Characterization of Common Variable Immunodeficiency: Identification of a Subset of Patients With Distinctive Immunophenotypic and Clinical Features

By John J. Wright, David K. Wagner, R. Michael Blaese, Claire Hagengruber, Thomas A. Waldmann, and Thomas A. Fleisher

The peripheral blood lymphocyte surface markers and clinical features of 38 patients with common variable immunodeficiency (CVID) were assessed. These studies identified a subset of CVID consisting of 14 of the 38 patients with a distinctive T-cell immunophenotype and clinical findings. The phenotypic changes were characterized by an abnormally low CD4/CD8 ratio (<0.9) due primarily to a significant increase in CD8 T cells. In addition, there was an expansion in CD8 T cells coexpressing CD57 and increased expression of the activation markers HLA-DR and interleukin-2 receptor (IL-2R) by these cells. This group of immunophenotypically abnormal CVID patients also had characteristic clinical features, including splenomegaly (P < .02) and in vivo T-cell dysfunction based on the evaluation of delayed-type hypersensitivity (P < .05).

Approximately 71% of these patients had splenomegaly and 42% were anergic in contrast to the remaining group of CVID patients, in which 29% had splenomegaly and 7% were anergic. These findings define a subgroup of CVID patients that have specific immunophenotypic features and functional T-cell abnormalities.

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COMMON VARIABLE immunodeficiency (CVID) is a primary immunodeficiency disorder characterized by decreased or absent levels of serum immunoglobulins (Ig) and increased susceptibility to infection with high-grade encapsulated bacterial organisms. Although categorized as a humoral immunodeficiency with the hallmark feature of hypogammaglobulinemia, there is evidence that in addition to B-cell dysfunction, T-cell abnormalities may contribute to the underlying pathogenesis of CVID in at least some patients. Peripheral blood T-lymphocyte subset abnormalities and specifically low CD4/CD8 ratios have been described in some patients, and increased T suppressor cell function has also been implicated as possibly contributing to the hypogammaglobulinemia. We extended these previous findings by performing extensive immunophenotyping of the peripheral blood lymphocytes from 38 CVID patients with a number of monoclonal antibodies. Included among these were several recently described reagents that further distinguish lymphocyte subsets. The results of these in vitro studies were then correlated with clinical features of these patients to evaluate for possible links between immunophenotypic abnormalities and disease characteristics.

MATERIALS AND METHODS

Patients. Patients with CVID referred to the National Institutes of Health were studied after informed consent was obtained. The study protocol was approved by the National Cancer Institute (NCI) Research Subpanel and the Director of the Clinical Center. The 38 patients consisted of 18 men and 20 women ranging in age from 19 to 67 years. The diagnosis of hypogammaglobulinemia involving the three major serum immunoglobulin classes had been established in all patients after childhood. At the time of evaluation, none of the patients had evidence of acute infection. Thirty patients were receiving replacement therapy with intramuscular or intravenous gamma globulin preparations and two of the untreated 8 patients had never received immunoglobulin replacement therapy. Delayed-type hypersensitivity skin testing was performed using the CM1 Multitest (Merieux Institute, Miami, FL) according to the manufacturer's instructions; patients without reactivity to any of the six test antigens were categorized as anergic. The diagnosis of splenomegaly was established when the spleen size was greater than 13 cm by technetium liver-spleen scan. A total of 8 CVID patients with noncaseating granulomas were identified based on characteristic histopathologic features in various tissue biopsy samples obtained from skin, liver, bone marrow, and/or lymph node. Gastrointestinal infection with either Helicobacter pylori or Giardia lamblia was documented by microbiologic culture or microscopic examination, respectively, of patient stool samples. Statistical comparison of the two CVID patient groups was assessed by $x^2$ analysis.

Immunophenotypic evaluation. Pan T-cell markers used in this study included CD3 (Leu 4), CD2 (T1 l), and CD5 (Leu 1). T-cell subset markers used were CD4 (Leu 3), CD8 (Leu 2), CD45R (2H4), anti-HLA-DR, and CD57 (Leu 7); natural killer (NK) cell specific marker used was CD16 (Leu 11); B cell markers used were CD19 (B4), CD20 (B1), CD5 (Leu 1), CD23 (Leu 17), and Leu 8. The presence of monocytes was determined using CD14 (Leu M3). The Leu reagents and anti-HLA-DR were obtained from Becton-Dickinson (Mountain View, CA); the reagents T11, 2H4, B1, and B4 were obtained from Coulter Immunology (Hialeah, FL).

Purified lymphocytes from fresh venous blood of the CVID patients were prepared and stained as previously described. A FACScan Analyzer (Becton-Dickinson) equipped with a Consort 30 was used to collect volume, side scatter (SSC), and fluorescent signals. Log amplification was used for the fluorescent signals and compensation for green-red overlap was established for each experiment using stained cells. Rectilinear gating was accomplished using electronic volume and SSC to define the lymphocyte gate, and included a relatively distinct population. List mode parameters were collected for $10^4$ cells and quadrants were defined on the basis of isotype reagent controls. In addition to the percentage and number of cells in each quadrant, the mean channel and coefficient of variation were obtained from single histograms for each of the reagents. Statistical analysis of the percent positive and number of events for each reagent was performed using Student's t test.

Soluble interleukin-2 receptor measurement. The enzyme-linked immunosorbent assay for the detection of soluble IL-2...
Table 1. T-Lymphocyte Populations in CVID Patients

<table>
<thead>
<tr>
<th></th>
<th>Normal Controls</th>
<th>CD4/CD &gt; 0.9*</th>
<th>CD4/CD ≤ 0.9†</th>
</tr>
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<tr>
<td>Lymphocytes</td>
<td>1,759 ± 490/mm³</td>
<td>1,530 ± 648/mm³</td>
<td>2,247 ± 785/mm³</td>
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<tr>
<td>CD3⁺ cells</td>
<td>76.6 ± 7.1%</td>
<td>78.9 ± 11.2%</td>
<td>81.7 ± 10.6%</td>
</tr>
<tr>
<td></td>
<td>1,380 ± 425/mm³</td>
<td>1,235 ± 757/mm³</td>
<td>1,812 ± 611/mm³</td>
</tr>
<tr>
<td>CD4⁺ cells</td>
<td>48.3 ± 8.3%</td>
<td>47.6 ± 8.6%</td>
<td>27.0 ± 10.1%</td>
</tr>
<tr>
<td></td>
<td>839 ± 276/mm³</td>
<td>668 ± 411/mm³</td>
<td>596 ± 250/mm³</td>
</tr>
<tr>
<td>CD8⁺ cells</td>
<td>26.2 ± 6.0%</td>
<td>29.0 ± 5.7%</td>
<td>53.0 ± 9.8%</td>
</tr>
<tr>
<td></td>
<td>473 ± 196/mm³</td>
<td>452 ± 300/mm³</td>
<td>1,184 ± 490/mm³</td>
</tr>
</tbody>
</table>

All percentages and cell numbers are presented as the group mean ± 1 SD.

* n = 24.
† n = 14.

Receptors (IL-2R) has been previously described. The undiluted reference sample was assigned a value of 1,000 IL-2R U/mL.

RESULTS

T lymphocytes. Examination of the percent and absolute numbers of T cells and CD4 and CD8 T-cell subsets revealed that certain CVID patients deviated from normal. The characteristic that most clearly distinguished the patients was the presence of an abnormal (≤0.9) CD4/CD8 ratio (14 of 38), whereas the remaining CVID patients all had a normal ratio (>0.9). The patients with abnormal CD4/CD8 ratios had an increase in total lymphocytes (Table 1) that resulted from a large elevation in the percent and absolute number of CD8 T cells (Fig 1), with more than a twofold increase in the mean values for this subset (Table 1). In addition, the total number of CD4 T cells was normal, but the percent of these cells was somewhat decreased due to the overall increase in the CD8 lymphocyte numbers (Table 1 and Fig 1). The 24 patients with normal CD4/CD8 ratios had a minimally decreased total mean lymphocyte count with normal mean results for total T cells (Table 1) and T-cell subpopulations (Fig 1).

Additional evaluation of CD8 T cells included enumeration of the cells coexpressing CD57, a relatively small
subpopulation in normals (5.8 ± 3.2%). These cells were present in levels that exceeded three standard deviations above the normal mean in 12 of the 14 patients with decreased CD4/CD8 ratios (Fig 2). In contrast, of the 19 patients with normal CD4/CD8 ratios studied, only 2 had an increased CD8/CD57 population (Fig 2).

In both patient groups there was increased expression of the T-cell activation marker HLA-DR on CD3-positive cells. Further evaluation revealed that the increase in this activation marker was found predominantly on the CD8-positive T cells. The magnitude of this increase was greater in those with low CD4/CD8 ratios (Fig 3), whose group mean (11.7%) was four times greater than the other CVID patients (2.8%) and eight times greater than the normal controls (1.4 ± 0.5%). Additional evidence for T-cell activation was demonstrated by the elevation of serum IL-2R levels in the majority of patients with increased HLA-DR expression (mean level 1,088 U/mL, normal 95% confidence interval 112 to 502 U/mL).

The CD4 lymphocyte subpopulation was examined for CD45R, a marker associated with naive/memory status. Within the normal phenotype group, this marker was expressed on approximately half of the CD4 T cells, which is similar to controls. In contrast, the patient group with low CD4/CD8 ratios had a significantly decreased percent and absolute number of CD4 cells expressing CD45R (Fig 4). Additional studies of the CD4 population were directed at evidence of cell activation based on HLA-DR expression and no difference was found between either of the patient groups and normal controls.

These T cell immunophenotypic changes were stable as demonstrated by comparable results on reevaluation of 11 CVID patients 2 to 7 months after initial assessment. The repeat studies included patients from both the normal and decreased CD4/CD8 subgroups.

B lymphocytes. B lymphocyte populations in the CVID patients were evaluated by studying the normally concordant pan-B-cell markers CD19 and CD20. Approximately 21% of the patients (8 of 38) had B-cell levels that were more than 2 standard deviations below the normal range (<2%); thus, about 75% had normal B-cell levels as has been previously observed. The patients with decreased B cells did not segregate into either of the established T-cell patient groups.

To further examine potential abnormalities in B-cell populations, we evaluated the expression of CD5, CD38, and Leu in selected CVID patients with total B cells in the normal range. These studies showed no significant difference in expression of these three surface antigens when compared with normal controls (n = 19). Thus, although there is a numerical decrease in some patients, most CVID patients appear to have circulating B cells with normal expression of a number of B cell–specific and associated antigens.

NK cells. NK cells were identified by CD16 expression. These studies showed that all the CVID patients had normal NK cell numbers, except for three individuals with a significantly increased percentage of NK cells (greater than 30% v normal of 11.8 ± 5.5%). However, there were no abnormalities in the B-cell or T-cell subpopulations of these three patients.

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Fig 2. Percent (left panel) and absolute number (right panel) of CD8+/CD67+ T cells in CVID patients. Dashed line represents the upper limit of the 95% confidence interval for normal controls.
**Clinical features.** Several clinical features were identified that distinguished the two immunophenotypically distinct CVID patient groups (Table 2). The finding of splenomegaly segregated according to the immunophenotypic grouping. Enlarged spleens, identified by nuclear imaging scans, were observed in 10 of 14 CVID patients with low CD4/CD8 ratios, whereas this was noted in only 7 of 24 patients with a normal ratio. Evaluation of delayed-type hypersensitivity revealed that 5 of 12 patients with low CD4/CD8 ratios were anergic, whereas only 1 of 15 immunophenotypically normal CVID patients failed to respond to the recall antigens. There was also a suggestion that granulomas were more common among the abnormal group, although this feature did not reach statistical significance. In contrast, no differences were observed in the use of immunoglobulin replacement therapy, the incidence of chronic gastrointestinal infection, or the sex distribution between the two groups of CVID patients.

**DISCUSSION**

Common variable immunodeficiency is a primary disorder of the immune system for which the underlying etiology at the molecular or cellular level has yet to be fully established. Previous studies of peripheral mononuclear cells including immunophenotypic evaluation and assessment of functional activity have demonstrated a heterogeneous pattern of cellular abnormalities. Alterations in B-cell maturation and/or differentiation have been observed in the majority of CVID patients. T cells also may play a role in the pathogenesis of CVID in a subgroup of patients whose T cells suppressed in vitro polyclonal Ig synthesis when cocultured with normal B-lymphocytes. B-cell dysfunction as a result of T-cell suppression also has been observed in avian agammaglobulinemia. In this chicken model, T cells are capable of completely inhibiting B-cell immunoglobulin production in vivo, inducing a persistent antibody deficiency. In addition, decreased helper T-cell activity has been observed in occasional CVID patients. There is also evidence of T-cell dysfunction in CVID patients based on impaired response to recall skin test antigens and altered in vitro proliferation after lectin stimulation.

We evaluated T cells in a group of CVID patients using two-color flow cytometry with a panel of monoclonal antibodies that identify T-cell subpopulations and activated T cells.
The majority of our CVID patients had normal numbers of T-lymphocytes. However, approximately one third of the patients we studied had significantly altered T-cell subpopulation profiles with an increased percent of CD8 and decreased percent of CD4 cells, resulting in markedly depressed CD4/CD8 ratios (≤0.9). These changes differ from the findings in human immunodeficiency virus (HIV) infection, in which the decreased CD4/CD8 ratio results primarily from marked depression of CD4 T cells.

These CVID patients were noted to have increased T-lymphocyte numbers as a result of the significant elevation of CD8 T cells. In addition, within this expanded CD8 T-cell subpopulation there was a significant increase in cells co-expressing CD8 and CD57. Expansion of the CD8/CD57 T-cell subpopulation has been previously observed early in the course of HIV infection and post-allogeneic bone marrow transplantation, although the immunologic significance of these findings is unknown. The CD8 T cells also demonstrated increased expression of HLA-DR. HLA-DR is upregulated and becomes detectable following T-cell activation and thus, the identification of HLA-DR positive T cells suggests in vivo activation. Soluble IL-2R rises in serum after T-cell activation and elevated levels of soluble IL-2R were observed in those CVID patients that also had increased HLA-DR expression. In selected patients with elevated soluble receptor there was also increased surface CD25 (IL-2R) expression on circulating lymphocytes. These observations establish that the expanded CD8/CD57 T-cell subpopulation in the CVID patients appears to have been activated in vivo. Elevation of HLA-DR expressing lymphocyte subpopulations have been described in a number of immunologically associated diseases, including autoimmune disorders (eg, systemic lupus erythematosus, multiple sclerosis, giant cell arteritis), graft-versus-host disease, aplastic anemia, and type I diabetes.

The clinical features of the two CVID patient subgroups differed significantly. Approximately 71% of the patients with the abnormal phenotypic pattern had splenomegaly and almost half of this subgroup had evidence of depressed in vivo T-cell response to recall antigens. In addition, there appeared to be an increase in granuloma formation among these patients. However, the true incidence of granuloma is uncertain in light of the requirement for a biopsy to establish this diagnosis, as well as the possibility for sampling error. Thus, there appears to be a correlation between altered T-cell subpopulations and T cell functional abnormalities in CVID. Taken together, our data provide evidence for at least two different CVID patient subgroups based on T-cell immunophenotype and clinical features.

Table 2. Selected Clinical Features of CVID Patients

<table>
<thead>
<tr>
<th>Incidence</th>
<th>CD4/CD8 &gt; 0.9</th>
<th>CD4/CD8 ≤ 0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenomegaly</td>
<td>7/24</td>
<td>10/14*</td>
</tr>
<tr>
<td>Anergy</td>
<td>1/15</td>
<td>5/12†</td>
</tr>
<tr>
<td>Granuloma</td>
<td>4/24</td>
<td>4/14</td>
</tr>
<tr>
<td>Ig replacement</td>
<td>18/24</td>
<td>12/14</td>
</tr>
<tr>
<td>GI infection</td>
<td>10/24</td>
<td>2/14</td>
</tr>
</tbody>
</table>

*P < .02 between the two CVID patient groups.
†P < .05 between the two CVID patient groups.

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REFERENCES


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Characterization of common variable immunodeficiency: identification of a subset of patients with distinctive immunophenotypic and clinical features [see comments]

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