Increased Activation of Protein Kinase A Type I Contributes to the T Cell Deficiency in Common Variable Immunodeficiency

Pål Aukrust, Einar Martin Aandahl, Bjørn S. Skålhegg, Ingvild Nordøy, Vidar Hansson, Kjetil Taskén, Stig S. Frøland and Fredrik Müller

*J Immunol* 1999; 162:1178-1185; http://www.jimmunol.org/content/162/2/1178

**References**

This article cites 38 articles, 15 of which you can access for free at:
http://www.jimmunol.org/content/162/2/1178.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Increased Activation of Protein Kinase A Type I Contributes to the T Cell Deficiency in Common Variable Immunodeficiency

Pål Aukrust,‡ Einar Martin Aandahl,† Bjørn S. Skålhegg,† Ingvild Nordøy,* Vidar Hansson,† Kjetil Taskén,† Stig S. Frøland,* and Fredrik Müller*

The molecular mechanisms underlying the T cell dysfunction often present in common variable immunodeficiency (CVI) are not established. cAMP-dependent protein kinase A type I (PKAI) is an important inhibitor of T cell proliferation after Ag stimulation. We therefore investigated the possibility that activation of PKAI may be involved in the development of T cell dysfunction in CVI. An exogenously added PKAI-selective antagonist (Rp-8-Br-cAMPS) induced a significant increase in anti-CD3-stimulated proliferation of 20 CVI patients compared with no effect in 15 controls. Purified T cells from 7 CVI patients with strictly defined T cell deficiency had elevated endogenous cAMP levels compared with controls. Treatment of T cells from these CVI patients with Rp-8-bromo-cAMP-phosphorothioate markedly improved anti-CD3-stimulated proliferation (up to 3.7-fold), particularly in CD4⁺ lymphocytes, reaching proliferation levels comparable to control values. No effect of cAMP antagonist on T cell proliferation was seen in controls. In these CVI patients, cAMP antagonist also increased IL-2 production in anti-CD3-stimulated T cells. However, exogenously added IL-2 at concentrations comparable to the achieved increase in IL-2 levels after addition of cAMP antagonist had no effect on T cell proliferation. Furthermore, the stimulatory effects of exogenously added IL-2 at higher concentrations and cAMP antagonist on T cell proliferation were additive. Our findings indicate that increased PKAI activation may be an important molecular basis for the T cell defect in CVI and suggest that the cAMP/PKAI system may be a potential molecular target for immunomodulating therapy in these patients. The Journal of Immunology, 1999, 162: 1178–1185.

Cyclic AMP is a key negative regulator of lymphocyte proliferation (1–3). With few exceptions all known actions of cAMP are mediated through cAMP-dependent protein kinases (PKA) (4, 5). Enhanced cAMP levels completely abolish early tyrosine phosphorylation following engagement of the Ag receptor as well as T cell proliferation induced through the TCR/CD3 complex (2, 3). This regulation seems mainly to be mediated through PKA type I (PKAI), and this isoenzyme of PKA redistributes and colocalizes with the Ag receptor during T cell activation (6). This serves to establish PKAI as an acute negative modulator of T cell Ag responses and clonal expansion. Triggering of the TCR/CD3 complex in itself leads to the production of cAMP (7, 8), suggesting that PKA activation following TCR/CD3 stimulation may represent a negative feedback control mechanism. In support of this, impaired PKAI has recently been demonstrated in T cells from patients with systemic lupus erythematosus, possibly contributing to persistent immune activation in these patients by lack of inhibition (9).

Common variable immunodeficiency (CVI) is a heterogeneous group of B cell deficiency syndromes characterized by defective Ab production, recurrent sinopulmonary bacterial infections, and a high rate or incidence of lymphoid and gastrointestinal malignancies, nonmalignant lymphoid hyperplasia, and granulomatous inflammation (10, 11). Although B cell defects are the immunologic hallmark of CVI, T cell abnormalities such as abnormal distribution of T cell subsets, impaired proliferative response to recall Ags, and dysregulated cytokine production, have been reported in a significant subset of patients (12, 13). These T cell abnormalities may be of importance both for the defective Ab production and for the clinical manifestations in CVI. In fact, the finding that B cells from CVI patients can proliferate and produce Igs if appropriately stimulated in vitro (14, 15) suggests that B cells in many CVI patients may not be intrinsically defective and that inappropriate T cell help is of importance for induction of the immunodeficiency.

The molecular mechanisms underlying the T cell dysfunction in CVI are not established. However, studies in a subgroup of CVI patients have suggested a defect in the early phase of T cell activation after triggering the TCR/CD3 complex and before activation of protein kinase C (PKC) (16–18). As cAMP through activation of PKAI exerts an early inhibitory effect on signaling through the TCR/CD3 complex (3, 19, 20), we investigated the possibility that the cAMP/PKAI system may be involved in the development of T cell dysfunction in CVI.

Materials and Methods

Patients and controls

Twenty consecutively recruited patients with the diagnosis of CVI based on established criteria (10, 11) were included in the study (Table I). Based on previously defined criteria (21, 22), 10 patients had splenomegaly, 6 had...
Table I. Clinical and immunologic characteristics of the study group

<table>
<thead>
<tr>
<th>Control (n = 15)</th>
<th>CVI (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in years (range)</td>
<td>41 (22–65)</td>
</tr>
<tr>
<td>Males/females</td>
<td>7 (47%)/8 (53%)</td>
</tr>
<tr>
<td>CD19+ lymphocytes (×10^9/l)</td>
<td>170</td>
</tr>
<tr>
<td>CD8+ lymphocytes (×10^9/l)</td>
<td>380</td>
</tr>
<tr>
<td>CD4+ lymphocytes (×10^9/l)</td>
<td>760</td>
</tr>
<tr>
<td>Monocytes (×10^9/l)</td>
<td>260</td>
</tr>
<tr>
<td>Serum neopterin (nmol/l)</td>
<td>9.1 (5.9–10.1)</td>
</tr>
</tbody>
</table>

* Lymphocyte subsets and monocytes were analyzed in peripheral blood. Data are given as medians and 25th to 75th percentiles if not otherwise stated. † p < 0.05; ‡ p < 0.10; § p < 0.001 vs controls.

Bromo-deoxy-uridine (BrUrd) incorporation as determined by flow cytometry

Determination of BrUrd incorporation in lymphocytes was performed as described previously (24) with some modifications. Briefly, negatively selected CD3+ T cells were cultured in 24-well plates (Costar; 10^6 cells/ml, 1 ml/well) precoated with anti-CD3 Ab (clone Sp2/3b; final dilution, 1/1500) or without coat (unstimulated), with or without preincubation with different concentrations of 8-Br-cAMPS. After 48 h, BrUrd (Sigma; final concentration, 30 μg/ml) was added to cell cultures. Sixteen hours later, the cells were washed once in PBS; resuspended in PBS with 5% mouse serum (Sigma), 5% human Ig (Octagam, Octapharma, Vienna, Austria), 2% BSA, and 0.1% sodium azide; and stained for membrane Ags by phycoerythrin-conjugated Abs (CD4, clone SK 3; CD8, clone SK 1; Becton Dickinson, San Jose, CA) for 30 min at 4°C. Thereafter, cells were washed in staining buffer (PBS with 1% BSA; Life Technologies), fixed in 1% paraformaldehyde (Sigma) and PBS with 0.01% Tween-20 (Sigma) overnight at 4°C, washed in PBS, and incubated in RPMI with 50 Kunitz units/ml DNase-I (Sigma) at 37°C for 45 min. Cells were then incubated in 150 μl of PBS, with 10% BSA and 0.5% Tween-20, and 20 μl of FITC-conjugated anti-BrUrd (Becton Dickinson) at 20°C for 45 min; washed twice; and finally resuspended in PBS. Samples were analyzed using a FACS-Scan (Becton Dickinson) with CellQuest software (Becton Dickinson). List mode files were collected from 10,000 cells from each sample.

Determination of IL-2 levels

For determination of IL-2 levels, negatively selected CD3+ T cells (10^6/ml, 200 μl/well) were cultured in medium alone or were stimulated with anti-CD3 Abs (clone Sp2/3b; final dilution 1/125,000) with or without preincubation with different concentrations of Rp-8-Br-cAMPS. The anti-CD3 Abs were cross-linked with immunomagnetic beads as described above. After 20 h of culture, cell-free supernatants were harvested and stored at −80°C until analysis. IL-2 levels in supernatants were determined by ELISA (R&D Systems, Minneapolis, MN).

cAMP determination

Extraction of cAMP and analyses of intracellular cAMP contents by RIA (Amersham, Aylesbury, U.K.) in negatively selected CD3+ T cells were performed as previously described (25). Basal levels of cAMP were stable at 4°C in both PBMC suspensions and negatively selected CD3+ T cells for >120 min, i.e., the time required for isolation of CD3+ T cells (data not shown).

Miscellaneous

The numbers of CD4+, CD8+, and CD19+ lymphocytes in peripheral blood were determined by immunomagnetic quantification (22). Serum levels of neopterin were measured by RIA (IMMU test Neopterin, Henning Berlin, Berlin, Germany).

Statistical analysis

For comparison of two groups of individuals, the Mann-Whitney U test (two-tailed) was used. For comparison of parameters within the same individuals, the Wilcoxon signed rank test for paired data (two-tailed) was used. Coefficients of correlation were calculated by the Spearman rank test. Curve-fit analyses were performed using Sigma plot (Jandel, Erkrath, Germany). Data are given as medians and 25th to 75th percentiles if not otherwise stated. The p values are two-sided and are considered significant when <0.05.

Results

Effect of cAMP antagonist on PBMC proliferation

To address the possible role of the cAMP/PKA system in the impaired T cell function in CVI, we first examined whether a sulfur-substituted cAMP analogue (Rp-8-Br-cAMPS), working as a full antagonist for PKA (26), could improve anti-CD3-stimulated proliferation of PBMC in 20 consecutively recruited CVI patients and 15 healthy controls. Confirming previous results (22, 27), stimulated lymphocyte proliferation was significantly impaired in this CVI population compared with that in control subjects (14,980 (9,100–21,340) cpm vs 63,340 (49,100–84,300) cpm; p < 0.001;
CVI patients and controls, respectively). Furthermore, while antagonist did not significantly alter the proliferation of lymphocytes obtained from normal blood donors, Rp-8-Br-cAMPS induced a significant and concentration-dependent improvement of anti-CD3-stimulated proliferation in the CVI group (fold increase with maximal Rp-8-Br-cAMPS concentration (1000 μM), 1.63 (1.39–2.37) vs 1.07 (1.05–1.12); p < 0.001; CVI patients and controls, respectively). However, single patient data from the CVI group revealed heterogeneity. Whereas a >100% increase in anti-CD3-induced lymphocyte proliferation was found in seven of the CVI patients, five of the patients had a <40% increase in proliferation when the cAMP antagonist was added to cells in vitro. Of note, the patients with the most marked increase in lymphocyte proliferation after stimulation with the cAMP antagonist were those with the most severely depressed proliferative response after anti-CD3 stimulation (r = −0.85; p < 0.001).

Bacterial products and endotoxins may influence the proliferation of PBMC, but CVI patients with chronic rhinosinusitis and bronchiectasis did not differ from other patients with respect to either proliferation or the effect of cAMP antagonist (data not shown). Furthermore, when examining the effect of cAMP antagonist on anti-CD3-stimulated proliferation of PBMC from three patients with X-linked agammaglobulinemia (XLA), a subgroup of primary hypogammaglobulinemia not characterized by T cell deficiency (11, 12), all had a <20% increase in lymphocyte proliferation after addition of cAMP antagonist, although the duration of replacement therapy and clinical symptoms was longer, and the occurrence of chronic infectious complications increased in these XLA patients compared with those in the CVI group (data not shown).

Effects of cAMP agonist and antagonist in purified T cells

CVI patients represent a heterogeneous group of patients, and T cell deficiency is a significant feature in only a subgroup of patients. When further examining the possible role of cAMP/PKAI in the T cell deficiency in CVI, we therefore in subsequent experiments studied CVI patients characterized by markedly and persistently impaired T cell function based on the following criteria: 1) anti-CD3-stimulated lymphocyte proliferation <15% of median levels in healthy controls, and 2) this impaired T cell function should have been confirmed at least three times during the last 5 yr. Seven of the CVI patients fulfilled these criteria, and these patients were compared with eight of the healthy controls. These CVI patients with T cell deficiency were not different from the other CVI patients with respect to Ig dosage, duration of Ig replacement therapy, duration of symptoms, or occurrence of infectious complications. However, they had significantly higher serum neopterin levels as a marker of monocyte hyperactivity (22) than CVI patients without strictly defined T cell deficiency (38.6 ± 26.3–65.8 μM) vs 19.3 (10.8–28.7) nmol/l; p < 0.01). Furthermore, while all CVI patients with T cell deficiency had splenomegaly, this was found in only three of the other 13 CVI patients.

When analyzing cAMP levels in negatively selected purified T lymphocytes from CVI patients with T cell deficiency and controls, we found significantly higher cAMP levels in the CVI group (Fig. 1A). The sensitivity to cAMP-dependent inhibition of T cell proliferation was also increased in CVI, showing the positive cooperative effect of endogenous cAMP levels (Fig. 1B), and this may be a more stable and reliable marker of endogenous cAMP levels than the actual cAMP level as determined by RIA. The results presented in Table II show such an effect of 8-CPT-cAMP on cell proliferation in all CVI patients with impaired T cell function compared with the effect in the eight control subjects. This significant increase in sensitivity to inhibition of cell proliferation by exogenously added 8-CPT-cAMP in the CVI group was reflected in a marked decrease in IC50 values in these patients, primarily due to a change in the slope for the inhibition curve (Hill coefficient; Table II and Fig. 1B).

To further address the specificity of the inhibition of anti-CD3-stimulated T cell proliferation we used a cAMP agonist (Sp-8-Br-cAMPS) and its complementary PKAI-selective antagonist (Rp-8-Br-cAMPS). In healthy controls the inhibitory effect of the cAMP agonist was completely reversed by its complementary antagonist (Fig. 2A), but the antagonist alone did not further enhance T cell proliferation (Fig. 2B and Table II). In contrast, we found a concentration-dependent increase in anti-CD3-stimulated proliferation in CVI patients (>100% increase in three patients and reaching levels within the normal range in two patients) when Rp-8-Br-cAMPS was added to cell cultures (Fig. 2, C and D, and Table II). Thus, it seems that in CVI patients, T cells with impaired anti-CD3-stimulated proliferation are characterized by chronically elevated endogenous cAMP levels, and treatment with a selective PKAI antagonist markedly improves anti-CD3-stimulated proliferation in these cells, reaching proliferation levels comparable to
control values (25 and 75% of levels in healthy controls, with and without Rp-8-Br-cAMPS, respectively).

Effects of cAMP antagonist on proliferation of CD4$^+$ and CD8$^+$ T cells

We next, by flow cytometric analysis of BrdUrd incorporation, examined anti-CD3-stimulated T cells DNA synthesis in the presence and the absence of Rp-8-Br-cAMPS in subsets of CD4$^+$ and CD8$^+$ T cells from the seven CVI patients with impaired T cell function (see above) and the seven controls. In CVI patients there was a significant increase in the percentage of BrdUrd$^+$ CD4$^+$ T cells when cAMP antagonist was added to cell culture (61.3% 33.6 – 86.0%) vs 80.0% (47.0 – 95.8%), without and with antagonist, respectively; $p$, 0.05).

In most patients the maximal increase was found at the highest concentration of Rp-8-Br-cAMPS (1000 $\mu$M). No effect of Rp-8-Br-cAMPS on DNA synthesis was seen in CD4$^+$ T cells from healthy controls.

FIGURE 2. Modulation of T cell proliferation by cAMP agonist and antagonist in CVI patients with impaired T cell function and in healthy controls. Inhibition of anti-CD3-stimulated proliferation of T cells by the cAMP agonist (Sp-8-Br-cAMS) and reversal of inhibition by its complementary PKA type I-selective antagonist (Rp-8-Br-cAMPS) are shown in one healthy control (A) and one representative CVI patient (C). The effect of increasing concentrations of Rp-8-Br-cAMPS on anti-CD3-stimulated T lymphocyte proliferation was also examined separately, and the results from the same control and CVI patient shown in A and C are given in B and D, respectively. Data are given as the mean value for triplicate determinations ± SD. For statistics between the CVI group and controls, see Table II.

AGONIST ± ANTAGONIST

<table>
<thead>
<tr>
<th>Patient</th>
<th>Anti-CD3 Stimulated Proliferation (cpm)</th>
<th>Highest Proliferation When Adding Rp-8-Br-cAMPS (cpm)</th>
<th>Increase in Proliferation by Rp-8-Br-cAMPS (fold increase)</th>
<th>Inhibition of Proliferation by 8-CPT-cAMP (IC$_{50}$, $\mu$M$^\dagger$)</th>
<th>Inhibition of Proliferation by 8-CPT-cAMP (-Hill coefficient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^a$</td>
<td>4,870</td>
<td>11,932</td>
<td>2.45</td>
<td>2.98</td>
<td>1.47</td>
</tr>
<tr>
<td>2$^b$</td>
<td>26,968</td>
<td>98,972</td>
<td>3.67</td>
<td>2.26</td>
<td>1.26</td>
</tr>
<tr>
<td>3$^c$</td>
<td>35,224</td>
<td>60,938</td>
<td>1.73</td>
<td>3.68</td>
<td>1.34</td>
</tr>
<tr>
<td>4$^d$</td>
<td>36,740</td>
<td>77,154</td>
<td>2.10</td>
<td>3.36</td>
<td>1.38</td>
</tr>
<tr>
<td>5$^e$</td>
<td>66,050</td>
<td>123,513</td>
<td>1.87</td>
<td>3.16</td>
<td>2.03</td>
</tr>
<tr>
<td>6$^f$</td>
<td>78,080</td>
<td>104,627</td>
<td>1.34</td>
<td>2.66</td>
<td>1.39</td>
</tr>
<tr>
<td>7$^g$</td>
<td>109,567</td>
<td>150,107</td>
<td>1.37</td>
<td>2.34</td>
<td>1.46</td>
</tr>
<tr>
<td>Median</td>
<td>36,740$^j$</td>
<td>98,972</td>
<td>1.87$^j$</td>
<td>2.98$^j$</td>
<td>1.39$^j$</td>
</tr>
<tr>
<td>(25–75th percentiles) in CVI</td>
<td>(26,968–78,080)</td>
<td>(60,938–123,513)</td>
<td>(1.37–2.45)</td>
<td>(2.34–3.36)</td>
<td>(1.34–1.47)</td>
</tr>
<tr>
<td>Median</td>
<td>132,484</td>
<td>129,530</td>
<td>1.01</td>
<td>4.59</td>
<td>1.59</td>
</tr>
<tr>
<td>(25–75th percentiles) in controls</td>
<td>(121,181–138,453)</td>
<td>(119,316–160,692)</td>
<td>(0.93–1.14)</td>
<td>(4.04–5.82)</td>
<td>(1.40–1.81)</td>
</tr>
</tbody>
</table>

$^a$ The individual values for the actual variables are given in the seven CVI patients ordered according to anti-CD3-stimulated T cell proliferative response. The group values for the actual variables are given in the CVI and the control group as medians and 25th to 75th percentiles.

$^j$ IC$_{50}$ denotes the concentration of cAMP analog necessary to produce a half-maximal inhibition of anti-CD3-stimulated T cell proliferation.

$^\dagger$ $p < 0.005$; $^j p < 0.02$ vs controls.

Superscript letters $a$–$g$ denote single patients also evaluated for IL-2 release (see Table III).
controls (data not shown). For CD8+ T cells there was no significant increase in the percentage of BrdUrd+ cells after addition of cAMP antagonist in either CVI patients or controls, although a modest increase was seen in three CVI patients (data not shown).

Effect of cAMP antagonist on IL-2 levels in T cell supernatants

IL-2 plays a pivotal role in the growth and function of T cells (28), and decreased IL-2 production from these cells may play an important role in the immunopathogenesis of CVI (29, 30). cAMP decreases IL-2 production in T cells (31), and to further elucidate the mechanism(s) of cAMP-induced inhibition of T cell proliferation in CVI, we examined the effect of Rp-8-Br-cAMPS on IL-2 levels in supernatants from anti-CD3-stimulated T lymphocytes in the seven CVI patients with impaired T cell function and the eight controls. Compared with control subjects, T cells from CVI patients released significantly lower IL-2 levels into supernatants (Table III), and we found a marked and concentration-dependent increase in IL-2 levels in the presence of cAMP antagonist (Table III and Fig. 3). The effect of Rp-8-Br-cAMPS on IL-2 levels of CVI T cells was largely similar to that on proliferation. However, despite the dramatic increase in IL-2 levels after addition of Rp-8-Br-cAMPS to cell cultures in CVI patients, the IL-2 level was still markedly lower than that in control subject (Table III). Thus, in this subgroup of CVI patients, T cell proliferation is normalized to a greater extent than IL-2 secretion by addition of cAMP antagonist to cells in vitro.

Effect of exogenously added IL-2 in combination with cAMP antagonist on anti-CD3-induced T cell proliferation

To further examine the role of IL-2 in the enhancement of T cell proliferation by addition of cAMP antagonist, we examined the effect of exogenously added IL-2, either alone or in combination with Rp-8-Br-cAMPS, on anti-CD3-stimulated T cell proliferation in the seven CVI patients with T cell deficiency and the eight controls. After addition of IL-2 to cell culture there was a marked increase in proliferation in both CVI patients and controls (Fig. 4). However, at IL-2 concentrations comparable to the achieved increase in IL-2 levels after addition of cAMP antagonist (~0.20 ng/ml; Fig. 3B), no significant effect was seen on proliferation in either CVI patients or controls (Fig. 4). In fact, the enhancing effect of cAMP antagonist in CVI patients was comparable to the effect of 10 ng/ml IL-2, i.e., a 50-fold higher concentration (Fig. 4). Furthermore, in CVI patients the enhancing effects of Rp-8-Br-cAMPS and IL-2 on T lymphocyte proliferation were additive at all concentrations tested (Fig. 4).

Table III. Effect of cAMP antagonist (Rp-8-Br-cAMPS) on IL-2 levels in supernatants from anti-CD3-stimulated T cells seven CVI patients with impaired T cell function and eight healthy controls*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Anti-CD3-Stimulated IL-2 Release (pg/ml)</th>
<th>Highest IL-2 Level when Adding Rp-8-Br-cAMPS (pg/ml)†</th>
<th>Increase in IL-2 Level by Rp-8-Br-cAMPS (fold increase)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1c</td>
<td>25</td>
<td>166</td>
<td>6.64</td>
</tr>
<tr>
<td>2b</td>
<td>32</td>
<td>144</td>
<td>4.50</td>
</tr>
<tr>
<td>3e</td>
<td>79</td>
<td>210</td>
<td>2.66</td>
</tr>
<tr>
<td>4d</td>
<td>100</td>
<td>249</td>
<td>2.49</td>
</tr>
<tr>
<td>5f</td>
<td>121</td>
<td>293</td>
<td>2.42</td>
</tr>
<tr>
<td>6g</td>
<td>130</td>
<td>246</td>
<td>1.89</td>
</tr>
<tr>
<td>7h</td>
<td>131</td>
<td>232</td>
<td>1.77</td>
</tr>
<tr>
<td>Median</td>
<td>100†</td>
<td>232†</td>
<td>2.49†</td>
</tr>
<tr>
<td>(25–75th percentiles) in CVI</td>
<td>(32–130)</td>
<td>(166–249)</td>
<td>(1.89–4.50)</td>
</tr>
<tr>
<td>Median</td>
<td>600</td>
<td>799</td>
<td>1.28</td>
</tr>
<tr>
<td>(25–75th percentiles) in controls</td>
<td>(510–800)</td>
<td>(510–968)</td>
<td>(1.06–1.45)</td>
</tr>
</tbody>
</table>

* The individual values for the actual variables are given in seven CVI patients ordered according to anti-CD3-stimulated IL-2 levels in T cell supernatants. The group values for the actual variables are given in CVI and control group as medians and 25th to 75th percentiles.

† While all CVI patients had highest IL-2 levels when 1000 μM of Rp-8-Br-cAMPS was added to cell cultures, the most pronounced effect in controls was seen at a lower antagonist concentration (100 μM).

‡ p < 0.005 vs controls.

Superscript letters a–g denote single patients also evaluated for T cell proliferation (see Table II).
Discussion

The present study demonstrates for the first time increased endogenous cAMP levels in T cells from CVI patients with impaired T cell function and, even more importantly, that a selective inhibition of PKAI could markedly improve or in some cases even fully restore anti-CD3-induced T cell proliferation in this subgroup of CVI patients. These findings indicate a possible intracellular mechanism for the T cell defect in CVI and suggest that the cAMP/PKAI system may be a potential target for immunomodulating therapy in these patients.

Some reports have previously studied the intracellular basis for the T cell defect in CVI. Eibl and co-workers found impaired proliferation, decreased formation of inositol 1,4,5-triphosphate (Ins(1,4,5)P$_3$), and reduced capacity to mount an increase in intracellular Ca$^{2+}$ after TCR stimulation in T cells from CVI patients. These findings indicate a possible intracellular mechanism for the T cell defect in CVI and suggest that the cAMP/PKAI system may be a potential target for immunomodulating therapy in these patients.

We have suggested that cAMP leading to activation of PKAI is an important inhibitor of normal T cell proliferation after Ag stimulation (3, 6, 20). Although cAMP agonists may inhibit T cell function at more than one site (31, 34), several lines of evidence indicate that PKAI is an early inhibitor of T cell activation after TCR/CD3 stimulation (18, 32, 33). Together, these studies suggest that the T cell defect in a subgroup of CVI patients is caused at least in part by an early defect in T cell activation after triggering of the TCR/CD3 complex before the generation of Ins(1,4,5)P$_3$ and PKC activation.

We have suggested that cAMP leading to activation of PKAI is an important inhibitor of normal T cell proliferation after Ag stimulation (3, 6, 20). Although cAMP agonists may inhibit T cell function at more than one site (31, 34), several lines of evidence indicate that PKAI is an early inhibitor of T cell activation after TCR/CD3 stimulation. PKA activation inhibits TCR/CD3-dependent hydrolysis of inositol phospholipids to Ins(1,4,5)P$_3$ and diacylglycerol as well as early tyrosine phosphorylation (20). Furthermore, T cell activation by direct PKC stimulation by phorbol esters seems to be insensitive to inhibition by cAMP/PKAI (20). Finally, PKAI colocalizes to the TCR/CD3 complex upon activation and capping, and may release kinase activity that, through phosphorylation, might uncouple the TCR/CD3 complex from the intracellular signaling pathway (6).

FIGURE 4. Modulation of T cell proliferation by IL-2 and cAMP antagonist in CVI patients with impaired T cell function and in healthy controls. The effect of increasing concentrations of IL-2 (2 U/ml) with or without 1000 μM of Rp-8-Br-cAMPS (Rp) on anti-CD3-stimulated T cell proliferation are shown for one healthy control (A) and one representative CVI patient (B). Data are given as the mean value for triplicate determinations ± SD.
PKA I and T Cell Deficiency in CVI

PKAI system in T cells from CVI patients may not necessarily be related to the basic molecular defect(s) that leads to CVI, these findings strongly suggest a defect in the early phase of T cell activation after TCR/CD3 triggering in these patients and, more importantly, that increased cAMP/PKAI activation may contribute to the molecular basis for this defect.

CVI represents a heterogeneous group of patients, both clinically and immunologically (35). As for the T cell defect, both subgroups with predominantly CD4+ and predominantly CD8+ T cell involvement have been reported (29, 36). Although the CVI patients in the present study had decreased CD4+ and tended to have increased CD8+ T cell counts compared with controls, our flow cytometry data analyzing the effect of cAMP antagonist on these T cell subsets separately, suggest that the enhanced PKAI activity in T cells from CVI patients with T cell deficiency does not merely reflect altered distribution of CD4+ and CD8+ subsets. In fact, our findings suggest that increased PKAI activity in this subgroup of CVI patients mostly affects CD4+ lymphocytes, which in these patients represent a smaller proportion of T cells than in controls.

Our findings of impaired IL-2 production from T cells in CVI in this study confirm previous reports (17, 18, 29), and the results suggest that increased PKAI activity may contribute to this impairment. However, the induction of enhanced T cell proliferation in CVI, mediated by cAMP antagonist, appears not to be dependent on increased IL-2 release. Exogenously added IL-2 had no effect on T cell proliferation at concentrations comparable to the achieved increase in IL-2 levels after addition of cAMP antagonist. Furthermore, the stimulatory effects of IL-2 and cAMP antagonist on TCR/CDR-stimulated T cell proliferation were additive at all concentrations tested and did not saturate each other. Thus, the effect of IL-2 and cAMP antagonist on T cell proliferation in CVI are distinct and possibly mediated by separate mechanisms. An additive effect between IL-2 and cAMP antagonist may be of particular interest also from a therapeutical point of view. Both in vivo and in vitro studies suggest that IL-2 may improve, but not fully correct, the T cell function in CVI (17, 18, 37), and it is tempting to hypothesize that the combination of IL-2 therapy and therapeutic interventions that down-regulate PKAI activity may be an interesting approach to immunomodulation in CVI.

Immunologic abnormalities in CVI might be secondary phenomena to infectious complications and therapy. However, we could not find any association between the presence of chronic bacterial complications and abnormalities in the cAMP/PKAI system in T cells from CVI patients. IgG may alter several immunologic functions both in vitro and in vivo (38), but in the present study blood samples were collected just before Ig substitution to minimize the effect of such therapy. Moreover, s.c. Ig administration, which was used by all CVI patients in the present study, in contrast to i.v. Ig therapy, does not affect lymphocyte and monocyte functions (39). Furthermore, among CVI patients there was no association between either Ig dosage or duration of Ig therapy and the magnitude of response to cAMP antagonist. Finally, although the duration of replacement therapy and clinical symptoms was longer and the occurrence of chronic infectious complications increased in XLA patients compared with those in the CVI group, the effect of cAMP antagonist on lymphocyte proliferation in XLA patients was comparable to the effect in healthy controls.

We have previously demonstrated a persistent monocyte activation in a subgroup of CVI patients (22, 40), and monocyte products such as IL-1 and PGE2, which appear to be elevated in CVI (P. Aukrust, F. Müller, and S. S. Frøland, unpublished observations), may increase cAMP levels in T cells (2, 31). In fact, CVI patients with T cell deficiency and enhanced PKAI activity had significantly higher serum neopterin levels, as a marker of monocyte hyperactivity (22), than other CVI patients. Furthermore, these T cell-deficient CVI patients had increased occurrence of splenomegaly, a finding that has previously been associated with monocyte hyperactivity and T cell deficiency in CVI (27, 41). Thus, there may well be a pathogenic link between monocyte hyperactivity and cAMP/PKAI-mediated T cell dysfunction in CVI, and studies examining this possibility are ongoing in our laboratory. Furthermore, retroviral peptides and other viral components have been found to increase cAMP levels in lymphocytes (42), and several authors have suggested that CVI may develop as a result of a chronic viral infection in genetically susceptible individuals (36, 43), possibly representing a slowly progressive, not stagnant, disorder of the immune system. Immunologic similarities to HIV infection (12, 13, 21, 27), as also demonstrated in the present study, i.e., abnormalities in the cAMP/PKAI system (44), may further support such an idea. Whatever the reasons, although several mechanisms may be involved in the pathogenesis of T cell deficiency in CVI, the demonstration of an up to 3.7-fold increase in T cell proliferation after addition of cAMP antagonist in CVI patients with impaired T cell function, clearly suggests that the increased PKAI activity may represent an important feature of T cells in these patients.

While CAMP is mitogenic in differentiated endocrine cells, in vitro studies suggest that the cAMP/PKAI system generally delivers an off signal for many functions in the immune system (2–4). The results of the present study in addition to recent reports demonstrating impaired cAMP/PKAI activity in T cells from patients with systemic lupus erythematosus and increased cAMP/PKAI activity in T cells from HIV-infected patients (9, 44) further support an important immunoregulatory role for the cAMP/PKAI system in vivo in human disorders. Further studies addressing the role of increased PKAI activation in the pathogenesis of T cell deficiency in CVI may be of great interest.

Acknowledgments
We thank Bodil Lunden, Lisbeth Wikeyby, and Vigdis Bjerkeli for excellent technical assistance.

References