

ORIGINAL ARTICLES

Elevated levels of activated CD4 T cells in common variable immunodeficiency: association with clinical findings

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ABSTRACT

Background. Common variable immunodeficiency (CVID) is a very heterogeneous syndrome defined by impaired immunoglobulin production. The primary defect remains unknown, but many reports describe peripheral blood T and B lymphocyte dysfunctions in a substantial proportion of CVID patients. Immunophenotypic alterations on memory B lymphocytes correlate with clinical findings. A B-cell-oriented classification principle of the patients has been proposed.

Methods and results. We investigated the expression of activation surface molecules on CD4 and CD8 T-cells from 14 patients with CVID, 6 non-CVID hypogammaglobulinemic patients with recurrent infections, 47 asymptomatic HIV-positive patients without AIDS defining conditions and 23 healthy subjects. Lymphocyte subsets were analysed by three-colour flow cytometry. Monoclonal panel: CD38-FITC/HLADR-PE/CD4 or CD8-PerCP. In CVID patients serum levels of CD4 T-cells co-expressing the activation marker HLA-DR [CD4+ DR + (34 %),

CD4+ CD38+ DR+ (18 %)] were significantly elevated compared with controls. Significant increases in CD8+ DR+ (54 %), CD8+ CD38+ (43 %) and CD8+ CD38+ DR+ (29 %) T-cells were observed in comparison with healthy controls. CVID patients with splenomegaly, lower pre-infusion IgG levels (< 600 mg/dl), autoimmune or lymphoproliferative conditions demonstrated even higher levels of CD4+ CD38+ DR+ T cells (22, 22, 21 and 21 % respectively) compared with other CVID patients (13, 13, 15 and 15 % respectively).

Conclusion. These findings indicate a state of ongoing T lymphocyte activation which is associated with clinical findings frequently observed in CVID.

Key words: Immunodeficiency. Common variable immunodeficiency. CD4. Lymphocyte activation. Classification.

INTRODUCTION

Common variable immunodeficiency (CVID) is the most clinically relevant primary antibody deficiency in the adult age¹⁻². Hypogammaglobulinemia and defective antibody production are the hallmark of CVID syndrome, predisposing patients to recurrent bacterial infections. An increased frequency of lymphoproliferative disease, splenomegaly, granulomatosis and or autoimmune diseases is also observed in CVID patients¹⁻³. Many reports describe peripheral blood T and B lymphocyte dysfunctions in a substantial proportion of CVID patients⁴⁻⁵. Recent studies

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point toward defects at various stages of B-cell differentiation in CVID subgroups and support the value of a B-cell-oriented classification principle⁶⁻¹². A good correspondence between a low number of memory B cells and splenomegaly, lymphoid proliferation, granulomatous disease and/or autoimmune diseases has been suggested⁶⁻¹². In this study, we investigated if the alterations in peripheral T-cell activated lymphocyte subsets correlated with clinical findings in CVID patients.

MATERIAL AND METHODS

In a transversal descriptive study we analyzed activated CD4+ and CD8+ T cell-subset percentages in 14 CVID patients (21-68 years old, mean age 37.4 years, 8 men and 6 women). All patients had been diagnosed as having CVID based on diagnostic criteria for primary immunodeficiencies¹³. The clinical characteristics of the patients are summarized in table I. At the time of the immunological study all the patients were receiving substitutive therapy with intravenous immunoglobulin (IVIG), at a dose of 400 mg/kg every 3 weeks. Blood samples were obtained immediately before IVIG administration. Increased levels of lymphocyte activation markers can be observed during chronic infections. For that rea-

son, in this study we compared the percentages of lymphocyte activated subsets in CVID patients with the percentages observed in 47 asymptomatic HIV-positive patients without clinical criteria of AIDS; and with 6 non-CVID hypogammaglobulinemic patients who had chronic bacterial infections and who were receiving IVIG. We also compared lymphocyte subsets percentages in CVID patients against values observed in a health control group composed of 23 healthcare workers. Splenomegaly and lymphoid proliferation were confirmed by computed tomography. Increased catabolism of serum IgG antibodies was suspected in those patients that did not reached IgG levels greater than 600 mg/dl despite conventional dosis of IVIG. None of the patients had protein losing diseases. Patients with acute infections or opportunistic infections were not included in the study.

Activated CD4 and CD8 T-cell subsets were quantitated by three color flow cytometry (FACScan, Becton & Dickinson, San Jose, CA, USA). We enumerated T-cell subsets using FITC/PE/PerCP combinations of CD38/HLA-DR/CD4 or CD8 and isotype controls. Percentages of activated CD4 and CD8 T cell subsets are expressed as a percentage of total CD4 and CD8 lymphocytes. Details of adquisition and analysis of lymphocyte subsets have been reported elsewhere¹⁴. T-cell activated percentages were compared between groups using the non-parametric Mann-Whitney test.

Table 1
Summary of clinical characteristics of 14 CVID patients

Patient N.º	Infections	Splenomegaly	Autoimmune diseases	Lymphoid proliferation	IgG hipercatabolism
1	B,G,GI,O,P,S	+	+	+	+
2	P,C	+	-	-	-
3	P,G, GI	-	-	+	-
4	GI	-	+	+	-
5	P, GI, S, C	-	-	-	-
6	P, GI	-	+	-	-
7	P	+	+	+	+
8	P, GI	-	-	-	-
9	S, O, G	+	-	+	+
10	P, S, GI	-	-	+	+
11	B, P	-	-	-	-
12	-	-	+	-	-
13	P, S	-	-	-	-
14	P	-	-	-	-

B: bronchitis; G: giardiasis; GI: gastrointestinal; O: otitis; P: pneumonia; S: sinusitis; C: conjunctivitis; CD: celiac disease; AHA: autoimmune hemolytic anemia, ITP: idiopathic thrombocytopenic purpura; AD: autoimmune diabetes.

RESULTS

Mean concentration of serum IgG at the time of diagnosis of CVID was 127 mg/dl (15 to 441 mg/dl) and at the time of the immunological study it was 837 mg/dl (384 to 1320 mg/dl). Table II shows the mean percentages of the functionally distinct activated CD4 and CD8 T-cell subsets in the study groups. In CVID patients serum levels of CD4+ T-cells co-expressing the activation marker HLA-DR [CD4+ DR+ (34 %), CD4+ CD38+ DR+ (18 %)] were significantly elevated compared with all control groups. A tendency toward a negative correlation between the percentage of CD4+ CD38+ DR+ T-cells and the concentration of serum IgG at the time of the diagnosis of CVID was observed (Spearman Coefficient, $r = -0.48$ ($p = 0.08$)). CVID patients showed higher percentages of CD8 + DR + T-cells (54 %) than HIV-negative controls. Significant increases of CD8+ CD38+ (43 %) and CD8+ CD38+ DR+ (29 %) T-cells were observed in CVID patients as compared with healthy controls. CVID patients with splenomegaly, lower pre-infusion IgG levels (< 600 mg/dl), autoimmune or lymphoproliferative conditions demonstrated even higher levels of CD4 + CD38 + DR + T cells (22, 22, 21 and 21 % respectively) compared with other CVID patients (13, 13, 15 and 15 % respectively).

Interestingly enough, the group of CVID patients without any complication ($n = 5$) showed higher percentages of CD4 + DR + (27 %), CD4+ CD38+ DR+ (12 %), CD8+ DR+ (49 %) and CD8+ CD38+ DR+ (22 %) T-cells than healthy controls.

DISCUSSION

Recent studies have proposed a new classification of CVID patients using class-switched memory B-cell counts determined by flow cytometry in peripheral blood^{8-9,11}. CD27+ (memory) B lymphocytes can be divided into two distinct subpopulations: IgD-CD27+, which produces IgG, IgM or IgA (class-switched memory phenotype), and IgD+ CD27+, which predominantly produces IgM (non-class-switched memory phenotype). A good correspondence has been observed between a low number of memory B cells and splenomegaly, lymphoid proliferation and/or autoimmune diseases which are common complications in CVID patients^{1-2,8-9,11}.

Table II

Percentages of functionally distinct activated CD4+ and CD8 + lymphocyte subsets

Lymphocytes	*CVID	Non CVID	HIV+	Healthy controls
CD4+ HLADR+	34 ± 3 ^{a,b,c}	19 ± 3	19 ± 2	9 ± 2
CD4+ CD38+	45 ± 4 ^b	45 ± 8	57 ± 2	44 ± 2
CD4+ CD38+ DR+	18 ± 2 ^{a,b,c}	10 ± 2	10 ± 1	4 ± 1
CD8+ HLADR+	54 ± 5 ^{a,c}	36 ± 5	51 ± 2	18 ± 2
CD8+ CD38+	43 ± 5 ^{b,c}	29 ± 9	62 ± 2	30 ± 3
CD8+ CD38+ DR+	29 ± 5 ^{b,c}	16 ± 4	38 ± 2	6 ± 1

Percentages of activated CD4 and CD8 T-cell subsets are expressed as a percentage of total CD4 and CD8 T-lymphocytes.

^aSignificantly higher percentages than non-CVID hypogammaglobulinemic patients

^bSignificantly higher percentages than asymptomatic HIV+ patients.

^cSignificantly higher percentages than healthy controls.

CVID: common variable immunodeficiency patients; Non CVID: hypogammaglobulinemic without diagnostic criteria of CVID; HIV+: asymptomatic HIV+ patients.

Several T-cell abnormalities have also been observed in CVID patients including decreased lymphocyte proliferation in response to mitogens and antigens, a tendency towards increased T-cell apoptosis and elevated levels of T-cell activation markers^{12,15-20}. However, few studies have correlated T-cell functional abnormalities with clinical findings^{12,16,20}. Studies performed in acquired states of immunodeficiency, such as in HIV infection, have demonstrated that the increase in the percentages of CD4 and CD8 T-cell activated subsets provided additional independent prognostic information on the progression of the disease¹⁴. We hypothesized that increased levels of CD4 and CD8 T-cells might be associated with clinical findings commonly observed in CVID.

Previous studies have compared the values of the functionally distinct subsets of T-cells observed in CVID patients with healthy controls. Since it is not easy to completely exclude the presence of infections in CVID patients, in this study we have compared the percentages of CD4 and CD8 T-cell activated subsets in CVID patients with the percentages observed in patients with a viral chronic infection (HIV + asymptomatic patients) or with chronic bacterial infections (non-CVID hypogammaglobulinemic patients with bacterial infections). Interestingly, CVID patients disclosed significantly higher percentages of activated CD4 T-cells than controls with chronic infections, which suggest that CD4 lymphocyte acti-

vation does not merely reflect a chronic infection state in CVID patients. Furthermore, when we split our patients into two groups according to the presence or absence of complications (splenomegaly, autoimmune disease and/or lymphoid proliferation), we found higher percentages of CD4+ CD38+ DR+ T-cells in CVID patients with such complications. In addition to previous studies, we have observed significantly higher percentages of CD4+ CD38+ DR+ T-cells in patients with suspected IgG hypercatabolism.

CVID patients showed increased percentages of activated CD8 T-cell subsets than healthy controls. It has been recently reported that the expression of the HLA-DR molecule on CD8 T-cells was restricted to patients with clinical complications²⁰. In our study we observed higher percentages of CD8+ DR+ in CVID patients with clinical complications (splenomegaly, autoimmune disease and/or lymphoid proliferation), but the difference was not significant (data not shown). Since the percentage of CD8+ DR+ T-cells in CVID patients was similar to that observed in HIV positive controls, we speculate that a subclinical or undetected infection could at least partially explain the higher percentage of activated CD8+ T cells observed.

The results presented in this study indicate a state of ongoing T lymphocyte activation which is associated with clinical findings frequently observed in CVID patients. Further studies with a greater number of patients are necessary to establish if the evaluation of CD4 and CD8 T-cell activated subsets might be useful to identify subgroups of patients for etiological studies and classification.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Jose Miguel Benito for performing lymphocyte subset analysis of HIV + and healthy controls. They also acknowledge Dr. Juana Gil (Cellular Immunity Section) and Dr Margarita Rodriguez-Mahou (Autoimmunity Section) for laboratory diagnostic evaluation of the patients.

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