Impaired catecholaminergic signalling of B lymphocytes in patients with chronic rheumatic diseases

M Wahle, S Kölker, A Krause, G R Burmester, C G O Baerwald

Abstract

Objective—To investigate further the influence of the autonomic nervous system on chronic rheumatic diseases.

Methods—The density and affinity of \( \beta_2 \) adrenergic receptors (\( \beta_2 \)R) on CD19+ lymphocytes in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis (SSc), as well as intracellular cAMP levels in patients with RA and SLE, were determined. Human peripheral blood mononuclear cells were separated from venous blood of patients and healthy controls by Ficoll-Hypaque density centrifugation. CD19+ lymphocytes were purified by magnetic cell sorting, and \( \beta_2 \)R were determined by a radioligand binding assay with \([\text{125I}]\text{iodocyanopindolol}\). Intracellular cAMP levels and \( \beta_2 \)R agonist induced cell death were measured by a radioligand assay and flow cytometry using annexin-V binding, respectively. Systemic disease activity of the patients was evaluated using multifactorial scoring systems.

Results—The density of \( \beta_2 \)R on peripheral blood lymphocytes in patients with RA, SLE, and SSc was significantly decreased in patients with RA, SLE, and SSc compared with healthy controls. In patients with RA and SSc, \( \beta_2 \)R density was negatively correlated with systemic disease activity. Furthermore, although basal intracellular cAMP levels were raised in patients with RA and SLE, the increase of cAMP upon stimulation of \( \beta_2 \)R was significantly reduced in these patients compared with control subjects. Preliminary data suggest that \( \beta_2 \)R agonist induced cell death is diminished in patients with RA exhibiting decreased \( \beta_2 \)R densities.

Conclusions—The results of this study show a reduction of \( \beta_2 \)R densities on B lymphocytes mirrored by an impaired intracellular cAMP generation in patients with chronic rheumatic diseases, indicating a decreased influence of the autonomic nervous system on B cells in these conditions.

(Ann Rheum Dis 2001;60:505–510)

Although the cause of inflammatory autoimmune diseases like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis (SSc) is unknown, it is well accepted that cellular and humoral immune responses underlie the distinct pathology of these diseases. A hallmark of chronic rheumatic diseases is the formation of autoantibodies—for example, rheumatoid factor in RA, antibodies against double stranded DNA (dsDNA) and Smith antigen (Sm) in SLE, and against topoisoerase-II (Scl-70) in SSc. Additionally, immune complexes and hypergammaglobulinemia are common features, indicating a B cell hyperactivity.

A growing body of evidence points towards a modulation of immune responses in vitro as well as in vivo and the inflammatory activity of autoimmune diseases by the autonomous nervous system. It has been shown that lymphocytes and noradrenergic varicosities form synapse-like conjunctions in lymphoid organs and that \( \beta_2 \) adrenergic receptors (\( \beta_2 \)R) are expressed on various lymphocyte subpopulations.

Previous studies of our group showed that in patients with chronic rheumatic diseases the density of \( \beta_2 \)R is decreased on peripheral blood mononuclear cells (PBMC). Further investigations showed that \( \beta_2 \)R are modulated differentially on lymphocyte subsets because \( \beta_2 \)R densities were reduced on CD8+ lymphocytes but not on CD4+ cells. However, little is known about \( \beta_2 \)R on B lymphocytes in these disease entities.

Therefore we investigated the characteristics of \( \beta_2 \)R on peripheral blood B lymphocytes (CD19+ mononuclear cells) in patients with RA, SLE, and SSc, together with the systemic disease activity, and determined the coupling of \( \beta_2 \)R to the intracellular signal transduction cascade.

Methods

PATIENTS AND CONTROL SUBJECTS

Patients with RA (n=24), SLE (n=13), and SSc (n=6) according to classical diagnostic criteria and a group of healthy blood donors (n=16) were included in the study. We excluded patients in whom other factors were supposed to influence \( \beta_2 \)R (that is, infectious and atopic diseases, hyperthyroidism/hypothyroidism, hypertonia, treatment with sympathomimetic/sympatholytic agents, cancer). Patients were examined by taking a history, physical examination, and laboratory findings (erythrocyte sedimentation rate (ESR), C reactive protein (CRP), haemoglobin, packed cell volume, leucocytes, lymphocytes, platelets, autoantibodies, creatinine). Inflammatory disease activity in RA, SLE, and SSc was determined by different multifactorial scoring systems. For patients with RA we used a modified total disease activity score according to Farr et al., (TAI) which includes morning stiffness, the Ritchie articular index, ESR,
CRP, and haemoglobin. For patients with SLE the SLAM was used. 14 For the patients with SSc a new scoring system was adapted from the SLAM and called the SSc score (SSS, table 1). Table 2 summarises the clinical characteristics of patients and control subjects. Treatment with non-steroidal antirheumatic drugs or steroids up to 7.5 mg prednisolone equivalent a day were allowed in all patient groups (RA 15/24 patients, range 2–7.5 mg prednisolone equivalent a day, SLE 9/13, range 2.5–7.5 mg, SSc 5/6, range 2–7.5 mg). No patient received disease modifying antirheumatic drugs.

**Table 1** Measures used to obtain the disease activity score of patients with systemic sclerosis, called the systemic sclerosis score (SSS). Each variable was graded from 0 to 3, points were then added to the total activity index with a maximum of 78 points.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
<th>Score</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>General progression</td>
<td>Limited</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Weight loss</td>
<td>&lt;10% Body weight</td>
<td>&gt;10% Body weight</td>
<td>Functional limitation</td>
</tr>
<tr>
<td>Fatigue</td>
<td>No limits on activity</td>
<td>Functional limitation</td>
<td></td>
</tr>
<tr>
<td>Sclerodactyly</td>
<td>Oedematous state</td>
<td>Sclerotic state</td>
<td>Atrophic state</td>
</tr>
<tr>
<td>Cutaneous expansion</td>
<td>Face or extremity</td>
<td>Face and extremity</td>
<td>Global</td>
</tr>
<tr>
<td>Respiratory insufficiency</td>
<td>Partial</td>
<td>Face, extremity, and trunk</td>
<td></td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>Dry cough</td>
<td>Shortness of breath with exercise</td>
<td></td>
</tr>
<tr>
<td>Dysphagia</td>
<td>Present</td>
<td>Complications</td>
<td></td>
</tr>
<tr>
<td>Peptic oesophagitis</td>
<td>Gastro-oesophageal reflux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>Present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>Low pattern</td>
<td>Active pattern</td>
<td></td>
</tr>
<tr>
<td>Capillary microscopy</td>
<td>Compensated</td>
<td>Decompensated</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>90–105</td>
<td>105–115</td>
<td>&gt;115</td>
</tr>
<tr>
<td>Heart failure</td>
<td>Compensated</td>
<td>Retrospernal pain</td>
<td></td>
</tr>
<tr>
<td>Pericarditis</td>
<td>Signs in ECG</td>
<td>With haemodynamic effect</td>
<td></td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>Present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myalgia/myositis</td>
<td>Moderate</td>
<td>Severe functional impairment</td>
<td></td>
</tr>
</tbody>
</table>

For patients with SLE the SLE activity measure (SLAM) was used. 14

**Table 2** Clinical characteristics of patients and control subjects studied

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Sex (M:F)</th>
<th>Age* (years)</th>
<th>Duration of disease* (years)</th>
<th>Autoantibody positive patients (n)</th>
<th>Disease activity index† (points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with SLE</td>
<td>13</td>
<td>0/13</td>
<td>47 (23–69)</td>
<td>4 (1–15)</td>
<td>Anti-dsDNA (pos) 10</td>
<td>SLAM 3–11</td>
</tr>
<tr>
<td>Patients with SSc</td>
<td>6</td>
<td>3/3</td>
<td>53.5 (42–69)</td>
<td>6.5 (1–11)</td>
<td>Anti-Scl-70 (pos) 4</td>
<td>SSS 3–15</td>
</tr>
<tr>
<td>Control subjects</td>
<td>16</td>
<td>3/13</td>
<td>43 (27–56)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean (range).
†TAI = total disease activity score; SLAM = SLE activity measure; SSS = SSc score.
Impaired catecholaminergic signalling of B lymphocytes

Specific binding of ICYP was determined by subtracting unspecific binding from the total binding capacity. The maximum number of ICYP binding sites, representing the number of β2R, and dissociation constant (KD) for ICYP of β2R were calculated according to the method of Scatchard.18

DETERMINATION OF BASAL AND STIMULATED INTRACELLULAR CAMP

The concentration of basal and stimulated levels of cAMP in CD19+ lymphocytes was determined in patients with RA (n=5), SLE (n=4), and controls (n=8) by a radioimmunoassay. Aliquots of 1×10^6 CD19+ cells in PBS/BSA containing 50 mM theophylline and 10 μM ascorbic acid were either incubated for 15 minutes at 37°C to determine basal cAMP levels or incubated with 10 μM (-)-isoprenaline for 15 minutes to determine stimulated cAMP levels.19 20 After boiling and centrifugation, cAMP in the supernatant was determined in patients with RA (n=5), SLE (n=3), SSc (n=6) compared with healthy control subjects (controls, n=16) (p<0.001; one way analysis of variance followed by Tukey’s procedure; RA, SLE, or SSc v controls). No significant difference is seen between the three patient groups. Data are presented as plots of the receptor numbers of individual subjects, the bar representing the mean.

Figure 1 Density of β2 adrenergic receptors (β2R) on CD19+ lymphocytes. The density of β2R on CD19+ cells is significantly reduced in patients with chronic rheumatic diseases (rheumatoid arthritis (RA, n=24), systemic lupus erythematosus (SLE, n=13), systemic sclerosis (SSc, n=6) compared with healthy control subjects (controls, n=16) (p<0.001; one way analysis of variance followed by Tukey’s procedure; RA, SLE, or SSc v controls). No significant difference is seen between the three patient groups. Values are presented as means ± standard errors of the mean (SEM) if not otherwise indicated. Correlations between β2R characteristics and disease activity scores were calculated by the Pearson product moment correlation. A comparison of independent single variables between the groups was calculated by one way analysis of variance (ANOVA) followed by Tukey’s procedure. When normality test failed, Kruskal-Wallis one way ANOVA on ranks was used. A comparison of isoprenaline induced cell death in patients with RA and healthy controls was calculated by a paired t test. p Values less than 0.05 were considered to be significant.

STATISTICAL ANALYSIS

Values in tables and figures are given as means and standard errors of the mean (SEM) if not otherwise indicated. Correlations between β2R characteristics and disease activity scores were calculated by the Pearson product moment correlation. A comparison of independent single variables between the groups was calculated by one way analysis of variance (ANOVA) followed by Tukey’s procedure. When normality test failed, Kruskal-Wallis one way ANOVA on ranks was used. A comparison of isoprenaline induced cell death in patients with RA and healthy controls was calculated by a paired t test. p Values less than 0.05 were considered to be significant.

RESULTS

β2R DENSITY AND DISSOCIATION CONSTANTS

The number of β2R on CD19+ cells was significantly decreased in patients with chronic rheumatic diseases compared with control subjects (p<0.001). The mean (SEM) number of β2R was 1041 (44) binding sites (bs)/cell in patients with RA, 1278 (103) bs/cell in patients with SLE, and 1035 (83) bs/cell in patients with SSc, respectively. On CD19+ cells derived from healthy donors 2060 (106) bs/cell were detected (fig 1). No significant difference was found between the β2R density in the various patient groups.

The KD values of β2R for ICYP were reduced in the patient groups (RA 5.89 (0.53) pmol/l; SLE 6.36 (0.92) pmol/l; SSc 5.9 (0.76) pmol/l) compared with the control group (9.86 (1.36) pmol/l). However, when the Kruskal-Wallis test was used the difference between patient groups and control subjects did not reach significance (p=0.113).

Stratifying patients with RA and SLE according to the use of corticosteroid drugs did not disclose a difference in β2R characteristics between the respective groups: patients with RA receiving corticosteroid treatment 1036 (62.6) bs/cell (KD 5.9 (0.7) pmol/l; n=15) v patients with RA without corticosteroid drugs 1048 (56.2) bs/cell (KD 5.8 (0.8) pmol/l; n=9); and patients with SLE receiving corticosteroid drugs 1287 (140) bs/cell (KD 6.4 (1.2) pmol/l; n=9) v patients with SLE without corticosteroids 1273 (144) bs/cell (KD 6.3 (1.5) pmol/l; n=4).

CORRELATION OF β2R STATUS WITH DISEASE ACTIVITY IN RA, SLE, AND SSc

In patients with RA and SSc, β2R density on CD19+ lymphocytes showed a significant negative correlation with disease activity. In patients with RA a negative correlation between the β2R density and the TAI (r=−0.76,
was found. In the SLE group \( \beta_2R \) density on CD19+ lymphocytes was negatively correlated with SLAM, but the correlation was not significant \((r=-0.52, p=0.07)\). This was because one patient had high disease activity as well as high \( \beta_2R \) density (fig 2). When this patient was omitted a significant negative correlation between \( \beta_2R \) density and disease activity \((r=-0.79, p=0.002)\) was found. No significant correlation was found between the KD values of the various patient groups and the disease activity \((p>0.05)\).

**Discussion**

The results of our study demonstrate a profound modulation of \( \beta_2R \) on B lymphocytes (CD19+ lymphocytes) in chronic rheumatic diseases. The density of \( \beta_2R \) on B lymphocytes was decreased in patients with RA, SLE, and SSc compared with healthy control subjects, and a negative correlation between \( \beta_2R \) density and disease activity scores was found in all patient groups studied except the SLE group. In addition, inducible intracellular cAMP levels in response to stimulation of \( \beta_2R \) were reduced in...
patients with chronic rheumatic diseases, thus demonstrating the functional significance of \( \beta_2 \)R modulation.

Previous studies showed that \( \beta_2 \)R density was decreased on PBMC in patients with chronic rheumatic diseases.\(^7\)\(^{17}\)\(^{22}\) Recently, it was shown that in patients with RA, \( \beta_2 \)R were differentially regulated in lymphocyte subpopulations because \( \beta_2 \)R on CD8+ lymphocytes were down regulated in contrast with unchanged \( \beta_2 \)R numbers on CD4+ lymphocytes.\(^7\) When the results of these studies are taken together the picture emerges that in chronic rheumatic diseases the expression of \( \beta_2 \)R on T and B lymphocytes is a complex modulated process rather than simply down regulation.

Various mechanisms contributing to the observed differential modulation of \( \beta_2 \)R expression are possible. A long term increase of catecholamines decreases \( \beta_2 \)R density through the stimulation of the \( \beta \) adrenoceptor kinase.\(^{22}\)\(^{23}\) However, the differential regulation of \( \beta_2 \)R on CD4+ and CD8+ lymphocyte subsets in patients with RA argues against a simple down regulation of \( \beta_2 \)R due to an increase of systemic catecholamine concentrations,\(^7\) as can be seen in patients with pheochromocytoma.\(^{24}\) Moreover, a correlation with plasma catecholamine levels could not be shown either for systemic disease activity or for \( \beta_2 \)R density on PBMC in patients with RA.\(^6\)

The affinity of \( \beta_2 \)R in human neutrophils and the transcription of \( \beta_2 \)R mRNA in human lung cells have been shown to be increased by corticosteroids.\(^7\) In RA and SLE a decreased cortisol plasma concentration due to alterations in cortisol metabolism in SLE\(^{24}\)\(^{27}\) and a disturbed adrenal secretion of cortisol in RA\(^{28}\) have been proposed. Therefore, decreased cortisol concentrations are a possible mechanism for the down regulation of \( \beta_2 \)R in rheumatic diseases. However, low dose corticosteroids did not influence \( \beta_2 \)R characteristics in previous investigations, nor in the study presented here. Thus, at least in patients with chronic rheumatic diseases, low dose corticosteroids do not influence \( \beta_2 \)R characteristics of immune cells.\(^{17}\)\(^{29}\)

Other factors, such as a differential regulation of high and low affinity \( \beta_2 \)R and a defect in intrinsic \( \beta_2 \)R regulation, may also contribute to the disturbed \( \beta_2 \)R expression.\(^{30}\)\(^{32}\) Because it has been shown in this study and in previous studies that there was a striking negative correlation between \( \beta_2 \)R density and the disease activity,\(^6\)^{\text{**}} it is tempting to speculate that the density of \( \beta_2 \)R on PBMC is influenced by the inflammatory process. In particular, cytokines and eicosanoids like prostaglandin E\(_2\) are known to modulate \( \beta_2 \)R density in vitro.\(^{32}\)\(^{33}\) Furthermore, investigations of our group showed that interleukin 2 may be a key player in modulating \( \beta_2 \)R expression differentially in CD4+ and CD8+ cells.\(^34\)

The generation of cAMP after stimulation of \( \beta_2 \)R with isoprenaline was impaired in B lymphocytes of patients with RA and SLE. As has been shown previously, the increase of stimulated cAMP rather than basal levels is crucial for the intracellular signal transduction.\(^35\) It seems unlikely that changes in intracellular cAMP levels were induced by preparation of CD19 positive cells, as engagement of CD19 does not interfere with cAMP production in B cells.\(^36\)

As a functional consequence of \( \beta_2 \)R stimulation, cell death induced by isoprenaline was decreased in B lymphocytes of patients with RA and SLE. As has been shown previously, the increase of stimulated cAMP rather than basal levels is crucial for the intracellular signal transduction.\(^35\) It seems unlikely that changes in intracellular cAMP levels were induced by preparation of CD19 positive cells, as engagement of CD19 does not interfere with cAMP production in B cells.\(^36\)

In conclusion, our results show that the density of \( \beta_2 \)R and the agonist induced cAMP
production are impaired in B lymphocytes of patients with chronic rheumatic diseases. However, it is not clear whether this phenom-

en occurs in response to the inflammatory process or precedes exacerbations of chronic rheumatic diseases. In every case, changes in the regulation of β2R density and coupling to the intracellular signalling machine may be an accelerating factor for B cell dysregulation and predispose to the development of rheumatic diseases.


Impaired catecholaminergic signalling of B lymphocytes in patients with chronic rheumatic diseases

M Wahle, S Kölker, A Krause, G R Burmester and C G O Baerwald

Ann Rheum Dis 2001 60: 505-510
doi: 10.1136/ard.60.5.505