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SHORT COMMUNICATION

Blood dendritic cells in patients with chronic lymphocytic leukaemia

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Abstract

Myeloid and plasmacytoid dendritic cells (MDC, PDC) play a key role in the initiation of immune responses. We found a reduction of both DC subsets in 42 patients with chronic lymphocytic leukaemia (CLL) at diagnosis ($P<0.0001$ and 0.0001 vs. controls, respectively), likely related to the high secretion of CCL22 and CXCL12 ($P = 0.04$ and 0.008 vs. controls, respectively) by leukaemic cells. However, CD14+ monocytes from CLL patients could give rise to functional IL-12p70-secreting monocyte-derived DCs, capable of inducing a type 1 polarization immunostimulatory profile. These monocyte-derived DCs from CLL patients efficiently migrate in response to CCL19/MIP-3β chemokine, suggesting that functional autologous DCs can be generated for immunotherapeutic purposes to circumvent DC defects in CLL.

Keywords: Chronic lymphocytic leukaemia; Dendritic cells; Immunotherapy

Introduction

Dendritic cells (DCs) are responsible for the initiation and regulation of immune responses. At least two subsets of blood DCs have been characterized based on the differential expression of CD11c. Myeloid DCs (MDC) and plasmacytoid DCs (PDC) can promote polarization of naive T cells (Cella et al., 2000; Rissoan et al., 1999) and induction of an efficient immune response. Thus, DC functional defects may play a key role in tumour immune escape, and restoration of DC functions is a logical target for immunotherapy. In this study, we investigated the status of circulating DCs in the blood of 42 chronic lymphocytic leukaemia (CLL) patients. In addition, we tested the ability of CD14+ monocytes from CLL patients to give rise to functional DCs *in vitro*.

Patients, materials and methods

Patients and controls

CLL cell samples were obtained after informed consent from 42 patients at diagnosis. CLL diagnosis was

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performed at the Institut Paoli-Calmettes (Marseille, France) according to standard criteria (Table 1). Peripheral blood mononuclear cells (PBMC) from patients and healthy controls (Etablissement Français du Sang, Marseille, France) were separated on a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient. Plasma fraction was stored at −80 °C for later use. Normal, and leukaemic MDCs and PDCs were identified by three-color staining as previously described (Mohty et al., 2001). Plasma monocyte-derived chemokine (CCL22) and chemokine ligand CXCL12/SDF-1 levels were determined using specific ELISA (R&D Systems, Abingdon, UK).

In vitro DC generation

CD14+ monocytes from healthy donors and CD14+ monocytes (as determined by FACS analysis in peripheral blood) from CLL patients were immunomagnetically purified with CD14 mAb-conjugated microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and were cultured with GM-CSF and IL-4 as previously reported (Mohty et al., 2003). Final maturation of monocyte-derived DCs was induced by adding 75-Gy irradiated CD40L-transfected murine L-cells (2×10^5 well). Supernatants of DC cultures were harvested at day 5, and at day 7 after 2 days of maturation with CD40L. IL-10, IL-12p70 and TNF-α concentrations were measured using specific ELISA with the OptEIA sets (BD-Biosciences, Le Pont de Claix, France). Quantification was normalized to 10^6 cells.

Flow cytometry analysis

The following mAb were used for flow cytometry: anti-CD1a, CD14, CD80, CD83, HLA-DR and relevant isotypic controls, all from Beckman–Coulter (Marseille, France). CD86 was from BD-Biosciences. Samples were analysed on a FACSCanto cytometer and data were analysed using BD-FACSDiva software (BD-Biosciences).

T cell separation and mixed lymphocyte reaction (MLR)

CD4+ T cells were purified by depletion of adult blood PBMC using the CD4 Cell Isolation Kit II (Miltenyi Biotec). CD4+/CD45RA+ naïve T lymphocytes were immunomagnetically isolated from the CD4+ T fraction by CD45RA mAb-conjugated microbeads (Miltenyi Biotec). CD8+ T lymphocytes were immunomagnetically purified from PBMCs by positive selection using CD8 mAb-conjugated microbeads (Miltenyi Biotec). T cell proliferation capacity was evaluated as previously described (Mohty et al., 2003). Allogeneic CD4+CD45RA+ T cells or CD8+ T cells (10^5 cells/well) were co-cultured with DC (10^4 cells/well), cells were harvested after 7 days and replated in 96-well culture plates at 10^4 cells per well in triplicate in the presence of 25 ng/ml PMA (Sigma, St. Louis, MO) and

Table 1. Patients' characteristics

UPN	Age	Sex	Disease stage[a]	% CD5+/CD19+ leukaemic cells[b]
CLL1	67	M	C	93
CLL2	80	F	C	94
CLL3	84	F	C	45
CLL4	47	M	B	43
CLL5	76	M	A	72
CLL6	75	M	C	94
CLL7	58	F	C	94
CLL8	55	F	A	80
CLL9	76	M	A	90
CLL10	42	M	A	77
CLL11	61	F	A	84
CLL12	71	M	C	97
CLL13	82	M	A	91
CLL14	51	M	A	72
CLL15	45	M	A	71
CLL16	67	F	B	75
CLL17	49	F	A	70
CLL18	51	F	C	85
CLL19	52	M	A	71
CLL20	66	M	A	95
CLL21	49	M	A	78
CLL22	75	F	A	86
CLL23	35	M	A	89
CLL24	77	F	A	61
CLL25	76	F	B	91
CLL26	69	F	C	84
CLL27	66	M	A	94
CLL28	62	M	C	89
CLL29	36	M	A	74
CLL30	70	F	A	85
CLL31	42	F	A	63
CLL32	64	M	B	98
CLL33	65	F	A	71
CLL34	56	M	A	83
CLL35	73	M	A	91
CLL36	65	F	A	62
CLL37	70	M	A	64
CLL38	44	M	A	30
CLL39	59	F	B	54
CLL40	61	F	C	85
CLL41	65	F	A	50
CLL42	67	F	A	59

Abbreviations: F, female; M, male; CLL, chronic lymphocytic leukaemia; NA, not available.

[a]Disease staging was performed according to the Binet classification.

[b]As determined by flow cytometry in the peripheral blood.