Common variable immunodeficiency is associated with defective functions of dendritic cells

Running Head: Dendritic cells are defective in CVID patients

Jagadeesh Bayry, Sébastien Lacroix-Desmazes, Michel D. Kazatchkine, Lionel Galicier, Yves Lepelletier, David Webster, Yves Lévy, Martha M. Eibl, Eric Oksenhendler, Olivier Hermine, Srini V Kaveri

INSERM Unité 430 and Université Pierre et Marie Curie, Institut des Cordeliers, Paris, France; Department of Clinical Immunopathology, Hôpital Saint Louis, Paris, France; Department of Hematology and Centre National de la Recherche Scientifique Unité Mixte de Recherche 8603, Hôpital Necker, Paris, France; Department of Immunology, Royal Free Medical School, London, United Kingdom; Service d’Immunologie Clinique, Hôpital Henri Mondor, Créteil Cedex, France and Immunology Outpatient Clinic, Schwarzspanierstrasse, Vienna, Austria

Supported by Institut National de la Santé et de la Recherche Médicale (INSERM) and Centre National de la Recherche Scientifique (CNRS) France, by a grant from ZLB Bioplasma AG, Bern, Switzerland and Octapharma, Austria. J.B is a recipient of fellowship from the Fondation de la Recherche Médicale (France).

Correspondence to:
Srini V Kaveri,
INSERM U430,
Institut des Cordeliers,
15, rue de l’Ecole de Médecine
75006 Paris, France.
Tel: + 33 1 55 42 82 67
Fax: + 33 1 55 42 82 62
E-mail: srini.kaveri@u430.bhdc.jussieu.fr

Word count: 1224
Abstract: 149
Scientific heading: Immunobiology
Abstract
Common variable immunodeficiency (CVID) is characterized by hypogammaglobulinemia and defects in T-cell functions that could be primary or secondary. We addressed whether CVID is associated with impairment in the dendritic cell (DC) compartment, as DC play a central role in the development of adaptive immunity. We demonstrate that DC from CVID patients display severely perturbed differentiation, maturation and function, and expressed markedly reduced levels of the co-stimulatory molecules that are critical for T-cell stimulation. Patients’ DC induced weak proliferation of allogeneic T-cells and produced significantly low amounts of IL-12 upon CD40 signalling. Multiple defects in the immune system, including malfunctioning of DC, appear to be prominent features of CVID patients. Impairment in both the innate and adaptive compartments of the immune system may thus cumulatively account for the inability of CVID patients to eradicate pathogens through conventional immune pathways thus resulting in an increased risk for recurrent bacterial infections.

E-mail of corresponding author: srini.kaveri@u430.bhdc.jussieu.fr
**Introduction**

Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by hypogammaglobulinemia, and a number of T- and B-cell defects. The fact that some patients’ B-cells can secrete IgM and IgG in vitro, while patients being hypogammaglobulinemic in vivo, implies that B-cells may not receive appropriate signalling for class switch and affinity maturation and that perturbed cellular interactions in germinal centres may be involved in the pathogenesis of the disease. Under physiological conditions, immune responses are initiated in the T-cell areas of secondary lymphoid organs, where naive T-cells encounter dendritic cells (DC). DC-derived cytokines play a crucial role in the cascade of events leading to priming of naive T-cells. The T-helper cells in turn induce B-cell growth and antibody production. However, direct interactions between DC and B-cells also occur. As DC play a critical role in bridging innate and adaptive immunity, we addressed the issue of whether CVID is associated with an impaired DC compartment.

**Study design**

*Patients and dendritic cells*

Heparinized blood samples were collected from ten CVID patients at least 21 days following the last infusion of intravenous immunoglobulin (IVIg) (patients 1 to 10), and from two newly diagnosed naïve CVID patients prior to IVIg therapy (patients 11 and 12), upon approval by local ethical committees. CVID patients were heterogeneous in clinical presentation and were associated with recurrent pneumonia, granulomatous disease, lamblia or autoimmune diseases (Supplementary Table 1). As control, blood samples were obtained from three patients (patients 1, 2 and 3) with selective antibody deficiencies (deficiency in IgG4; IgG2 and IgG4; and IgG4 and IgA respectively) and one patient with hyper IgM syndrome (patient 4) who received IVIg similar to CVID patients and from six healthy controls. Monocyte-derived DC from patients’ blood and from control groups were generated as described with 10% autologous plasma. For allogeneic mixed lymphocyte reaction (MLR), DC from CVID patients and healthy donors were exposed to CD4+ T-cells of third party healthy donors. The CD4+ T-cells were isolated by MACS cell isolation kit (Miltenyi Biotech, Bergisch Gladbach, Germany).

**Results and Discussion**
DC of CVID patients expressed lower levels of CD1a (15.7 ± 10.6%) as compared with DC of healthy donors (46.1 ± 18.4%) and of control patients (42.3 ± 15.8%). The percentage of DC from CVID patients expressing CD83 and CD80 was also significantly lower than in control groups (P≤0.01, Mann-Whitney test) (Figure 1A and 1B). The expression of HLA-DR (mean fluorescence intensity, MFI: 131.6 ± 58.4), CD11c (224.4 ± 150.7) and CD40 (160.6 ± 72.4) (Figure 1C) on CVID patients’ DC was also significantly lower than that of DC from healthy donors (n=6) (201.5 ± 108.5; 397.7 ± 189.8 and 269.5 ± 112.9 for HLA-DR, CD11c and CD40 respectively) and control patients (n=4) (312.5 ± 182.7; 262.5 ± 27.4 and 323.8 ± 240.6 for HLA-DR, CD11c and CD40 respectively) (P<0.05; Mann-Whitney test) indicative of an impaired differentiation of DC in patients with CVID. The defective differentiation of DC from CVID patients was also observed in the presence of AB serum or serum-free medium, thus excluding the possible influence of patients’ plasma components on differentiation. The defects in DC were not due to IVIg replacement therapy since defects were also observed in newly diagnosed naïve CVID patients, and in patient having received IVIg six months prior to obtaining blood sample (patient 8). In addition, lymphoid DC of CVID patients also presented with down-regulated CD86 and HLA-DR expression while other markers were not altered (not shown).

A major function of DC is their ability to trigger the activation and proliferation of T-cells. DC from CVID patients displayed a markedly weaker stimulatory effect on allogeneic T-cells than DC from healthy donors (Figure 2A). To further investigate the functional properties of CVID patients’ DC, we tested their ability to mature and produce IL-12 upon stimulation with CD40L-transfected fibroblasts. There was a striking up-regulation of markers on DC from control patients (Figure 2B) whereas DC from CVID patients failed to up-regulate the maturation markers and co-stimulatory molecules (Figure 2B). We then measured the secretion of bioactive IL-12 (p70) from DC of CVID patients upon stimulation with CD40L. A significantly low amount of IL-12 was produced by DC of CVID patients as compared to healthy donors and control patients (Figure 2C). Defective IL-12 production was observed in DC of CVID patients whether naïve or under IVIg therapy. Together, our results demonstrate that despite heterogeneous clinical presentation, CVID is generally associated with defective DC functions. Although our results are in contrast to a previous report indicating an enhanced proportion of intracellular IL-12-positive monocytes in CVID patients, the discrepancies could be due to differences in the
methodologies (p70 versus p40; secretory versus intracellular IL-12; and isolated DC versus whole PBMC) or the stimuli used in the assays. As the expression of markers on DC can be modulated by several cytokines, we analyzed whether IL-10 was responsible for defective differentiation of DC from CVID patients. Although CVID patients’ DC produced small amounts of IL-10 (69 ± 93 pg/mL, n=6; Quantikine® kit, Immunotech, Marseilles, France), the addition of neutralizing anti-IL-10 mAbs (2 g/mL) during differentiation only marginally restored the phenotypes on DC. Furthermore, stimulation of cells with CD40 mAb (4 g/106 cells) or with several-fold higher concentration of GM-CSF and IL-4 during differentiation did not restore the normal phenotypes of DC from CVID patients. These results indicate that IL-10 may not be the exclusive factor responsible for defective differentiation. Co-stimulatory molecules on DC play a critical role in the cascade of events leading to T-cell priming. IL-12 production by DC polarizes CD4+T-cells into IFN-γ-producing Th1 cells. IFN-γ activates the anti-microbial activities of macrophages and together with IL-12, promotes T-cell differentiation into cytotoxic T-lymphocytes. Various T-cell defects, including anergy, impaired proliferation, reduced expression of the CD40L and impaired production of cytokines IL-2, IL-4 and IFN-γ have been reported in CVID patients. A subgroup of CVID patients also presents with defective macrophage functions; and dysregulated T-cell function or macrophage activation have been implicated in the formation of granulomas in CVID patients. In the present study, although the patients displayed a normal or higher percent of T-cells, they showed a defective proliferation (not shown). It is observed that majority of CVID patients display normal T-cell numbers in their peripheral blood. Therefore, it is the impaired function of T-cells that may be responsible for the pathogenesis in these patients. In addition, we report on the diminished production of IL-12 by DC and reduced expression of CD40, CD80 and other co-stimulatory molecules involved in DC-T-cell cross-talk. We thus suggest that impairment in both the innate and adaptive compartments of the immune system cumulatively account for the inability of CVID patients to eradicate pathogens through conventional immune pathways. CVID patients exhibit reduced serum levels of all immunoglobulin isotypes predisposing patients to frequent bacterial infections of respiratory tract. In addition to T-cell-stimulation, DC regulate B-cell growth and immunoglobulin secretion. A direct interaction between CD40-activated DC and B-cells promotes the development of mucosal immunity. DC together with IL-2 stimulate CD40-activated germinal center B-cell proliferation and drive their differentiation towards plasma
cells. Thus, defective DC function along with impaired T-cell activity will have serious repercussion on the humoral immune response of CVID patients. Multiple defects in the immune system, including defective DC function, thus appear to be prominent features of CVID.
References

Figure Legends

**Figure 1.** Dendritic cells from CVID patients display impaired differentiation. (A) Differentiation of DC is impaired in CVID patients. Flow cytometric analysis of the expression of differentiation markers on the surface of six-day old monocyte-derived DC of healthy donors (upper panels), CVID patients (patient 1; middle panels) and selective antibody deficient patients (patient 1; lower panels). Percentage of cells that are positive for the indicated markers are depicted and mean fluorescence intensities are indicated in parenthesis. (B) Comparison of percentage of cells expressing CD1a, CD83 and CD80 in twelve CVID patients (open circles; numbers 1-12 within each circle correspond to patients’ number), six healthy donors (filled circles), three selective antibody deficient patients (open squares; numbers 1-3 within each square correspond to patients’ number) and one patient with hyper IgM syndrome (open squares with number 4) after differentiation of monocytes for six days. The mean values are indicated with a horizontal bar for each marker. Statistical significance as determined by the non-parametric Mann-Whitney test is indicated (*, P<0.01). (C) Variations among CVID patients with respect to the expression of DC maturation markers in six-day old DC. Each symbol in the figure denotes corresponding CVID patient. MFI: mean fluorescence intensity.

**Figure 2.** Dendritic cells from CVID patients display impaired functional properties. (A) Dendritic cells from CVID patients are impaired in their allogeneic CD4+ T-cell stimulatory capacity in an MLR, as measured by [3H]-thymidine uptake. Graded doses of DC were seeded with 1x10^5 responder T-cells in RPMI 1640 medium supplemented with 10% human AB serum. After 4 days, the cells were pulsed for 16 h with 1 µCi of [3H]thymidine. Radioactive incorporation was measured by standard liquid scintillation counting and results expressed as counts per minute (cpm, mean ± SD of triplicate values) after subtracting values from culturing stimulator cells alone. The level of [3H]-thymidine uptake by T cells in medium alone were in the range of 1320 ± 160 cpm and was less than 1000 cpm in stimulator cells alone when 10,000 cells were tested. (B) Dendritic cells from CVID patients display altered capacities for maturation. Six-day old DC from CVID patients (thick lines) and selective antibody deficient patients (thin lines) were stimulated with CD40L-transfected fibroblasts (10:1) for 48 hr. The mean fluorescence intensity for one of the four CVID patients (patient 5, bold numbers) and one of the three selective antibody deficient patients (patient 3, light numbers) analysed are indicated. (C) Dendritic cells from CVID patients (n=4, patients 3, 4, 5 and 12) produce significantly lower amounts of IL-12 as compared with that of DC from healthy donors (n=3) (filled bar) and selective antibody deficient patients (n=3, patients 2, 3 and 4) (open bar). Cytokine secretion was measured in a cell-free culture supernatant by Quantikine® kit (R&D Systems, Abingdon, Oxon, UK) following stimulation of cells with CD40L-transfected fibroblasts for 48 hr. The level of IL-12-production by above four CVID patients was in the range of 5 to 86 pg/mL/0.5x10^6 cells. DC of patient 3 produced 29 pg/mL, patients 4 and 5 produced ≤ 5 pg/mL and the cells of patient 12 secreted 86 pg/mL of IL-12 upon CD40 stimulation. Statistical significance as determined by the non-parametric Mann-Whitney test is indicated (*, P < 0.01).
Figure 1: Graphs showing the expression of various markers on dendritic cells from different patient groups.
Figure 2: Bayry et al.
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