Research article

Altered dendritic cell distribution in patients with common variable immunodeficiency

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Abstract

Recent data suggest a critical role for dendritic cells (DCs) in the generation of immunoglobulin-secreting plasma cells. In the work reported herein, we analyzed the frequency of peripheral blood plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) in a cohort of 44 adults with common variable immunodeficiency (CVID) classified according to their CD27 membrane expression status on B cells. A deep alteration in the distribution of DC subsets, especially of pDCs, in the peripheral blood of CVID patients was found. Patients with a reduced number of class-switched CD27+IgD-IgM- memory B cells and patients with granulomatous disease had a dramatic decrease in pDCs (P = 0.00005 and 0.0003 vs controls, respectively) and, to a lesser extent, of mDCs (P = 0.001 and 0.01 vs controls, respectively). In contrast, patients with normal numbers of switched memory B cells had a DC distribution pattern similar to that in controls. Taken together, our results raise the possibility that innate immunity contributes to pathogenesis in CVID.

Introduction

Common variable immunodeficiency (CVID) is a primary immunodeficiency disease characterized by a low concentration of serum immunoglobulins and an impaired antibody response to challenging antigens [1]. Although the pathophysiology of CVID is heterogeneous and largely unknown, several causes leading to an alteration of immunoglobulin concentrations in the blood have already been identified. These include a failure of B-cell maturation, including altered somatic hypermutation [2]; defective cell-membrane signaling [3]; T-cell abnormalities such as reduced expression of key membrane-expressed molecules (CD40 ligand, ICOS (inducible costimulator protein), L-selectin) [4]; impaired cytokine production [5]; and a reduced generation of antigen-specific memory T cells [6].

Whereas the antigen-presenting function of DCs has been reported to be normal in CVID [7,8], their involvement in the origin of some CVID cannot be ruled out, as these cells are known to interact directly with B cells, to present antigen to T cells, and to produce cytokines implicated in B-cell differentiation [9]. Two major pathways of differentiation generating DCs are thought to exist, according to their membrane expression of the β-integrin CD11c [10]. Myeloid DCs (mDCs) include skin Langerhans’ cells and interstitial DCs and express CD11c at their surface. In contrast, plasmacytoid DCs (pDCs), which do not express CD11c, are CD123+. Recent data suggest a role for DCs in B-cell growth and differentiation, as the release by mDCs of soluble factors such as IL-12 and IL-6 and/or membrane molecules such as BAFF/APRIL induces the activation and the differentiation of normal B cells [11,12]. In addition, the observation that pDCs directly induce the differentiation of plasma cells into immunoglobulin-secreting plasma cells suggests that pDCs are critically involved in humoral responses [13,14]. Altogether, these new data prompted us to examine the blood distribution of DC subsets in 44 patients with CVID.

CVID = common variable immunodeficiency; Ig = immunoglobulin; IL = interleukin; mDC = myeloid dendritic cell; pDC = plasmacytoid dendritic cell.
Materials and methods
Patient characteristics
Forty-four patients with CVID (17 to 77 years of age; 28 women and 17 men) were enrolled in this study after they had given their informed consent and following the approval of the local Ethics Committee. All patients were diagnosed as having CVID, on the basis of a specific medical history of recurrent bacterial infections associated with hypogammaglobulinemia (serum immunoglobulin (Ig)G and IgA and/or IgM at least two standard deviations below the normal mean) [15]. At the time of evaluation, none of the patients showed evidence of acute infection. As is frequently observed in CVID, 13 of the 45 patients had autoimmune diseases, 14 had splenomegaly, 5 had lymphoid hyperplasia, and 7 presented with a chronic granulomatous disease.

All the patients included in this study had blood CD19+CD3- B-lymphocyte counts above 1% of peripheral blood lymphocytes. The CVID patients were divided into two groups according to the detection of switched memory B cells (CD27+IgD-) as recently proposed by Warnatz and colleagues [16]. Group 1 (n = 22), comprising patients whose proportion of switched memory B cells was less than 0.4% of their total peripheral blood lymphocytes, was further subdivided according to whether they had increased (group 1a; n = 13) or normal (group 1b; n = 9) numbers of CD19+CD21- immature B cells. Group 2 (n = 15) comprised patients whose proportion of switched memory B cells was more than 0.4% of total peripheral blood lymphocytes. As Warnatz and colleagues excluded from their classification patients with granulomatous disease, we classified these patients in a distinct group (group 3; n = 7). Control patients were healthy Caucasian blood donors and health-care workers (n = 12, median 36 years; 8 women and 4 men); they were not matched with patients for sex or age.

Quantitation of blood DC precursors by flow cytometry
DC subsets were measured using the DC kit purchased from BD Biosciences (Pont-de-Clai, France). Samples were analyzed on a FACSCalibur flow cytometer (BD Biosciences) and 106 white blood cells were acquired. Peripheral blood mDC and pDC subsets were defined by the concomitant lack of lineage markers, HLA-DR expression, and mutually exclusive membrane expression of CD11c or CD123, respectively. Absolute numbers of blood DC precursor subsets were calculated as percentage of white blood cells or expressed per ml of peripheral blood. Membrane expression of chemokine receptors was assessed by flow cytometry using a triple combination of monoclonal antibodies: BDCA2-FITC (Milteny Biotech, Paris, France); and HLA-DR-PerCP; and CCR2-PE or CCR5-PE or CCR7-PE or CXCR3-PE or CXCR4-PE (BD Biosciences).

Statistical analysis
Median values found for blood cells were compared between groups using the nonparametric Mann–Whitney U test, with a level of significance of P = 0.05. The Spearman test was used to make correlations. The tests were performed with the statistical software Statistica Inc (Statsoft, Tucson, AZ, USA).

Results and discussion
The median absolute count of circulating pDCs was significantly lower in CVID patients than in controls (P = 0.002). Although CVID patients had a lower mean mDC count, their median absolute count was not statistically significantly different from that of controls (P = 0.1) (Fig. 1).

Table 1

| Absolute counts of peripheral blood DCs in controls and in patients with CVID |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Dendritic cells per ml        | Healthy controls (n = 12) | CVID patients a |
|                               |                  | Group 1a (n = 13) | Group 1b (n = 9) | Group 2 (n = 15) | Group 3 (n = 7) |
| Myeloid DCs                   |                  |                  |                  |                  |
| Total (median)                | 14,361           | 8,432*           | 13,475           | 15,267           | 5,568***         |
| Percentiles (25th, 75th)      | 11,470, 16,693   | 1,426, 11,330    | 8,378, 16,143    | 9,482, 18,590    | 4,315, 11,100    |
| Range                         | 8,941–22,204     | 342–21,895       | 3,039–19,385     | 6,178–28,472     | 3,787–22,994     |
| Plasmacytoid DCs              |                  |                  |                  |                  |
| Total pDCs (median)           | 9,829            | 2,367*           | 3,647            | 8,980            | 1,403**          |
| Percentiles (25th, 75th)      | 8,366, 16,555    | 353, 5,012       | 2,532, 11,590    | 4,096, 13,367    | 661, 2,448       |
| Range                         | 7,514–22,769     | 19–8,291         | 2,004–13,714     | 1,614–25,725     | 417–4,917        |

aPatients were grouped according to whether their proportion of switched memory B cells was <0.4% (groups 1a, 1b) or >0.4% (group 2) of total peripheral blood lymphocytes or they had granulomatous disease (group 3). Group 1 was subdivided according to whether numbers of CD19+CD21- immature B cells were increased (group 1a) or normal (group 1b). *P < 0.001 vs controls; **P < 0.009 vs group 2; ***P = 0.02 vs group 1b; ΦP < 0.01 vs controls; ΨP < 0.009 vs group 1b; ΦP < 0.02 vs group 2. Other comparisons between each population were not statistically significant. The nonparametric Mann–Whitney U test was used for comparisons. CVID, common variable immunodeficiency.
Hospital, directed by JL Pellegrin and JF Viallard. They were chosen because blood samples could easily be taken, in consideration of the short time-period available to perform new experiments with chemokine receptors. Of these 19 patients, 5 belonged to group Ia, 5 to group Ib, 6 to group II and 3 to group III. No differences were observed between the different groups with regard to the expression of other chemokine receptors including CCR2, CCR5, CXCR3, and CXCR4. This observation is in agreement with the already published data concerning the CCL19- and CCL21-dependent migration of mature pDCs to lymph nodes [17].

The most salient feature of the present study concerns patients in group 1a, who were at higher risk of autoimmune diseases (10 of 13 patients) and splenomegaly (9 of 13), and in group 3, both groups exhibiting a marked decrease in their peripheral pDC levels. Patients in group 2, who did not develop such complications, were similar to healthy individuals.

From these preliminary observations we can only speculate on the diminished pDC counts observed in the patients of groups 1a and 3. The migratory properties of DCs seem to be altered, leading to sequestration in tissue and/or secondary lymphoid organs due to an alteration of chemokine receptor membrane expression and maturation. However, we cannot rule out a defect in their function, such as has been suggested regarding DCs derived in vitro from monocytes [18].

At least in some patients, hypogammaglobulinemia could rely on an alteration of the innate immunity, since pDCs seem to play a critical role in the generation of antibody responses: they have been shown in vitro to control the differentiation of activated B cells into plasma cells through the secretion of interferon α and β and of IL-6 [13]. B cells activated with pDCs preferentially secrete IgG over IgM, suggesting that pDCs may specifically target memory B cells, whereas mDCs, which can enhance B-cell proliferation and isotype switching toward IgA [19], as well as plasma cell differentiation [20], could induce the differentiation of naive B cells through the secretion of IL-12 and IL-6 [11]. Granulocyte-colony-stimulating factor and FLT3 ligand, through their ability to mobilize dendritic cells, may be a new and valuable therapeutic alternative for CVID patients.

**Conclusion**

Our data provide evidence of a profound alteration in the distribution of subsets of DCs, especially pDCs, in the peripheral blood of CVID patients. Taken together, our results raise the possibility that innate immunity is involved in CVID pathogenesis.

**Competing interests**

The authors declare that they have no competing interests.
Authors’ contributions
JFV and FC were in charge of most of the CVID patients gathered in this study, performed the statistical analysis, analyzed the data, and contributed to the writing of the article. PB put together the data, supervised the laboratory examinations, analyzed the data, and contributed to the writing of the article. JFM analyzed the data, participated in the laboratory examinations, contributed to the editing of the article, and supervised the research group. JLP was, along with JFV, in charge of most of the CVID patients gathered in this study and contributed to the writing of the article. MA and FL were in charge of CVID patients and contributed to the writing and preparation of the article. All authors read and approved the final manuscript.

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