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.

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**COMMON VARIABLE** immunodeficiency (CVID) is characterized by heterogeneity in the clinical manifestations of the disease as well as in the underlying mechanisms leading to immunodeficiency. An impairment in specific antibody production and a decrease in all or some Ig isotypes are the characteristic features, and defects in B-cell differentiation and/or antibody secretion are common. Abnormal distribution of T-cell subsets has 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.

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**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. 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 lgs. 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 mononuclear 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.15 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 into 2-adherent cells (i.e., >95% CD2+ and >90% CD3+ cells) and non-T cells by rosetting with sheep erythrocytes treated with 2-aminooethoxyisothiuronium bromide (Sigma Chemicals, St. Louis, MO) as described earlier.13

Lymphocyte proliferation. Triplicate cultures containing adherence-purified monocytes (1 × 10^5/well) and T-enriched cells (1 × 10^6/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 fluid units/mL; Swiss Serum and Vaccine Institute, Berne, Switzerland); heat-inactivated E coli 089:H10 (5 × 10^8 E. coli/mL), staphylococcal enterotoxins (SEs) SEA, SEC3, and SED (10 ng/mL; Toxin Technology, Madison, WI); PHA (1:1250; Wellcome, Dartford, UK); anti-CD3 MoAb (10 ng/mL OKT3; Ortho, Raritan, NJ); or anti-TCRα/β (2 µg BMA-031 were coated on 4 × 10^6 sheep ant moisue Ig-coated beads in a total volume of 1 mL [Dynabeads M450; Dynal, Oslo, Norway] and used at a final concentration of 1 × 10^6 beads/1 × 10^6 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% fetal calf serum (FCS; [Sigma Chemicals] and 5 pg/mL ionomycin [Calbiochem, San Diego, CA] added to the T cells after investigation of the superantigen-induced Ca++ flux served as a positive control inducing maximal Ca++ release.

Induction and measurement of IL-2 and IFN-γ in T-cell supernatants. Cultures of macrophages (1 × 10^5/mL) and T-enriched cells (1 × 10^6/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.16 Concentrations of IFN-γ in culture supernatants were determined using a commercial enzyme-linked immunosorbent 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, MNC 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/L × 10^6 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 × 10^6 beads/1 × 10^6 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-I; Dynal). The remaining cells were collected, washed twice, and analyzed by flow cytometry. Cell purity was 90% to 95% CD4+ cells.

Assay of intracellular free Ca++ concentration. The increase in intracellular free Ca++ observed after stimulation of T cells was measured by flow cytometry. In brief, T-enriched cells (2 × 10^6/mL) were loaded with two fluorochromes, the Ca++-sensitive dye Fluo-3 (final concentration, 1 µmol; Molecular Probes, Eugene, OR), and the Ca++-insensitive dye Snarf-1 (final concentration, 0.2 µmol; Molecular Probes) dissolved in buffer A (Isco’s modified Dulbecco’s medium [IMDM; Sigma Chemicals] containing 10 mMol/L HEPES [Sigma Chemicals], pH 7.0). Fluronic F-127 (BSF; final concentration, 37.5 g/L; Wyandotte, OR) was used to increase the uptake of the two fluorochromes by the cells. Cells were incubated for 30 minutes in a CO2 incubator at 37°C. Next, an equal volume of buffer B (IMDM containing 10 mMol/L HEPES and 10% fetal calf serum [FCS; HyClone Laboratories, Inc, Logan, UT], pH 7.4) was added to the cells suspended in buffer A, and the cells were incubated for an additional 10 minutes at 37°C. Cells were then washed twice and resuspended in buffer C (IMDM containing 10% FCS, 10 mMol/L HEPES and 10 µg/mL DNAse [Sigma Chemicals]) at a final concentration of 2 × 10^6/mL. Autologous monocytes (2 × 10^5/mL) that had been preincubated at 37°C for 3 hours with a cocktail of SEs (final SE concentrations: SEA, 5 pg/mL; SEB, 10 pg/mL; SEC1, 10 µg/mL; SEC2, 5 µg/mL; SEC3, 5 µg/mL; SEC4, 5 µg/mL; SEC5, 1 µg/mL) were added to Fluo-3- and Snarf-1-loaded T cells. The mixture of monocytes and T cells was immediately centrifuged for 20 seconds at 200g in an Eppendorf cup (Eppendorf, Hamburg, Germany), and the cells were gently resuspended before fluorescence-activated cell sorter (FACS) analysis. The flow cytometric analysis of intracellular Ca++ was performed using a FACSscan (Becton Dickinson) interfaced to a Hewlett Packard computer system using Lysis and Chronus software (Becton Dickinson). Forward and right angle light scatter were used to selectively gate the lymphocyte population. For kinetic analysis of Ca++ changes, events were continuously monitored with the Chronus software, and the mean fluorescence intensity of events measured at 10-second intervals was calculated. Relative intracellular Ca++ increase was calculated in computed parameters with the Chronus software using the increase in Fluo-3--specific fluorescence intensity measured in FI1 compared with the stable Snarf-1--specific fluorescence intensity measured in FI3 by the formula: FI1/FI3* = FI1 /FI3 t × (FI1 t / FI3 t1)). In every experiment, unpulsed autologous macrophages were included as a control for T-cell activation induced by autologous monocytes. The combination of PMA + IM (1 µg/mL PMA [Sigma Chemicals] and 5 µg/mL ionomycin [Calbiochem, San Diego, CA]) added to the T cells after investigation of the superantigen-induced Ca++ flux served as a positive control inducing maximal Ca++ release.

Induction and assessment of B-cell Ig secretion in patients’ MNCs. A total of 1 × 10^6 MNCs were stimulated with pokeweed mitogen (PWM; GIBCO, Grand Island, NY), anti-IgM MoAb plus recombinant IL-2 (rIL-2; Genzyme), or Staphylococcus aureus Cowan 1 (SAC; Calbiochem). The anti-IgM MoAb (kindly provided by Dr O. Majdic, Institute of Immunology, University of Vienna) was coupled to Dynabeads M 450 (2 µg anti-IgM MoAb were coated on 4 × 10^6 sheep antimouse IgG-coated beads in a total volume of 1
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 results in impaired IL-2 and IFN-γ release and 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, Vβ8a, 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-03; 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 TCR-mediated 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,53 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+ T cells (ie, >40% CD8+ lymphocytes) as well as in those with normal numbers of CD8+ T cells. To assure that the defect observed was not caused by suppression by CD8+ cells, purified CD4+ cells (purity between 90% and 95%) were prepared by depletion of CD8+ and CD56+ cells from the T-enriched population. Patients’ CD4-enriched cells as well as their T cells responded normally to PHA stimulation (Fig 3) or anti-CD3 MoAb stimulation (controls [n = 6]; 3H-thymidine incorporation (dpm), 84127 ± 54962 and IFN-γ release, 157 ± 149 U/mL; patients [n = 7]; 3H-thymidine incorporation (dpm), 108944 ± 40176 and IFN-γ release, 175 ± 82 U/mL; mean ± SD). In contrast, purified CD4+ T cells of the patients were unable to respond to substantial proliferation and with IL-2 and IFN-γ production to the recall antigen tetanus toxoid. Addition of exogenous rIL-2 to the patients’ CD4+ T cells significantly increased antigen-induced T-cell proliferation and IFN-γ release as compared with tetanus toxoid (P < .005) or IL-2 (P < .01) alone, but proliferative responses and IFN-γ release in the patients were still significantly lower than that in the controls (Fig 3).
Impaired intracellular free Ca\textsuperscript{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\textsuperscript{2+} after triggering of the TCR. T cells from healthy controls responded with a significant Ca\textsuperscript{2+} flux to triggering by superantigens 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\textsuperscript{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\textsuperscript{2+} from intracellular and extracellular stores, resulted in a significant Ca\textsuperscript{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\textsubscript{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.\textsuperscript{7,12} Sneller et al\textsuperscript{7} described four patients with CVID whose T
cells had a normal phenotype but were unable to express detectable levels of IL-2, IL-4, IL-5, and IFN-γ mRNA after lectin (PHA) stimulation. The same patients’ PHA-stimulated T cells showed a normal proliferative response and expressed adequate amounts of IL-2 receptor and c-myc mRNA. These investigators discussed the possibility of an impairment in the interaction of nuclear transcription factors with specific DNA sequences located in the 5' regulatory regions of lymphokine genes. In a subsequent report, these investigators clarified that the lymphokine production defect observed in their four patients with CVID was a primary abnormality of CD4+ T cells that might be related to an impairment of a membrane receptor-dependent signaling pathway. However, antigen-induced T-cell responses have not been studied.

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 both in 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+ 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)</th>
<th>Controls (N = 10)</th>
</tr>
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<tbody>
<tr>
<td>Proliferation (dpm)</td>
<td>2,060 ± 2,040</td>
<td>1,233 ± 463</td>
</tr>
<tr>
<td>IL-2 (U/mL)</td>
<td>1.3 ± 0.5</td>
<td>1.1 ± 0.9</td>
</tr>
<tr>
<td>IFN-γ (U/mL)</td>
<td>3.9 ± 3.6</td>
<td>15 ± 2.7</td>
</tr>
</tbody>
</table>

T-enriched cells (1 x 10^6/well) and autologous monocytes (1 x 10^6/well) were cocultured and stimulated with tetanus toxoid (10 IU/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 are 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 = .00067.

Statistical differences as compared with controls are as follows: for proliferation, P = .0011; for IL-2, P = .00091; 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 x 10^6 cells/well) were cocultivated in the presence of autologous monocytes (1 x 10^6 cells/well) and stimulated with PHA (1:1250) or tetanus toxoid (T.T.; 10 IU/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 = .0005; 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).
IMPAIRED TCR-MEDIATED T-CELL ACTIVATION IN CVID

Fig 4. Decreased Ca++ mobilization in T lymphocytes of CVID patients after stimulation with superantigen. T-enriched cells (2 x 10^6/mL) were loaded with Fluo-3 (1 µmol) and Snarf-1 (0.2 µmol) and were subsequently stimulated with autologous monocytes (2 x 10^6/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; SED, 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 (F11/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).

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. 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 TCRα-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. 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. 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 TCRα-β molecule. The possibility that T-cell activation by superantigen and by antigen follows different signal transduction pathways has been indicated but needs further clarification. The question of Ca++ mobilization in T cells after superantigen stimulation is controversial. Oyaizu et al, 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 PB 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, may preferentially stimulate T-cell activation without
Table 2. B Cells of CVID Patients Secrete 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>
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</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>
</tr>
<tr>
<td>SAC</td>
<td>7.95 ± 7.30</td>
<td>6.38 ± 5.68</td>
</tr>
<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>
</tr>
<tr>
<td>IgG + IL-2</td>
<td>0.25 ± 0.18*</td>
<td>4.73 ± 3.28</td>
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</table>

Patients’ PBMNC (1 × 10⁶/mL) were stimulated with PWM (1:500), anti-IgM coupled to Dynabeads M450, 5 lg anti-IgM coated on 1 × 10⁶ sheep antimouse IgG beads/mL in combination with rIL-2 (100 U/mL) or SAC (1:5000). After 7 days of cultivation the levels of IgM and IgG were determined by ELISA. Values represent mean ± SD.

* Statistically significant difference as compared with the controls is as follows: for PWM, P = .00073; for SAC, P = .00074; and for IgG + IL-2, P = .00057.

inducing measurable Ca++ flux. Our experiments followed the experimental design described by Fleischer et al and Chatila et al and confirm that, in resting PB T cells of healthy individuals, bacterial superantigens applied at micromolar concentrations induce a measurable Ca++ flux. The patients’ T cells were clearly deficient in mounting a Ca++ flux after superantigen stimulation in this system. Although it is unlikely, our results cannot rule out a delayed Ca++ response after triggering of the TCR with superantigen in the patients. The combination of PMA and IM, agents that bypass receptor-mediated signal mechanisms, was shown to induce an appropriate and immediate increase in cytosolic free Ca++ concentration in the patients’ lymphocytes and also triggered normal proliferation and cytokine release (data not shown). Therefore, it appears likely that the impairment in T-cell activation observed in our patients is caused by a defect in early signaling events after triggering of the TCR. Although our results indicate that TCR-coupled generation of second messengers such as intracellular free Ca++ is defective in the patients, other TCR-coupled signaling pathways independent of Ca++ mobilization could also be impaired.

The impairment in T-cell activation found in this subset of CVID patients is not absolute. Although IL-2 mRNA and protein levels are decreased after TCR stimulation, possibly as a consequence of reduced Ca++ mobilization, other events triggered by TCR activation such as gene expression of IL-2 receptor, IL-3, and IL-4 are unimpaired (Hauber et al, manuscript submitted). This might be because of different signaling requirements, eg, different pathways and/or quantitative differences in the levels of second messengers produced. Furthermore, T cells from these patients are principally capable of producing IL-2 after triggering of surface receptors other than TCR (eg, mitogenic stimulation with PHA known to trigger both the TCR/CD3 complex and CD2) or by stimuli that bypass surface receptor-mediated signaling (eg, PMA + IM). 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. IL-2 is a potent inducer of 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

A defect in the early phase of T-cell receptor-mediated T-cell activation in patients with common variable immunodeficiency