A Defect in the Early Phase of T-Cell Receptor-Mediated T-Cell Activation in Patients With Common Variable Immunodeficiency


Common variable immunodeficiency (CVID) is characterized by an impairment of specific antibody production and a decrease in all or selected Ig isotypes. Abnormalities at the level of the B cells, T cells, and antigen-presenting cells have been described. In the present study, we have focused our attention on T-cell activation in CVID. T cells from 15 of 24 patients failed to respond to recall antigens (e.g., tetanus toxoid, *Escherichia coli*). Of these 15 patients, 11 were studied in detail and showed significantly decreased T-cell proliferative responses and/or decreased interleukin-2 and interferon-γ production on T-cell receptor-mediated stimulation with recall antigens and superantigens (staphylococcal enterotoxins [SE]); however, T-cell response to mitogens (anti-CD3 monoclonal antibody, phytohemagglutinin) was normal. The defect in interleukin-2 and interferon-γ release on tetanus toxoid stimulation could also be documented in purified CD4 T cells of the patients and was present in patients with high and normal CD8 counts alike. Furthermore, patients’ T cells failed to mount a significant elevation in free intracellular calcium (Ca++ flux) in response to superantigen, whereas the response to phorbol myristate acetate and ionomycin, bypassing receptor-mediated signaling, was unimpaired. These results indicate a defect in the early phase of T-cell activation after triggering of the T-cell receptor in a significant subgroup of CVID patients. © 1994 by The American Society of Hematology.

**MATERIALS AND METHODS**

Patients. At the time of the study, 24 patients with CVID as defined by the World Health Organization classification of primary immunodeficiencies were followed up by our immunology division. The patients had a history of recurrent bacterial infections of the upper and lower respiratory tract and reduced or absent serum IgG, IgA, and IgM levels. In 9 of the 24 patients, the T-cell response to recall antigen (*E coli* or tetanus toxoid) was comparable with the response in healthy individuals, and 2 of these 9 patients had low numbers of B cells in their peripheral blood. Fifteen patients had T lymphocytes incapable of responding to recall antigen presented by autologous antigen-presenting cells (APCs). One patient was shown to have a defect in APCs. Three further patients were excluded from the study because of severe concomitant disease (lymphoma, alcoholic cirrhosis of the liver, and eosinophilic gastroenteritis with severe gastrointestinal loss of protein). The remaining 11 patients, 5 men and 6 women ranging in age from 17 to 50 years, were enrolled in the study after informed consent had been obtained. These 11 patients showed hypogammaglobulinemia or agammaglobulinemia with a decrease in all Ig isotypes and consistently impaired production of specific antibodies in the presence of normal numbers of B cells, as well as impaired ability of their T cells to respond to recall antigens (e.g., tetanus toxoid, *E coli*). During the course of this study, all patients were on regular gammaglobulin replacement therapy (7 patients, intravenous administration of 400 mg IgG/kg body weight per month; 4 patients, intramuscular administration of 100 mg IgG/kg body weight per month). All patients had received tetanus toxoid vaccination or boosters within the last 6 to 12 months before the study. At the time of evaluation, none of the patients was

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suffering from severe infections requiring hospital admission. Blood samples were taken 2 to 4 weeks after the last administration of IGs. Phenotypic analysis of CVID patients' peripheral blood (PB) leukocytes showed that total numbers of T cells (CD3+ [Leu 4]), B cells (CD19+ [B4]), and natural killer cells (CD56+ [Leu 19]) were within the normal range. Five patients had normal CD8+ counts (32± 9% of lymphocytes; mean ± SD), whereas high CD8+ counts could be detected in 6 patients (52± 12% of lymphocytes). Healthy age-matched volunteers were investigated in parallel and served as controls. The vaccination history of the controls was comparable with that of the patients.

Isolation of monoclonal cells (MNCs) and preparation of a T-enriched lymphocyte population. PBMCs were isolated from heparinized whole blood (7.5 IU/mL of preservative-free heparin) by buoyant density gradient centrifugation.14 Monocytes were prepared by adherence to plastic surfaces as described previously.14 Adherent cells were washed several times and removed gently with a rubber policeman. Cell purity was 80% to 90% monocytes, as defined by flow cytometry with a CD14 MoAb. Nonadherent cells were removed and fractionated to 1-2 monoNome cells (CD45RA+ and >90% CD3+ cells) and non-T cells by rosetting with sheep erythrocytes treated with 2-aminothiophenyltrimethoxycarbonyl (Sigma Chemicals, St Louis, MO) as described earlier.15

Lymphocyte proliferation. Triplicate cultures containing adherence-purified monocytes (1×106/well) and T-enriched cells (1×106/well) were set up in flat-bottomed microtiter plates (Falcon Microtest II, Becton Dickinson, Lincoln Park, NJ), and one of the following stimuli was added: tetanus toxoid (10 Loeffler units/mL; Lederle, Pearl River, NY); anti-CD3 MoAb (5 µg/mL OKT3; Ortho, Raritan, NJ); or anti-TCR-β (2 µg BMA-031 were coated on 4×105 sheep anti-mouse Ig-coated beads in a total volume of 1 mL [Dynabeads M450; Dynal, Oslo, Norway] and used at a final concentration of 1×106 beads/1×106 T cells). Recombinant human IL-2 (Genzyme Corp, Cambridge, MA) was used at a final concentration of 20 U/mL. The cells were kept for 7 days (antigen), 5 days (superantigen, anti-TCR MoAb), or 3 days (PHA, OKT3) at 37°C in a CO2 incubator (5% CO2; humidified air) in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 2mM L-glutamine, 100 IU/mL penicillin, and 100 µg/mL streptomycin. 1H-thymidine incorporation was determined as described earlier.15 Results are shown as disintegrations per minute (mean ± SEM of the average values of individual experiments performed in triplicate).

Induction and measurement of IL-2 and IFN-γ in T-cell supernatants. Cultures of macrophages (1×106/mL) and T-enriched cells (1×106/mL) were set up in 24-well tissue culture plates and activated using the stimuli and culture media as described above. Culture supernatants were removed after 24 or 72 hours of incubation, were sterile-filtered, and were analyzed for their cytokine content. The IL-2 concentration was determined using the murine cytotoxic lymphoid cell line CTL-2 as described earlier.15 Concentrations of IFN-γ in culture supernatants were determined using a commercial enzyme immunoassortant assay (ELISA) kit (IFN-γ Medgenix, Brussels, Belgium). IFN-γ concentrations were calculated using a standard curve derived by linear regression of the log-transformed concentrations of the cytokine standards supplied with the ELISA kits versus the respective log-transformed ELISA-optical density (OD) values.

Separation of CD4+ T lymphocytes. CD4+ T cells were isolated by negative magnetic immunoselection. In brief, MNCs were adhered to plastic surfaces to remove monocytes; nonadherent cells were E- rosetted and the E+ cells were incubated with saturating concentrations of CD8 MoAb (Leu 2a) and CD56 MoAb (Leu 19; Becton Dickinson, used at a final concentration of 2.5 µg/1×106 cells) for 30 minutes at 4°C under continuous rotation. Cells were washed twice, mixed with sheep antimouse IgG-coated beads (Dynabeads M450; at a final concentration of 1×106 beads/1×106 cells) and incubated for another 30 minutes at 4°C under continuous rotation. Cells that were reactive with the two MoAbs and thus bound to the magnetic beads were removed with a powerful permanent cobalt-samarium magnet (HPC-1; Dynal). The remaining cells were collected, washed twice, and analyzed by flow cytometry. Cell purity was 90% to 95% CD4+ cells.
In CVID patients with severe T-cell impairment, the expression of Vp2, Vp3, VP5a and c, as well as the levels of Vp6a, VP8a, VP12a, VP13 and VP17 (data not shown), were comparable to those of the controls. Furthermore, patients' T cells released significantly reduced amounts of IL-2 and IFN-γ when stimulated with an MoAb specific for the constant region of the TCR β-chain (BMA-031; see Fig 2). When the patients' T cells were stimulated via CD3, proliferation and IL-2 release were comparable with that of the controls (Fig 2), and IFN-γ production was slightly (but not statistically significantly) decreased (P = .096 as compared with that of the controls; see Fig 2). Levels of T-cell proliferative responses and IL-2 and IFN-γ production after stimulation with PHA were comparable in patients and controls (for statistical comparison between patients and controls see legend of Fig 2).

The defect in T-cell activation can be detected in purified CD4+ T cells and cannot be corrected by exogenous rIL-2. Because increased numbers of CD8+ cells have been described to be associated with impaired T-cell function in CVID,24 we examined whether the impairment of T-cell activation observed in our patients correlated with the levels of CD8+ cells in the PB. As shown in Table 1, antigen-induced T-cell proliferation as well as IL-2 and IFN-γ release were equally depressed in patients with high numbers of CD8+ cells (ie, >40% CD8+ lymphocytes) as well as in those with normal numbers of CD8+ T cells.

Impaired T-cell response to recall antigen is expressed in a relevant subset of CVID patients. At the time of the study, 24 patients with CVID were followed up by our immunology division. As shown in Fig 1, all 24 patients responded to PHA with substantial proliferation. In contrast, analysis of the response to recall antigen (tetanus toxoid or E coli) showed two distinct populations. Of the 24 patients recently (within 1 year before the study) vaccinated with tetanus toxoid, 15 showed an impairment of tetanus toxoid-induced T-cell activation in the presence of functionally intact autologous macrophages (Fig 1). T cells of these 15 patients were also unresponsive to stimulation with E coli (data not shown). The remaining 9 patients responded normally to tetanus toxoid (Fig 1) or E coli (data not shown).

The defect in T-cell activation can be observed after triggering of the TCR by different stimuli. Further studies were designed to characterize the T-cell activation defect observed in the patients unresponsive to recall antigens. Superantigens such as SEs are an appropriate tool for studying TCR-mediated T-cell activation. Because they do not have to be processed by APCs to be presented in context with the major histocompatibility complex class II molecule, they stimulate the T cells by binding to the TCR-Vβ chain outside the recognition site for antigen and activate between 1 in 5 and 1 in 20 PB T cells. In contrast, conventional antigens stimulate fewer than 1 in 10,000 PB T cells. Although patients' T cells expressed comparable levels of Vβ2, Vβ3, Vβ5a and c, Vβ6a, Vβ8, VP8a, Vβ12a, Vβ13, Vβ17, and Vβ19 (data not shown), their proliferative response as well as IL-2 and IFN-γ production after stimulation with SEA, SEC3, or SED (1 ng/mL) were significantly impaired (Fig 2). Furthermore, patients' T cells released significantly reduced amounts of IL-2 and IFN-γ when stimulated with an MoAb specific for the constant region of the TCR β-chain (BMA-031; see Fig 2). When the patients' T cells were stimulated via CD3, proliferation and IL-2 release were comparable with that of the controls (Fig 2), and IFN-γ production was slightly (but not statistically significantly) decreased (P = .096 as compared with that of the controls; see Fig 2). Levels of T-cell proliferative responses and IL-2 and IFN-γ production after stimulation with PHA were comparable in patients and controls (for statistical comparison between patients and controls see legend of Fig 2).
Impaired intracellular free Ca\(^{2+}\) in patients' T cells after triggering of the TCR by superantigens. A cocktail of seven different superantigens as described in Materials and Methods was used to examine the increase in intracellular free Ca\(^{2+}\) after triggering of the TCR. T cells from healthy controls responded with a significant Ca\(^{2+}\) flux to triggering of the TCR by superantigen presented on the surface of autologous monocytes. In contrast, patients' T cells were significantly (P < .001) depressed in their capacity to mount an increase in intracellular free Ca\(^{2+}\) after triggering with superantigens (Fig 4). Stimulation of the same cells with the combination of PMA + IM, which thus bypassed receptor-mediated signal mechanisms by direct activation of diacylglycerol (DAG) and mobilization of free Ca\(^{2+}\) from intracellular and extracellular stores, resulted in a significant Ca\(^{2+}\) flux in the patients' cells to levels that were comparable with that of the controls (Fig 4). These results indicate a defect in the early phase of T-cell activation after triggering via the TCR, before the generation of the second messengers DAG and inositoltriphosphate (IP\(_3\)).

B cells of CVID patients produce IgM, but not IgG, after in vitro stimulation. PB B cells in patients whose T cells were unresponsive to recall antigen were normal in numbers (data not shown), but Ig secretion in vitro was impaired. Although stimulation of PB lymphocytes with PWM, SAC, or anti-IgM plus rIL-2 resulted in levels of IgM secretion that were comparable with that of controls, none of the stimulants induced IgG release in the patients' B cells (Table 2).

**DISCUSSION**

The results presented here confirm and extend our previous observation made in three patients with CVID by showing that T cell activation on triggering of the TCR is defective in a significant subset of CVID patients (15 of 24 patients). Furthermore, the results of the present study provide evidence that a major subgroup of CVID patients expresses a defect in the early phase of T-cell activation after triggering of the TCR.

Impaired T-cell activation, including defective IL-2 production on activation with mitogenic lectins or anti-CD3, has been observed previously by several investigators.\(^7\) Sneller et al\(^7\) described four patients with CVID whose T
Conflicting data have been reported with regard to the role of immunoregulatory abnormalities such as an increase in CD8+ T cells in the impaired response of T cells to antigen in CVID patients. A report by Wright et al. described 5 of 12 CVID patients with high CD8 counts who were anergic as determined by skin testing of delayed-type hypersensitivity (DTH) reaction, whereas, in the group of CVID patients with normal numbers of CD8+ T cells, only 1 of 15 patients was anergic to recall antigens. These data suggest that, in CVID patients with high levels of CD8+ T cells, antigen unresponsiveness is more frequent than in CVID patients with normal numbers of CD8+ T cells. Jaffe et al. reported that CD4+ T-cell function (the principal T-cell subset responding to antigen) of patients with high numbers of CD8+ T cells was unimpaired.

The defect in T-cell antigen responsiveness observed in the subgroup of CVID patients we studied was expressed in both patients with high and with normal CD8 counts, and no correlation could be found between CD8 counts and tetanus toxoid-specific T-cell proliferation (linear regression analysis; data not shown). Purified CD4+ cells expressed the T-cell activation defect both in the absence or presence of CD8+ T cells alike. Therefore, it appears unlikely that the defect described is caused by immunoregulatory abnormalities such as an increase in CD8+ T cells leading to excessive suppression at the T-cell level.

Defective ligand recognition by TCR is not likely to be the only explanation for the impaired response to antigen. The combination of antigen and exogenously added rIL-2 induced significantly higher proliferative response and lymphokine secretion in the patients’ T cells than did either antigen or IL-2 alone. Furthermore, antigenic stimulation induced normal production of IL-2 receptor, IL-3, and IL-4 mRNA transcripts in these patients’ T lymphocytes in the

Table 1. Antigen-Induced T-Cell Activation Is Depressed in Patients With High as Well as in Those With Normal Numbers of CD8+ T Cells

<table>
<thead>
<tr>
<th>T-Cell Response to the Recall Antigen Tetanus Toxoid</th>
<th>Patients (N = 6) With High CD8+</th>
<th>Patients (N = 5) With Normal CD8+</th>
<th>Controls (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation (dpm)</td>
<td>2,060 ± 2,040</td>
<td>1,233 ± 463</td>
<td>75,219 ± 13,698</td>
</tr>
<tr>
<td>IL-2 (IU/mL)</td>
<td>1.3 ± 0.5</td>
<td>1.1 ± 0.9</td>
<td>46.8 ± 16.7</td>
</tr>
<tr>
<td>IFN-γ (IU/mL)</td>
<td>3.9 ± 3.6</td>
<td>1.5 ± 0.7</td>
<td>56.1 ± 24.8</td>
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T-enriched cells (1 × 10^6/well) and autologous monocytes (1 × 10^6/well) were cocultivated and stimulated with tetanus toxoid (10 LIF/mL). After 3 days, culture supernatants were removed and assayed for IL-2 and IFN-γ content. 3H-thymidine incorporation was determined after 7 days of culture as described in Materials and Methods. Values represent mean ± SD. Statistical comparison between patients with high levels of CD8+ T cells and patients with normal levels of CD8+ T cells is as follows: for proliferation,  P = .23; for IL-2,  P = .32; and for IFN-γ,  P = .25.

*Statistically significant differences as compared with controls are as follows: for proliferation,  P = .00067; for IL-2,  P = .00045; and for IFN-γ,  P = .0023.

Fig 3. Impaired antigen-induced T-cell activation can be observed in purified CD4+ T cells obtained from CVID patients and cannot be corrected by addition of rIL-2. CD4-enriched T cells (1 × 10^6 cells/well) were cocultivated in the presence of autologous monocytes (1 × 10^6 cells/well) and stimulated with PHA (1:1250) or tetanus toxoid (T.T.; 10 LIF/mL) with or without IL-2 (10 IU/mL). 3H-thymidine incorporation was determined after 3 days for PHA or 7 days for antigen, as described in Materials and Methods. Supernatants of patients’ and controls’ T-cell cultures were collected after 24 hours (PHA) and 72 hours (antigen), and IL-2 and IFN-γ levels were determined as described in Materials and Methods. Values represent mean ± SD; statistically significant differences between patients and controls are marked with an asterisk.

Statistical comparison between patients and controls is as follows: for PHA: proliferation,  P = .2; IL-2,  P = .057; IFN-γ,  P = .087; for T.T.: proliferation,  P = .0013; IL-2,  P = .01; IFN-γ,  P = .0051; for T.T. + IL-2: proliferation,  P = .012; IFN-γ,  P = .0071; and for IL-2: proliferation,  P = .074; IFN-γ,  P = .07. (□) Controls (n = 6); (●) patients (n = 7).
presence of impaired IL-2 and IFN-γ gene expression (Hauber et al, manuscript submitted), clearly indicating that the antigen had been recognized by the T cell and delivered a signal. In addition, T-cell response to superantigen stimulation was impaired. Superantigens stimulate the T cell by binding to the TCR-β chain apart from the antigen binding site. No evidence for narrowing of the TCR-β repertoire could be found, because T cells of this group of patients with CVID expressed comparable levels of Vβ2, Vβ3, Vβ5a and c, Vβ6a, Vβ8, Vβ8a, Vβ12a, Vβ13, Vβ17, and Vβ19 (data not shown), as has also been described by others.18 Even though binding of superantigen to the TCR has not been assessed directly, the normal expression of the TCR-β repertoire makes impaired binding of superantigens unlikely. Finally, stimulation via the monomorphic region of the TCR with anti-TCR MoAb resulted in impaired T-cell activation as shown by significantly reduced cytokine release, whereas expression of the TCR-α-β molecule on the T-cell surface was normal (data not shown). The reduced response to antigen, superantigen, and anti-TCR MoAb binding to distinct sites of the TCR points to defective TCR-mediated signaling and makes impaired ligand recognition unlikely.

Increasing experimental evidence points to the possibility that signaling events in T-cell activation on stimulation of the TCR/CD3 complex might follow at least two different pathways.20,21 Examples of this dichotomy also come from experiments of nature. T cells of patients with Wiskott-Aldrich syndrome were shown to function normally when triggered with antigen but showed a defect in activation when triggered with anti-CD3.22 T cells from our CVID patients could be activated normally on anti-CD3 triggering but could not be activated to express IL-2 after stimulation of the TCR-α-β molecule. The possibility that T-cell activation by superantigen and by antigen follows different signal transduction pathways has been indicated23 but needs further clarification. The question of Ca++ mobilization in T cells after superantigen stimulation is controversial. Oyaizu et al24 when studying human T-cell lines and their response to superantigen, did not observe a significant Ca++ flux, whereas two groups using resting human T cells from the PB25,26 were able to induce Ca++ flux on stimulation with superantigens. These discrepancies could be explained by differences in the cell population studied or the nature and concentration of the superantigen applied. It is feasible that superantigens applied at lower concentrations, as in the study by Oyaizu et al.,24 may preferentially stimulate T-cell activation without

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**Fig 4.** Decreased Ca++ mobilization in T lymphocytes of CVID patients after stimulation with superantigen. T-enriched cells (2 x 10⁶/mL) were loaded with Fluo-3 (1 μmol) and Snarf-1 (0.2 μmol) and were subsequently stimulated with autologous monocytes (2 x 10⁶/mL) that had been pulsed with a cocktail of different superantigens (final SE concentrations: SEA, 5 μg/mL; SEB, 10 μg/mL; SEC1, 10 μg/mL; SEC2, 5 μg/mL; SEC3, 5 μg/mL; SEE, 1 μg/mL; SEE, 5 μg/mL; SEE, 1 μg/mL). Changes in the levels of intracellular free Ca++ in patients’ and controls’ T-enriched lymphocytes were determined using a cytofluorograph as described in Materials and Methods. Stimulation with a combination of PMA (1 μg/mL) and IM (5 μg/mL) resulted in a maximum increase in intracellular free Ca++ and served as a positive control in these experiments. Results are depicted as relative intracellular Ca++ increase (F1/F13; mean ± SD) calculated as described in Materials and Methods. The relative intracellular Ca++ increase observed after addition of unpulsed autologous monocytes to patients’ or controls’ T cells did not exceed 1.10. Statistically significant differences between patients and controls (Student’s t-test, P < .001) are marked with an asterisk. (□) Controls (n = 10); (○) patients (n = 8).
A defect in antigen-induced T-cell activation leading to decreased lymphokine release can have several important implications for the pathogenesis of CVID. T cells act on isotype-uncommitted or naive B cells by signaling via CD40 and modulate switch mechanisms at the DNA level. In addition, T cells support the expansion of committed B cells that already have undergone Ig isotype switching. A defect in antigen-induced human B-cell proliferation and differentiation, and deficient production of IL-2 by T cells on antigenic stimulation may contribute to the B-cell abnormalities observed in these CVID patients. In addition, decreased IFN-γ production, whether primary or secondary to the decreased IL-2 release, may also have implications for B-cell differentiation. In agreement with this notion, patients with impaired TCR-mediated T-cell activation showed a rather uniform pattern with respect to B-cell numbers and function. B-cell numbers were normal, and the B cells secreted normal amounts of IgM in response to several stimuli (PWM, SAC, IgM + IL-2) but were unable to produce IgG. A defect in the production of specific antibodies is common to all patients with CVID. The impairment of TCR-mediated signaling leading to reduced IL-2 and IFN-γ production is likely to contribute to the pathophysiology of the disease in a significant subset of these patients.

**REFERENCES**


**Table 2. B Cells of CVID Patients Secret IgM But Not IgG**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Patients (N = 10)</th>
<th>Controls (N = 6)</th>
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<tbody>
<tr>
<td>IgM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>0.17 ± 0.13</td>
<td>0.21 ± 0.12</td>
</tr>
<tr>
<td>PWM</td>
<td>10.43 ± 9.01</td>
<td>7.80 ± 5.61</td>
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<tr>
<td>SAC</td>
<td>7.95 ± 7.30</td>
<td>6.36 ± 5.68</td>
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<tr>
<td>IgM + IL-2</td>
<td>2.78 ± 2.50</td>
<td>2.19 ± 2.18</td>
</tr>
<tr>
<td>IgG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>0.18 ± 0.03</td>
<td>0.20 ± 0.18</td>
</tr>
<tr>
<td>PWM</td>
<td>0.28 ± 0.16*</td>
<td>5.99 ± 2.13</td>
</tr>
<tr>
<td>SAC</td>
<td>0.12 ± 0.06*</td>
<td>6.67 ± 3.04</td>
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<tr>
<td>IgM + IL-2</td>
<td>0.25 ± 0.18*</td>
<td>4.73 ± 3.28</td>
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* Statistically significant difference as compared with the controls is as follows: for PWM, \( P = .00073 \); for SAC, \( P = .00074 \); and for IgM + IL-2, \( P = .00057 \).
A defect in the early phase of T-cell receptor-mediated T-cell activation in patients with common variable immunodeficiency