer cells selected from our volunteers were Leu $2a^-$, Leu 7^+, Leu 11^+, and Leu 19^+. The Leu 12^+ B-cells and the Leu $M3^+$ contained < 1% contaminating cells.

The number of β-adrenoceptors in each MNL subset was assessed by radioligand binding as previously described (DeBlasi *et al.*, 1986). Cyclic AMP accumulation was determined by adding 0.1 ml of cells ($2–10 \times 10^5$, ice-cold) to 0.9 ml of medium at 37° C. The final reaction mixture contained 100 μM isobutylmethylxanthine and 100 μM Ro 20-1724 to inhibit cyclic nucleotide phosphodiesterase, 10 μg ml^{-1} each of superoxide dismutase and catalase to prevent oxidation of isoprenaline (Mahan & Insel, 1984), and in some tubes 10 μM isoprenaline, 10 μM prostaglandin E_1, or 20 μM forskolin. The reactions were terminated after 2 min by centrifuging at 10,000 *g*, aspirating the supernatant, resuspending the pellet in 100 μM of 50 μM sodium acetate (pH 4.0) containing 0.2 mM isobutylmethylxanthine, and placing the tubes in a boiling water bath for 5 min. The tubes were then frozen and aliquots were later assayed for cAMP using a commercially available radioimmunoassay (Amersham). All assays were performed in quadruplicate.

Using these methods we found that T_{helper}-cells had the smallest (949 ± 91 sites/cell) and natural killer cells the greatest (2575 ± 261 sites/cell) density of β-adrenoceptors. $T_{suppressor/cytotoxic}$- and B-lymphocytes also had a high number of β-adrenoceptors (1797 ± 304), and the β-adrenoceptor density in monocytes was intermediate (1460 ± 18). As our sequential preparation protocol partially depleted Leu $2a^+$ Leu 7^+ (see above), we isolated natural killer cells prior to depletion of Leu $2a^+$ cells in several subjects. This yielded an even greater density of β-adrenoceptors on natural killer cells. The affinities of IPIN for β-adrenoceptors on T_{helper}-, $T_{suppressor/cytotoxic}$-, and natural killer cells ranged from 40–80 pM, while that on B-cells was 130 ± 30 pM.

Basal cAMP accumulation was similar in all subjects. Isoprenaline-stimulated cAMP accumulation was only roughly correlated with the density of β-adrenoceptors in each subset, i.e. was smallest in T_{helper}-cells (4.2 ± 1.9 pmol/10^6 cells) and greatest in $T_{suppressor/cytotoxic}$- and natural killer cells (15.0 ± 3.4 and 30.0 ± 3.3 pmol/10^6 cells, respectively). B-cells, however, which have many β-adrenoceptors only had little cAMP accumulation in response to

isoprenaline (4.8 ± 1.0 pmol/10^6 cells). Prostaglandin E_1 stimulated cAMP accumulation in $T_{suppressor/cytotoxic}$-cells much more than in any other subset (300 ± 33 pmol/10^7 cells).

In conclusion, subsets of circulating cells differ considerably in their sensitivity towards β-adrenoceptor stimulation *in vitro*. This differential sensitivity can only partially be explained by differences in receptor number. It appears that different efficacies of signal transduction in MNL subsets play a major role in the determination of sensitivity towards β-adrenoceptor stimulation. B-cells appear to have a particularly poor signal transduction following β-adrenoceptor occupation by agonists.

Regulation of circulating MNL subsets by terbutaline treatment

We studied 12 healthy drug-free men aged 24–43 years with no history of diabetes, hypertension, cardiac or thyroid disease. None of them showed signs of viral or bacterial infections. After the first blood withdrawal, the volunteers were given 5 mg terbutaline three times daily for 7 days. We have previously shown that this regimen down-regulates mixed MNL β-adrenoceptors (Maisel & Motulsky, 1987). All subjects noted increased tremulousness during the first 24–36 h after drug intake, but this abated and all subjects were able to complete the study. On the morning of day 8 (12 h after the last terbutaline dose), blood was withdrawn again. The numbers and subset distribution of white blood cells were determined by fluorescence-activated cell counting.

Treatment with terbutaline decreased the white blood cell count without altering the number of neutrophils or monocytes. Thus, the number of circulating lymphocytes decreased in 11 out of 12 volunteers by an average of 18%. Looking at lymphocyte subsets, the number of natural killer- and T-cells decreased, whereas the number of B-cells did not change. The number of circulating natural killer-cells was reduced in 8–10 out of 11 volunteers (depending on which antibody was used to define these cells), and the average decrease among all subjects was 35–48%. The reduction in the number of pan T-cells (10 out of 11 volunteers was predominantly due to a decrease in $T_{suppressor/cytotoxic}$-cell number (10 out 11 volunteers, 34% average decrease, $P = 0.0014$) with only a small decrease in T_{helper}-cells (15% average decrease, $P = 0.0990$). Accordingly, the T_{helper}-/$T_{suppressor/cytotoxic}$-cell ratio increased in 7 out of 10 volunteers from 1.2 ± 0.1 to 1.6 ± 0.1.

These data demonstrate that treatment with a β-adrenoceptor agonist decreases the number of circulating cells most in those lymphocyte subsets which have the greatest β-adrenergic sensitivity *in vitro*. As the number of B-cells (which have many β-adrenoceptors but generate cAMP only poorly) was not reduced, the decrease correlates better with the isoprenaline-stimulated cAMP generation than with the β-adrenoceptor number. As various investigators have shown that β-adrenoceptor stimulation can inhibit lymphocyte proliferation *in vitro* (Bourne *et al.*, 1974; Kammer, 1988), an anti-proliferative effect of β-adrenoceptor agonists on lymphocytes *in vivo* is the most likely explanation of these data. It is interesting that the number of monocytes and neutrophils did not decrease although these cells are quite sensitive to β-adrenoceptor stimulation *in vitro*. It may well be that β-adrenoceptor stimulation can inhibit their function (e.g. Mueller *et al.*, 1988) but does not inhibit neutrophil proliferation.

Alterations of circulating lymphocytes in heart failure patients

We studied 36 patients from the San Diego Veterans Administration Medical Center (age range 35–75 years, mean ± s.e. mean: 58 ± 2) with stable CHF NYHA class I–IV. None of them showed signs of viral or bacterial infections. Each patient had a clinical history of CHF for at least 6 months; four patients were in class IV, 12 in class III, 17 in class II, and three in class I. The latter three patients had had more severe heart disease, but were now classified as I after treatment with vasodilators. Seventeen patients were known to have occlusive coronary artery disease as documented either by coronary arteriography or a well documented myocardial infarction; five patients had presumed alcoholic cardiomyopathy, and 14 were classified as idiopathic. The ischaemic cardiomyopathy patients were either 'burnt out CAD' patients or had undergone angioplasty or bypass surgery after large anterior wall myocardial infarctions. Left ventricular ejection fraction, determined by equilibrium gated radionuclide ventriculography, averaged 30 ± 2%. All patients were receiving digitalis and diuretics, and most were on long-term therapy with vasodilator drugs (21 with nitrates, nine with hydralazine, and 27 with angiotensin converting enzyme inhibitors). Patients had not received adrenoceptor agonists or antagonists for at least 3 weeks before study. All cardiac medications were withdrawn for 12–24 h prior to study. These CHF patients were

compared with 31 healthy control subjects (age range 26–91 years, mean ± s.e. mean: 60 ± 4) with no history of cardiac impairment.

The number of circulating lymphocytes, but not that of total white blood cells was decreased in patients with CHF. This reduction was not randomly distributed, and affected only some lymphocyte subsets. The number of circulating natural killer-cells as assessed by three different monoclonal antibodies was decreased by an average of approximately 50%. The number of cells positive for Leu 7/2, a lymphocyte subset believed to be associated with anti-viral activity (Phillips & Lanier, 1986), was especially low in CHF patients (150 ± 26 *vs* 317 ± 37 cells μl^{-1} in control subjects, $P < 0.002$).

The number of circulating T-cells was also decreased in CHF patients. Among the T-cells, however, the reduction was again found in only some subsets. The number of $T_{suppressor/cytotoxic}$-cells was decreased by approximately 50%, whereas that of T_{helper}-cells was not significantly altered. Thus, T_{helper}-cells accounted for a higher percentage of T-cells in CHF patients (44.4 ± 1.8 *vs* 35.4 ± 1.7%, $P = 0.006$), and the calculated ratio T_{helper}-/$T_{suppressor/cytotoxic}$-cells was greater in CHF patients (2.3 ± 0.2) than in control subjects (1.2 ± 0.1, $P < 0.0001$).

To characterize further the alteration of circulating lymphocytes in CHF, we separated patients according to the aetiology and severity of their disease. If an immunological abnormality was responsible for cardiac dysfunction, one would expect to see differences only in those patients with idiopathic (presumably viral) cardiomyopathy. However, patients whose CHF was due to ischaemic heart disease ($n = 17$) had similar cell numbers of each lymphocyte subset as did patients with idiopathic CHF. When classified according to severity, patients with NYHA class III–IV had a more pronounced decrease of $T_{suppressor/cytotoxic}$- and natural killer-cells than did patients with NYHA class I–II. Similarly, plasma catecholamine levels (measured in 19 patients) were substantially higher in patients with more severe heart failure but did not differ between ischaemic or idiopathic CHF. These differences cannot be explained by different treatment modalities, since both groups were on similar drug regimens.

Taken together, the alterations of circulating lymphocytes in CHF patients are not related to the aetiology of the disease but rather to its severity and can be correlated to plasma noradrenaline levels. Moreover, the alterations in CHF patients match quite well those observed after treatment of healthy subjects with terbutaline. Thus while several neurohumoral parameters are altered in CHF patients, we consider enhanced sympathetic activity to be chiefly responsible for the alteration of circulating lymphocytes in CHF patients. Evidence against this hypothesis is the low β_2-adrenoceptor affinity of noradrenaline and the lack of adrenaline increases in our patients. It should be noted, however, that the elevation of plasma noradrenaline is only a reflection of enhanced release within tissues. As stated above, lymphocytes within the lymphoid tissues may very well be exposed to high local noradrenaline concentrations which are sufficient to act on β_2-adrenoceptors and thereby inhibit lymphocyte proliferation.

In summary, we have shown that lymphocyte subsets differ in their *in vitro* sensitivity to adrenergic stimulation and that this differential sensitivity is reflected by a distinct regulation of the number of circulating cells in each subset following treatment with a β-adrenergic agonist or in CHF. The relevance of these immunological alterations for heart failure and other disease states characterized by an increased sympathetic activity remains to be assessed.

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