Brief report

Abnormal interleukin-7 function in common variable immunodeficiency

Are Martin Holm, Pål Aukrust, Jan Kristian Damås, Fredrik Müller, Bente Halvorsen, and Stig S. Frøland

Common variable immunodeficiency (CVID) is characterized by low levels of circulating immunoglobulins, leading to frequent infections, particularly of the respiratory tract. Frequently, T-cell abnormalities are observed. Interleukin-7 (IL-7) is involved in the homeostasis of lymphocytes, and may be elevated in lymphopenia. Mutations of genes related to IL-7 may lead to severe immunodeficiency disorders. We report elevated plasma levels of circulating IL-7 in a subgroup of CVID. These patients have increased numbers of circulating $CD8^+$ T cells with decreased apoptosis and a predominance of CC chemokine receptor 7^- ($CCR7^-$) effector-memory T cells. Moreover, in some of these patients there is impaired response to IL-7 as assessed by in vitro proliferation and secretion of interferon γ and transforming growth factor β. These findings suggest novel pathogenic mechanisms and specific targets for further research in CVID. (Blood. 2005;105: 2887-2890)

Introduction

Common variable immunodeficiency (CVID) is a heterogeneous group of immunodeficiency disorders,[1] characterized by deficient B-cell function. However, about half the cases show signs of T-cell deficiency,[2] possibly contributing to the defective antibody production.

Interleukin-7 (IL-7) plays a critical role in lymphocyte development and homeostasis.[3] High plasma levels of IL-7 have been correlated with low $CD4^+$ T-cell counts (eg, in HIV infection), reflecting a homeostatic response to T-cell depletion.[4-6] However, high IL-7 levels may also lead to autoimmunity and ectopic lymphocyte infiltrates, as suggested in animal models examining the effects of exogenous IL-7 administration.[7] Deficiencies of the IL-7 receptor α chain (IL-7Rα) or the γ common chain (γc) may lead to severe combined immunodeficiency (SCID). Interestingly, a partial IL-7Rα deficiency results in a delayed clinical presentation of immunodeficiency, and may additionally cause hypogammaglobulinemia.[8]

Although IL-7 seems to be crucially involved in the function of human lymphocytes and defects in the IL-7 signaling system in humans have been described, the role of IL-7 in CVID has not been studied. Herein, we examine the expression of IL-7 and its role in T-cell function in CVID.

Study design

Patients

Patients with CVID[1] without clinically apparent infections were included (Table 1). Healthy donors were included as controls. Informed consent was obtained from all subjects before inclusion. The study was conducted according to the ethical guidelines at our hospital and the Helsinki declaration, and approved by the Rikshospitalet University Hospital's authorized representative.

Cell isolation and cultures

Negative selection of $CD3^+$ T cells was performed as previously described.[9] The $CD3^+$ T cells were resuspended in RPMI 1640 with 2 mM L-glutamine and 25 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) buffer (Gibco, Paisley, United Kingdom) supplemented with 10% fetal calf serum (FCS) and incubated in 96-well plates (10^6 cells/mL). The cells were stimulated with anti-CD3 (SpvT3b; final concentration, 40 ng/mL for optimal stimulation; suboptimal stimulation, 10 ng/mL; Zymed, San Francisco, CA), anti-CD28 (clone 15E8; CLB, Amsterdam, the Netherlands; final concentration 50 ng/mL for optimal stimulation; not added for suboptimal stimulation) with and without IL-7 (R&D Systems, Oxon, United Kingdom) and cross-linked.[10] For cytokine and lactate-dehydrogenase (LDH) analyses, cell-free supernatants were harvested after 24 (interferon γ [IFNγ] and IL-2) and 48 (transforming growth factor β1 [TGFβ1]) hours. Proliferation analyses were performed as previously described.[11]

Flow cytometry

Absolute numbers of circulating lymphocyte subsets were assessed using TruCount and TriTEST (Becton Dickinson, San Diego, CA). Other staining for flow cytometry was performed using fluorescein isothiocyanate (FITC)–conjugated anti-CD4; phycoerythrin (PE)–conjugated anti–IL-7Rα and anti-Ki67; peridinin chlorophyll-alpha protein (PerCP)–conjugated anti-CD3 and allophycocyanin (APC)–conjugated anti-CD8 (Becton Dickinson); and FITC-conjugated anti–CC chemokine receptor 7 (CCR7; R&D Systems). Apoptosis was estimated using annexin V/propidium-iodide staining (Becton Dickinson). Flow cytometry was performed as previously described.[10]

Cytokine and LDH assay

Cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA; R&D Systems). LDH activity was measured using Cytotoxicity Detection Kit (LDH; Roche-Applied-Science, Basel, Switzerland).

From the Section of Clinical Immunology and Infectious Diseases, Medical Department; the Research Institute for Internal Medicine; and the Department of Microbiology, Rikshospitalet/The National Hospital, Oslo, Norway.

Submitted June 28, 2004; accepted November 30, 2004. Prepublished online as *Blood* First Edition Paper, December 14, 2004; DOI 10.1182/blood-2004-06-2423.

Supported by the Norwegian Research Council.

Reprints: Are Martin Holm, Research Institute for Internal Medicine, The National Hospital, N-0027 Oslo, Norway; e-mail: a.m.holm@klinmed.uio.no.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

Table 1. Characteristics of CVID patients

No.	pI.IL-7, pg/mL	Age, y	Sex	Age at onset, y	Splenomegaly	Bronchiectasis	Autoimmune phenomena	Leukocytes, 10^6/mm^3		
								CD4	CD8	CD19
1	11.1	60	M	44	0	0	1	289	1486	47
2	12.4	52	M	45	0	0	1	922	2224	527
3	12.2	29	M	50	0	0	1	1107	600	199
4	12.4	38	M	3	1	1	1	632	199	124
5	16.3	31	M	13	1	1	0	1950	2396	442
6	12.4	30	M	10	0	0	0	915	595	336
7	10.9	63	M	10	1	1	0	1579	3979	21
8	14.9	22	M	12	1	1	1	3638	1629	1641
9	11.0	48	M	47	0	1	1	827	612	689
10	11.0	53	W	10	0	0	0	1046	1837	328
11	11.4	23	M	3	1	1	1	309	591	2
12	11.2	30	M	4	1	1	0	366	213	0
13	13.2	39	W	23	0	1	1	726	1219	30
14	7.3	70	M	27	1	1	1	225	722	106
15	1.9	26	W	19	1	1	1	1060	512	209
16	5.1	51	M	4	0	0	0	570	2297	153
17	1.1	64	W	52	0	1	0	385	520	185
18	4.9	57	W	31	1	1	0	731	295	30
19	3.1	43	W	40	0	1	1	1224	636	238
20	1.9	42	W	20	1	1	1	708	237	220
21	3.0	40	M	38	1	1	0	693	275	550
22	4.2	66	M	60	0	1	0	655	489	105
23	3.6	54	M	21	0	1	1	527	392	20
24	1.9	33	M	21	0	0	0	288	204	192

Individual data for all CVID patients selected for in vitro analyses (n = 24; note that not all analyses were performed on all patients due to limited amounts of available material).

Splenomegaly is verified by computed tomography (CT) scan or ultrasonographic examination; bronchiectasis is detected using high-resolution CT.

M indicates men; W, women.

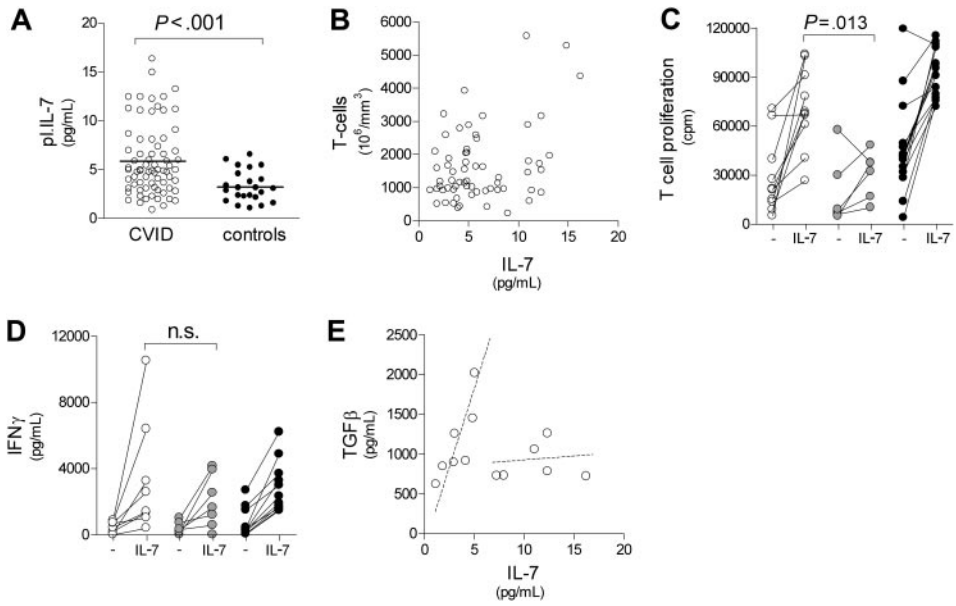

Figure 1. Elevated plasma levels and aberrant response to IL-7 in CVID. (A) Individual levels of plasma IL-7 (pg/mL) in 72 CVID patients (○) and 23 healthy controls (●). Horizontal lines indicate median values (median (25%-75%) for CVID, 4.9 pg/mL [3.05-7.65 pg/mL] and for controls, 3.0 pg/mL [2.15-4.85 pg/mL]). (B) The absent correlation between plasma IL-7 and numbers of circulating T cells in CVID patients (n = 72). (C) The effects of IL-7 (10 ng/mL) on in vitro proliferation in suboptimally stimulated (anti-CD3 [10 ng/mL]) T cells from CVID patients with normal plasma levels of IL-7 (n = 10; ○), CVID patients with high plasma levels of IL-7 (ie, > 10 pg/mL, n = 6; ◍), and healthy controls (n = 13; ●). The y-axis shows scintillation counts per minute (cpm; see "Study design"). (D) The effects of IL-7 (10 ng/mL) on in vitro secretion of IFNγ in suboptimally stimulated (anti-CD3 [10 ng/mL]) T cells from CVID patients with normal (n = 8) or elevated (ie, > 10 pg/mL; n = 7) plasma levels of IL-7 and healthy controls (n = 11); symbols as in panel C. n.s. indicates not significant. (E) Secreted levels of TGFβ1 in T cells from CVID patients (n = 13) stimulated with anti-CD3 [40 ng/mL], anti-CD28 [50 ng/mL], and IL-7 [10 ng/mL] related to plasma levels of IL-7. Note the positive correlation in patients with normal pI.IL-7 (r = 0.96; *P* = .003) and the absent correlation in patients with high pI.IL-7 (indicated by dotted lines).

Table 2. Characteristics of CVID patients with high, intermediate, and low pl.IL-7

	Age, y	Women, %	Age at onset, y	Splenomegaly, %	Bronchiectasis, %	Autoimmune phenomena, %	Leukocytes, 10^6/mm^3		
							CD4	CD8	CD19
High pl.IL-7 (> 10 pg/mL), n = 13	37	18	21	55	73	73	1190	1261	347
Intermediate pl.IL-7 (6.5-10 pg/mL), n = 11	38	45	22	36	45	18	580	564	161
Normal pl.IL-7 (< 6.4), n = 47	44	51	26	32	40	30	702	777	262

Summarized data for CVID patients stratified according to plasma levels of IL-7.

Statistical methods

Data are presented as median [25th-75th percentile]. The 2-tailed Mann-Whitney *U* test, Wilcoxon signed-rank test, chi-square test, and Spearman signed rank test were used as appropriate. Tests were considered significant when *P* is less than .05.

Results and discussion

We found significantly higher plasma levels of IL-7 in CVID patients (n = 72) compared with healthy controls (n = 23), with particularly high levels in 13 patients (> 10 pg/mL; hereafter termed CVID$^{IL\text{-}7high}$; Figure 1A). Except for a higher proportion of males in the CVID$^{IL\text{-}7high}$ group ($P = .026$), there was no association between IL-7 levels and clinical characteristics of the study group (Table 2). In contrast to previous reports in T-cell–depleted individuals, we did not find any correlation between IL-7 levels and CD3$^+$ or CD4$^+$CD3$^+$ T-cell counts in CVID (Figure 1B). In fact, the 13 CVID$^{IL\text{-}7high}$ patients had significantly higher CD8$^+$ T-cell counts than the remaining patients (1043 [299-2040] vs 531 [353-722] CD3$^+$CD8$^+$ cells/mm^3; $P = .02$). There were no different proportions of CD45RA$^+$ T cells, but the predominance of CCR7$^-$ effector-memory T cells[11,12] was significantly stronger in the CVID$^{IL\text{-}7high}$ patients (88% [75%-90%] vs 68% [45%-90%] CCR7$^-$ of CD3$^+$ cells; $P = .01$). There was no difference in the expression of the proliferation marker Ki67 between CVID and controls or between the CVID$^{IL\text{-}7high}$ and the remaining patients, indicating that the increased numbers of CD8$^+$ T cells were not due to increased proliferation in vivo. However, the proportion of apoptotic cells after in vitro isolation correlated inversely to IL-7 levels in the CVID patients ($r = -0.080$; $P = .055$), suggesting a protective effect of IL-7. There was no difference between CVID patients and controls in the expression of IL-7Rα on T cells, and there was no association between in vitro responses of IL-7 in proliferation or cytokine assays and the expression of IL-7Rα.

In further in vitro analyses of 16 randomly selected CVID patients, we found an inverse correlation between IL-7 levels and in vitro T-cell proliferation in the CVID$^{IL\text{-}7high}$ patients ($r = -0.55$, $P = .034$). While IL-7 had no effect on T-cell proliferation when given alone or in combination with optimal stimulation in patients or controls, it strongly enhanced proliferation in suboptimally stimulated T cells in healthy controls (Figure 1C). In contrast, while there was a heterogeneous response among CVID patients with normal IL-7 levels, none of the CVID$^{IL\text{-}7high}$ patients reached similar levels (Figure 1C).

While the addition of IL-7 in vitro increased IFNγ secretion in optimally stimulated T cells (fold change, ~ 2), there was a particularly strong response to IL-7 in suboptimally stimulated T cells in both CVID patients and controls (fold change, ~ 12; Figure 1D). Notably, in the CVID group there was a significant correlation between IL-7–stimulated proliferation and IFNγ response (fold change in suboptimally stimulated T cells; $r = 0.71$, $P = .01$), and all 4 individuals with poor IL-7 response in IFNγ secretion also had a poor IL-7 response in the proliferation assay at suboptimal stimulation.

We found that T cells from CVID patients spontaneously secreted less TGFβ1 than cells from healthy controls. However, while adding IL-7 significantly increased TGFβ1 secretion in T cells from healthy controls in all conditions, no consistent effect of IL-7 was seen in CVID, although again, the effect varied within the CVID group. Interestingly, TGFβ1 has been demonstrated to inhibit secretion of IL-7 by bone marrow stromal cells,[13] indicating a possible feedback mechanism from T cells for regulation of IL-7.[14] While there was a positive correlation between plasma levels of IL-7 and in vitro secretion of TGFβ1 in patients with normal levels of IL-7, this correlation was disrupted in the CVID$^{IL\text{-}7high}$ patients (Figure 1E), suggesting a deficient feedback mechanism for IL-7 secretion in these patients.

Finally, the impaired responses to IL-7 in T-cell proliferation and IFNγ secretion were not due to increased in vitro cell death as assessed by LDH levels in cell culture supernatants. In fact the LDH concentration was lower in CVID$^{IL\text{-}7high}$ patients suggesting protective effects of IL-7.

We describe a subgroup of CVID patients with high plasma levels of IL-7, associated with elevated numbers of CD8$^+$ T cells and increased proportion of terminally differentiated effector-memory T cells[11,12] that do not show signs of increased proliferation, but appear to have reduced apoptosis. Some of these patients also have reduced response to IL-7 in vitro. Our observations suggest abnormalities in IL-7–mediated lymphocyte homeostasis in some CVID patients, possibly involving mechanisms such as abnormal signaling pathways (similar to certain forms of SCID),[8] or impaired feedback to IL-7–producing stroma cells due to primary T-cell defects.[11,15-18] IL-7 also plays a role in APC-mediated T-cell stimulation.[3] Thus, the IL-7 dysfunction may also be involved in the defective dendritic cell function recently described in CVID.[19,20] Considering the role of IL-7 in both development and peripheral homeostasis of lymphocytes,[3] it is conceivable that the abnormal IL-7 function as demonstrated in this study may be involved in the skewing of lymphocyte subsets and in the development of an impaired T- and B-cell function in some CVID patients.

References

1. Rosen FS, Eibl MM, Roifman CM, et al. Primary immunodeficiency diseases: report of an IUIS Scientific Committee: International Union of Immunological Societies. Clin Exp Immunol. 1999; 118(suppl 1):1-28.
2. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999;92:34-48.
3. Fry TJ, Mackall CL. Interleukin-7: from bench to clinic. Blood. 2002;99:3892-3904.
4. Napolitano LA, Grant RM, Deeks SG, et al. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. Nat Med. 2001;7:73-79.
5. Bolotin E, Annett G, Parkman R, Weinberg K. Serum levels of IL-7 in bone marrow transplant recipients: relationship to clinical characteristics

and lymphocyte count. Bone Marrow Transplant. 1999;23:783-788.

6. Fry TJ, Connick E, Falloon J, et al. A potential role for interleukin-7 in T-cell homeostasis. Blood. 2001;97:2983-2990.
7. Storek J, Gillespy T III, Lu H, et al. Interleukin-7 improves CD4 T-cell reconstitution after autologous CD34 cell transplantation in monkeys. Blood. 2003;101:4209-4218.
8. Roifman CM, Zhang J, Chitayat D, Sharfe N. A partial deficiency of interleukin-7R alpha is sufficient to abrogate T-cell development and cause severe combined immunodeficiency. Blood. 2000;96:2803-2807.
9. Aandahl EM, Aukrust P, Skalhegg BS, et al. Protein kinase A type I antagonist restores immune responses of T cells from HIV-infected patients. FASEB J. 1998;12:855-862.
10. Holm AM, Aukrust P, Aandahl EM, et al. Impaired secretion of IL-10 by T cells from patients with common variable immunodeficiency-involvement of protein kinase a type I. J Immunol. 2003;170:5772-5777.
11. Holm AM, Sivertsen EA, Tunheim SH, et al. Gene expression analysis of peripheral T cells in a subgroup of common variable immunodeficiency shows predominance of CCR7 effector-memory T cells. Clin Exp Immunol. 2004;138:278-289.
12. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999;401:708-712.
13. Tang J, Nuccie BL, Ritterman I, et al. TGF-beta down-regulates stromal IL-7 secretion and inhibits proliferation of human B cell precursors. J Immunol. 1997;159:117-125.
14. Fry TJ, Mackall CL. Interleukin-7: master regulator of peripheral T-cell homeostasis? Trends Immunol. 2001;22:564-571.
15. Jaffe JS, Strober W, Sneller MC. Functional abnormalities of CD8+ T cells define a unique subset of patients with common variable immunodeficiency. Blood. 1993;82:192-201.
16. Grimbacher B, Hutloff A, Schlesier M, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. Nat Immunol. 2003;4:261-268.
17. North ME, Webster AD, Farrant J. Primary defect in CD8+ lymphocytes in the antibody deficiency disease (common variable immunodeficiency): abnormalities in intracellular production of interferon-gamma (IFN-gamma) in CD28+ ('cytotoxic') and CD28- ('suppressor') CD8+ subsets. Clin Exp Immunol. 1998;111:70-75.
18. Serrano D, Becker K, Cunningham-Rundles C, Mayer L. Characterization of the T cell receptor repertoire in patients with common variable immunodeficiency: oligoclonal expansion of CD8(+) T cells. Clin Immunol. 2000;97:248-258.
19. Bayry J, Lacroix-Desmazes S, Kazatchkine MD, et al. Common variable immunodeficiency is associated with defective functions of dendritic cells. Blood. 2004;104:2441-2443.
20. Scott-Taylor TH, Green MR, Eren E, Webster AD. Monocyte derived dendritic cell responses in common variable immunodeficiency. Clin Exp Immunol. 2004;138:484-490.