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.

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. Several distinct blood DC populations have been recognized. 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-/CD123+/HLA-DR+ myeloid and CD123+/Lin-/CD33+/CD123+/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). 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.

Based on promising results of tumor vaccination in animal models DC have been introduced as vectors for clinical anti-cancer therapy. 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
in percentages and absolute numbers of CD11c⁺ and CD123⁺ blood DC in CLL patients already at diagnosis (Stage Rai 0). The number of CD11c⁺ 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⁺ 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⁺ blood DC and reduced acquisition of CD40 surface expression on CD123⁺ blood DC when matured in suspension cultures 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 macrophagocytosis 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., who described normal numbers but defective function of blood DC from myeloma patients. In contrast, Ratta et al. 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.

Rezvany et al. 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 allo-geneic T cells in a primary mixed lymphocyte reaction after in vitro maturation (data not shown). Orsini et al. 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 pathophysiological interest but might also help to improve vaccination strategies.

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References


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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 (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.