Chronic Lymphocytic Leukemia

Dendritic cell deficiency in early B-cell chronic lymphocytic leukemia

We compared the frequency, phenotype and function of blood dendritic cells (DC) and their precursors in patients with untreated B cell chronic lymphocytic leukemia (CLL) and in normal individuals. The numbers of CD11c$^+$ and CD123$^+$ blood DC were significantly reduced in CLL patients already at diagnosis and these cells showed diminished antigen uptake and perturbed maturation. The number of monocytes was normal and the number of CD34$^+$ progenitor cells increased in the CLL patients' blood.

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Dendritic cells form a complex system of professional antigen presenting cells and are detectable at low frequencies in peripheral blood and virtually all solid organs.[1] Several distinct blood DC populations have been recognized.[2] Based on their constitutive expression of MHC class II, CD11c or CD123, together with the lack of white blood cell lineage (Lin)-restricted markers and the lack of the progenitor cell antigen CD34, CD11c$^+$/Lin$^-$/CD13$^+$/CD33$^+$/CD34$^-$/HLA$^-$DR$^+$ *myeloid* and CD123$^+$/Lin$^-$/CD13$^-$/CD33$^-$/CD34$^-$/HLA$^-$DR$^+$ *lymphoid* blood DC can be distinguished. DC precursor cells in human blood are CD14$^+$ monocytes, which can give rise to CD11c$^+$ DC and CD34$^+$ hematopoietic progenitor cells (HPC).[2] The critical role of DC in the control of immunity is based on their function of capturing and processing antigens as immature cells in their resident environment and, then, upon migration and differential maturation, initiating specific immune responses.[1]

Based on promising results of tumor vaccination in animal models DC have been introduced as vectors for clinical anti-cancer therapy.[3] Observations of defective DC functions in patients with solid tumors and myeloid leukemia as well as the limited success of DC vaccinations have highlighted the need for better understanding of DC function during the development and progression of malignant diseases.

We measured DC in peripheral blood samples from 44 untreated CLL patients and 35 age-matched control individuals by flow cytometry and found a significant reduction

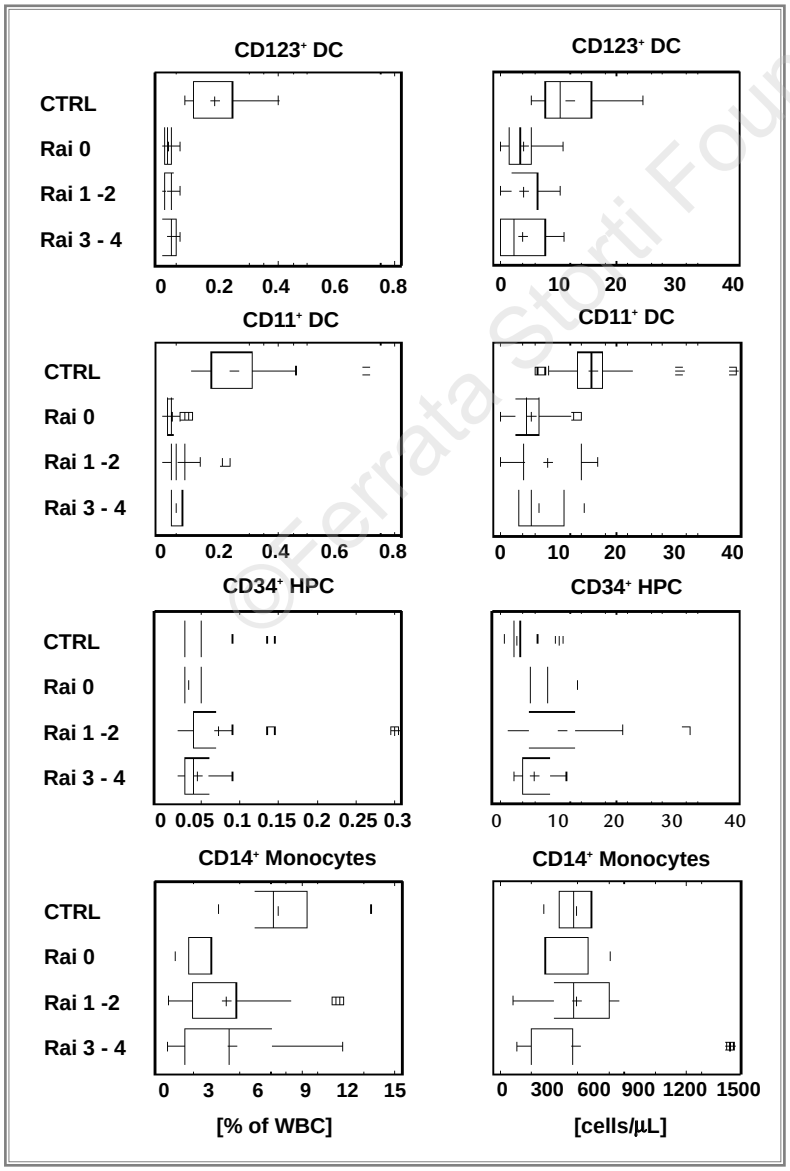

Figure 1. Enumeration of leukocyte subpopulations. Percentage and calculated absolute numbers of CD123$^+$ and CD11c$^+$ blood DC, CD34$^+$ hematopoietic progenitor cells and CD14$^+$ monocytes are shown in box-and-whisker plots comparing data from age-matched control individuals without evidence of malignant disease (CTRL, n = 35, mean age = 60 years) with data from untreated patients with early (Rai 0, Binet A, n = 19), intermediate (Rai 1-2, Binet A-B, n = 18), and high stage (Rai 3-4, Binet C, n=7) disease (mean age of CLL patients = 64 years). Statistical analysis revealed a significant reduction of both percentages and absolute numbers of CD123$^+$ and CD11c$^+$ blood DC, as well as a significant increase of absolute numbers of hematopoietic progenitor cells in CLL patients in comparison to controls (Mann-Whitney (Wilcoxon) W test; each *p* value < 0.005). Flow cytometry was performed immediately after red cell lysis of fresh heparinized blood using a FACSCalibur. Data were analyzed with CellQuestPro®, software (Becton Dickinson, San José, CA, USA).[4] Four-color analyses of blood DC were performed after adding CD34-FITC to the Lin-FITC cocktail (CD3, 14, 19, 20, 56; Becton Dickinson) to exclude CD34$^+$ progenitor cell contamination of DC populations but otherwise following the manufacturer's instructions for gating strategies. Absolute cell counts were calculated separately as percentages of CD11c$^+$/Lin$^-$/CD34$^-$/HLA-DR$^+$ and CD123$^+$/Lin$^-$/CD34$^-$/HLA-DR$^+$, CD34$^+$ and CD14$^+$ leukocytes × leukocyte count/100. Duplicate analyses of individual samples and multiple analysis of samples from selected patients and control individuals were performed at different time points as quality control.

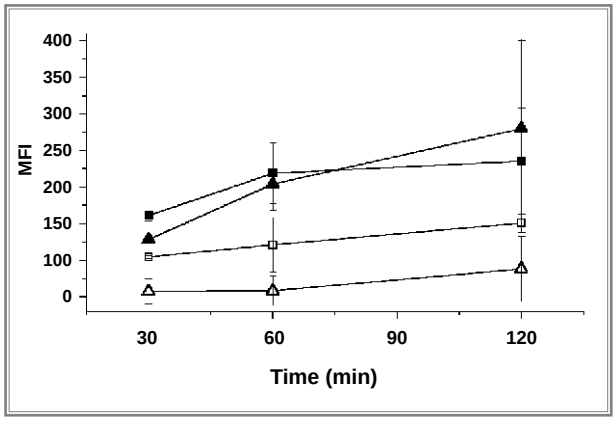

Figure 2. Antigen uptake function of blood DC. The antigen uptake function of fresh CD11c⁺ (squares) and CD123⁺ blood DC (triangles) from control individuals (filled symbols) was compared to that of untreated CLL patients (open symbols) in a time-resolved albumin-FITC internalization assay. Peripheral blood mononuclear cells were incubated with 1 μg/mL albumin-FITC (Molecular Probes, Eugene, OR, USA) at either 37°C or on ice for the time periods indicated. Antigen uptake was stopped by washing twice with ice-cold 0.1% sodium azide-1% FCS/PBS. Specific mean FITC fluorescence intensity (MFI) of CD11c⁺ and CD123⁺ DC was analyzed after staining with Lin-cocktail-PE HLA-DR-PerCP and CD11c-APC or CD123-APC with a FACSCalibur instrument equipped with CellQuestPro software (Becton Dickinson). Mean ± SEM data from 4 individual preparations are shown.

in percentages and absolute numbers of CD11c⁺ and CD123⁺ blood DC in CLL patients already at diagnosis (Stage Rai 0). The number of CD14⁺ monocytes was normal but absolute counts of circulating CD34⁺ HPC were increased in the CLL patients' blood (Figure 1).

MHC class II expression was lower and CD33 expression higher on CD11c⁺ blood DC from CLL patients and CD4 and CD11a were both lower on CD123⁺ CLL blood DC; otherwise blood DC from healthy donors had a similar phenotype. FACS-purified CD11c⁺ and CD123⁺ blood DC from CLL patients had comparable levels of adhesion and co-stimulatory molecules except for increased MHC class I reactivity on CD11c⁺ CLL blood DC and reduced acquisition of CD40 surface expression on CD123⁺ CLL blood DC when matured in suspension cultures[4] with GM-CSF/IL-4/CD40L or IL-3/TNF/CD40L, respectively. Purified CLL DC showed reduced cluster formation during *in vitro* culture, indicating functional deviations (*not shown*). Because antigen uptake is a prerequisite for the antigen-specific DC functions we next performed macropinocytosis assays. CLL DC showed reduced internalization of albumin-FITC and did not reach the same level of antigen accumulation as did blood DC from healthy controls in kinetic experiments (Figure 2). These data indicate a deficiency early in the cascade of DC functions, which could disturb DC-dependent immune responses in CLL patients.

So far, we have not found this reduction in blood DC in patients with lymphoma or myeloma at diagnosis. This is in accordance with published data from Brown *et al.*,[5] who described normal numbers but defective function of blood DC from myeloma patients. In contrast, Ratta *et al.*[6] observed a significant interleukin (IL)-6 dependent reduction of blood DC numbers in myeloma. These obviously contradictory published data may reflect a higher variability in DC alterations in myeloma than that observed in our study for CLL patients. Alternatively these differences might be due to variations in the DC enumeration protocols that still await standardization.[7]

Rezvany *et al.*[8] recently observed increased MHC class I expression on CD11c⁺ DC matured *in vitro* from monocytes, this expression being comparable to what we measured for CD11c⁺ blood DC of CLL patients. They also found that the CLL monocyte-derived mature DC had a normal allostimulatory function but reduced interferon γ gene expression. In our study, FACS-purified, *in vitro*-matured CD11c⁺ and CD123⁺ blood DC also showed a normal capacity to stimulate allogeneic T cells in a primary mixed lymphocyte reaction after *in vitro* maturation (*data not shown*). Orsini *et al.*[9] recently described a severely defective circulating DC compartment in CLL patients whose immature blood DC were unable to induce effective T-cell priming and were less potent stimulators of type 1 T-cell responses. They also found that CLL-derived IL-6 is a crucial inhibitor of DC differentiation from CLL blood monocytes.

It is obvious that the biological and clinical significance of the alterations in blood DC phenotype and function, described in this and other studies, await further clarification. Understanding the role of human DC during the development and progression of leukemia may not be only of pathophysiologic interest but might also help to improve vaccination strategies.

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