T-LYMPHOCYTES IN IgA DEFICIENCY

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A b s t r a c t

Common variable immunodeficiency (CVID) and IgA deficiency (IgAD) are primary humoral immune deficiencies with a similar genetic background, but clinical and laboratory manifestation of CVID is much more severe than that of IgAD. The aim of this study was to determine whether some abnormalities in T-lymphocyte subsets or function observed in CVID are also present in IgAD patients. T-lymphocyte subsets (CD3+, CD4+, CD8+, CD4+CD45RA+, CD4+CD45RO+ and CD8+CD57+) were studied in 61 adult IgAD patients and in 43 control, healthy subjects. In the IgAD patients, a significant decrease in CD4+ and an increase in CD8+ and CD8+CD57+ lymphocytes were observed but no significant changes in the proportion of CD45RA+ or CD45RO+ in CD4+ cells were detected. No significant changes in lymphocyte proliferation after stimulation with Phytohaemagglutinin, Concanavalin-A or tetanic toxoid were observed in 15 adult IgAD patients compared to 15 controls. Our study showed that T-lymphocyte abnormalities were present in IgAD patients, although not to such a broad extent as in CVID.

K e y w o r d s

IgA deficiency, T-lymphocytes, Lymphocyte subsets

I N T R O D U C T I O N

Common variable immunodeficiency (CVID) is the most frequent symptomatic primary immunodeficiency disease (1) which affects about 1 in 25 000 Caucasians (2). It usually manifests itself by frequent and complicated infections of the respiratory tract. The disease is defined by marked impairment of the specific antibody response to antigens, i.e., serum IgG and IgA levels are markedly decreased while IgM levels vary (3).

Selective IgA deficiency (IgAD) is the most frequent primary immunoglobulin deficiency in Caucasians with a prevalence of 1:408 in the Czech population (4). In contrast to CVID, IgAD is usually without clinical manifestation; only a minority of patients suffer from recurrent respiratory tract infections or are prone to autoimmune diseases (3). The specific antibody response is not impaired, serum IgG and IgM level are normal or increased.

Despite their clinical heterogeneity, the two diseases seem to be a distinct manifestation of the same or similar genetic background. This is supported by a link to the same HLA antigens, the occurrence of both diseases in one family and occasionally reported progression of IgAD to CVID (2).
In addition to decreased immunoglobulin levels, various T-cell abnormalities were reported in CVID patients. These include a decreased response to stimulation via T-cell receptors (TCR) \( (5,6,7) \) and abnormalities in T-lymphocyte subsets, namely, a decreased proportion of CD4+CD45RA+ (naive T-cells) in CD4+ lymphocytes \( (8,9) \) and an increased number of CD8+CD57+ lymphocytes \( (10,11) \) in a subset of CVID patients. On the other hand, no important T–lymphocyte abnormalities in IgAD have been reported yet.

In this study with IgAD patients, we determined T-lymphocyte subpopulations, which are most frequently changed in CVID patients, and measured the proliferation response of T-lymphocytes after stimulation though TCR by a specific antigen (tetanus toxoid) and non-specific proliferative stimuli.

**MATERIALS AND METHODS**

T-lymphocyte subsets were determined in 61 patients (34 females and 27 males), aged 18–65 years (mean \( \pm \) SD, 30.6 \( \pm \) 11.3), who had IgAD and in 43 control subjects (24 females, 19 males) aged 18–70 years (31.7 \( \pm \) 14.4). The lymphocyte proliferation response was determined in 15 adult IgAD patients (8 females, 7 males) aged 19–41 years (33.0 \( \pm \) 9.86) 6 to 8 weeks after booster vaccination against tetanus toxoid (Alteana, Sevac, Czech Republic). Fifteen control subjects (8 females, 7 males) aged 23–44 years (34.9 \( \pm \) 7.1) were also tested for the lymphocyte proliferation response 6 to 8 weeks after booster vaccination against tetanus. Blood was collected in a period without clinically apparent infection.

Lymphocyte subpopulations were determined by direct immunofluorescence, using a flow cytometer (Coulter EPICS XL). The following lymphocyte subpopulations were measured: CD3+ (pan-T-lymphocytes), CD4+ (helper T-lymphocytes), CD8+ (cytotoxic/suppressor T-lymphocytes), CD4+CD45RA+ (naive helper T-lymphocytes), CD4+CD45RO+ (activated/memory helper T-lymphocytes) and CD8+CD57+ (subset of cytotoxic T-lymphocytes), using monoclonal antibodies provided by Becton-Dickinson (San Jose, CA).

Lymphocyte proliferation tests were performed as follows: Peripheral blood mononuclear cells were isolated from heparinised blood (15 U/ml) diluted 1:2 in RPMI 1640 Medium (Sigma Chemicals Co., St Louis, MO) by density gradient centrifugation (Lymphoprep; Nycomed Pharma AS, Oslo, Norway). The cells were resuspended in RPMI 1640 medium supplemented with 10% (vol/vol) inactivated human AB serum, 2mmol/l L-glutamine (Sigma Chemicals Co.), 100 IU/ml penicillin and 100μg/ml streptomycin (both Sigma Chemicals Co.) at a final concentration of 10^6 cells/ml. The suspension was incubated (200 μl/well) in microtitration plates (Montegrotto Terme, Padova, Italy) in a CO2 incubator (5% CO2 in humidified air) at 37°C. One of the following stimuli was added: tetanus toxoid (kindly provided by Sevac, Prague, Czech Republic) at a final concentration of 5Lf/ml, Phytohaemagglutinin (PHA, Sigma Chemicals Co.) at a final concentration of 5μg/ml and 1μg/ml, or Concanavalin A (ConA, Sigma Chemicals Co.) at a final concentration of 5μg/ml and 1μg/ml. After 3 days (PHA, ConA) or 7 days (tetanus toxoid) of incubation, the cells were pulsed with 0.5 μCi 3H thymidine (Amersham, Aylesbury, UK) and harvested 16 h later. 3H-thymidine incorporation was determined by using a scintillation counter (Tricarb 2100 TR, Packard, Canberra Company, Meriden, USA). Each test was performed in triplicate and average values were used for calculation.

The Kolmzrov-Smirnov test was used to test the normal distribution of the data obtained; if necessary, a log-transformation was performed. Statistical comparison was performed by Student’s \( t \)-test.
RESULTS

T-lymphocyte subsets in patients with IgAD and in controls are shown in Table 1. There was no difference in the proportion of total T-lymphocytes (CD3+), but CD4+ lymphocytes were significantly decreased, while CD8+ T lymphocytes were increased. There were no significant changes in the proportion of naive (CD45RA+) and activated/memory (CD45RO+) lymphocytes in the total CD4+ lymphocytes, but the proportion of CD8+CD57+ subpopulation was significantly increased in the IgAD patients as compared to the controls.

The lymphocyte proliferation response to polyclonal mitogens (PHA, ConA) and the specific antigen (tetanus toxoid) in the IgAD patients and control subjects are expressed in Table 2 and Fig. 1. No significant differences between the IgAD patients and the controls were observed.

Table 1
Lymphocyte subpopulation subsets in patients with IgA deficiency (IgAD) and in control subjects

<table>
<thead>
<tr>
<th>Lymphocyte population</th>
<th>IgAD (n= 61)</th>
<th>Controls (n=43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+ /total lymphocytes (%)</td>
<td>70.6 ± 6.5</td>
<td>71.6 ± 8.8</td>
<td>0.49</td>
</tr>
<tr>
<td>CD4+ /total lymphocytes (%)</td>
<td>39.9 ± 8.1</td>
<td>45.4 ± 7.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8+ /total lymphocytes (%)</td>
<td>26.4 ± 6.8</td>
<td>23.3 ± 6.8</td>
<td>0.024</td>
</tr>
<tr>
<td>CD4+/CD8+</td>
<td>1.65 ± 0.60</td>
<td>2.11 ± 0.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4+CD45RA+/CD4+ (%)</td>
<td>44.4 ± 17.6</td>
<td>49.2 ± 18.8</td>
<td>0.176</td>
</tr>
<tr>
<td>CD4+CD45RO+/CD4+ (%)</td>
<td>52.4 ± 16.7</td>
<td>47.3 ± 20.1</td>
<td>0.183</td>
</tr>
<tr>
<td>CD8+CD57+/total lymphocytes (%)</td>
<td>5.2 ± 16.7</td>
<td>2.5 ± 3.2</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SD. Student’s t-test was used for statistical analysis.
* Log transformation was used before statistical analysis.

DISCUSSION

The aim of this study was to detect whether some T-lymphocyte abnormalities observed in CVID patients, i.e., lymphocyte subset alterations and disturbed stimulation via TCR, are also present in patients with IgA deficiency. Significant changes in major lymphocyte subpopulations were observed. The highly significant result was a decrease in CD4+ (helper) T-lymphocytes accompanied by a decrease in the proportion of CD8+ (cytotoxic/suppressor) T-lymphocytes. Similar changes have repeatedly been observed in several groups
Table 2
Lymphocyte proliferation response after stimulation with Phytohaemagglutinin (PHA), Concanavalin A (ConA) and tetanus toxoid (T.Tox).

<table>
<thead>
<tr>
<th>Lymphocyte population</th>
<th>IgAD (n= 61)</th>
<th>Controls (n=43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHA (5µg/ml)</td>
<td>56 600 ± 33 569</td>
<td>71 200 ± 47 822</td>
<td>0.390</td>
</tr>
<tr>
<td>PHA (1µg/ml)</td>
<td>4 876 ± 3 906</td>
<td>5 076 ± 2 892</td>
<td>0.884</td>
</tr>
<tr>
<td>ConA (5µg/ml)</td>
<td>20 242 ± 12 054</td>
<td>23 600 ± 15 100</td>
<td>0.547</td>
</tr>
<tr>
<td>ConA (1µg/ml)</td>
<td>5 475 ± 2 963</td>
<td>6 900 ± 4 687</td>
<td>0.377</td>
</tr>
<tr>
<td>T. Tox (5Lf/ml)</td>
<td>36 701 ± 37 863</td>
<td>61 553 ± 61 198</td>
<td>0.191</td>
</tr>
</tbody>
</table>

Results are expressed in cpm (counts per minute) as mean± SD.

Fig. 1:
Lymphocyte proliferation response after stimulation with Phytohaemagglutinin (PHA), Concanavalin A (ConA) and tetanus toxoid (T.Tox) in IgAD patients (P) and control subjects (C). Cpm = counts per minute. µ
of CVID patients (12,13). Surprisingly, very few data are available about T-lymphocyte subpopulations in IgAD patients. Only Melamed et al. observed an increased proportion of CD8+ (Leu2+) and a decrease in the CD4/CD8 ratio in IgAD patients compared with controls but, in contrast to our results, they found no significant change in the CD4+ subpopulation (14).

Although, in CVID patients, a decrease in CD4+ lymphocytes was attributed to a decrease in CD45RA+ (naive) T-lymphocytes that caused a relative increase in CD45RO+ (activated/memory) T-lymphocytes (8,9), we did not observe such changes in the IgAD patients. In our group of patients the proportion of CD4+CD45RA+ (naive) to CD4+ lymphocytes was comparable to that found in healthy subjects.

Similarly to a subgroup of CVID patients with low CD4/CD8 counts (10), the CD8+CD57+ subpopulation was increased in our IgAD patients. This subpopulation was increased in various immunopathological situations including viral infections, such as cytomegalovirus or HIV, and autoimmune diseases, for instance, rheumatoid arthritis, Felty’s syndrome, Crohn’s disease or ulcerative colitis (for review see 15). Although autoimmune diseases are frequent in IgAD patients (16), clinically manifested autoimmune diseases were not present in our group, with the exception of a mild form of rheumatoid arthritis in one patient. The increase in the CD8+CD57+ population in CVID is attributed to chronic viral stimulation (10). Although patients with IgAD are referred for immunological investigation more frequently than healthy subjects (4) and probably also suffer from respiratory tract infections more frequently, it is questionable whether this relatively mild increase in the frequency of infections can explain the increase in the CD8+CD57+ subpopulation found in our IgAD patients. The CD8+CD57+ subpopulation also increases with age (17); although our patients and controls were not directly matched, both groups showed a similar age range.

The normal response of IgAD patients to polyclonal mitogens PHA and ConA (used in optimal concentrations) corresponded to that observed in other studies (18,19); only Melamed et al. reported a decreased stimulation index after PHA, but not after ConA, stimulation (14). We did not observe any significant difference between the IgAD patients and the controls in their response to tetanus toxoid after a previous tetanus booster immunisation. This comparison was influenced by a relatively high variance of measured values, but as shown in Fig. 1, the proliferative response of both IgAD patients and controls was similar. In contrast to CVID patients in whom a subgroup of patients with markedly decreased lymphocyte proliferation to specific stimuli was observed (5), no such subgroup could be defined in our IgAD patients.

In general, our study shows that some abnormalities in T-cell immunity are similar in IgAD and CVID patients. This includes a decrease in CD4+ lymphocytes and an increase in CD8+CD57+ lymphocytes. Another typical
laboratory sign of CVID, a decrease in CD4+CD45RA+ lymphocytes, was not present in our study. No significant changes in proliferative responses to various stimuli were observed in our IgAD patients. Our results show that IgAD might be a model of some immunoregulatory changes present in CVID and that T-cell numbers and also their function in IgAD should be more extensively studied.

Acknowledgement
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T-LYMFOCYTY U PACIENTŮ SE SELEKTIVNÍM DEFICITEM IgA

Souhrn

Běžná variabilní imunodeficience (CVID) a selektivní deficit IgA (IgAD) jsou primárními imunodeficiencemi s obdobným genetickým základem, klinická manifestace i laboratorní příznaky CVID jsou však mnohem těžší než u IgAD. Cílem práce bylo zjistit, zda se některé abnormality T-lymfocytů, dříve popsané u CVID, objevují i u pacientů s IgAD. T-lymfocytární populace (CD3+, CD4+, CD8+, CD4+CD45RA+, CD4+CD45RO+, CD8+CD57+) byly vyšetřeny u 61 dospělých pacientů s IgAD a 43 zdravých kontrolních osob. U nemocných s IgAD bylo nalezeno znaménkové snížení CD4+lymfocytů, počet CD8+ a CD8+CD57+ lymfocytů byl zvýšen. Nebyly zaznamenány žádné změny zastoupení CD45RA+nebo CD45RO+uCD4+lymfocytů.

U 15 pacientů IgAD a 15 kontrolních osob jsme nezaznamenali žádné statisticky významné změny proliferace lymfocytů po stimulaci Phytohaemagglutininem, Concanavalinem-A a tetanickým toxoidem.

Naše studie ukazuje, že některé, ne však všechny, T-lymfocytární abnormality, které se objevují u CVID, je možné prokázat i u nemocných s IgAD.

REFERENCES


