Flow cytometric assessment of the reactivity of a panel of monoclonal antibodies (mAb) against two populations of human dendritic cells (DC)

ABSTRACT
The immunophenotyping assays demonstrated differences between the two DC populations as well as variations in the reactivity of the mAb at diverse time points, suggesting the existence of subpopulations within the Mo-DC and Mo-LC.

INTRODUCTION
Background Dendritic cells (DC) are a complex group of mainly bone marrow derived cells that play an important role in the afferent branch of the immune response. However, DC represents only a minute subpopulation of the peripheral blood mononuclear cells (PBMC), as well as of bulk cellular populations of the lung, intestine, genitourinary tissue, and lymphoid tissue. DC also has been found in the epidermis, dermis and mucous membranes and constituting about 2% of the total cellular population of the human epidermis. The Langerhans cells (LC) are a skin derived-DC, that have the capability to travel to the regional lymphoid organs after take up of antigen and undergo there an activation/maturation step. Thereafter, LC interacts and activates T cells. Because of such significant capability to take up soluble antigens, process and present them to responder cells in the lymphoid tissues in the context of the restricted MHC pathway, LC have been considered one of the most important elements in the afferent arm of the immune response. Recent, successful efforts to generate DC from PBMC derived monocytes or from CD34 blood precursors by utilizing GM-CSF and IL-4, as well as GM-CSF and/or TNF, has enabled us to obtain PBMC derived DC (Mo-DC)). In addition, an approach has been developed to generate LC from isolated monocytes (Mo-LC). Despite the successful efforts in the generation of DC from blood precursors, the characterization of surface markers on human DC has been a very difficult and elusive task because the lack of appropriate reagents with high specificity for DC identification. However, some molecules whose genes recently have been cloned and sequenced (e.g. CD83, DEC-205) have been found strongly associated with DC. In addition, a panel of monoclonal antibodies (e.g. CMRF-44) that recognize molecules on DC has been raised. There is a growing need for cluster and establishment of a common and comprehensive nomenclature for such DC associated molecules, as well as to clarify and define the lineage(s) of DC and the existence of DC subsets. These developments have prompted the set up of diverse approaches that evaluated the reactivity of a group of mAb against populations of DC. Therefore and within the scope of this study, we set up a flow cytometry approach and evaluated a panel of 20 mAb against two populations of DC aiming to determine the kinetics of expression of antigens on DC at diverse intervals of time and the likelihood to identify markers for DC subsets.

CONCLUSION
Conclusions The immunophenotyping assays described in this report enabled us to determine in human DC: (i) the existence of differences between Mo-DC and Mo-LC populations; (ii) the existence of subsets within the Mo-DC and Mo-LC populations; (iii) the kinetics of antigens expression at diverse intervals of time on DC; and (iv) specific markers for subpopulations of DC.