

Hepatic environment elicits monocyte differentiation into a dendritic cell subset directing Th2 response

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Background/Aims: Dendritic cells (DCs), which play a critical role during immune response, could present alternative differentiation patterns depending on tissue microenvironment. Our aim was to examine the influence of hepatic microenvironment on human monocyte differentiation into DCs.

Methods: Cytology, immunophenotyping, cytokine production and T-cell activation were analyzed in DCs differentiated from human monocytes co-cultured with rat liver epithelial cells (RLEC) or human cells from various tissue origins and compared to control DCs obtained on plastic with GM-CSF/IL-4.

Results: RLEC environment promotes DC differentiation in the presence of IL-4 without GM-CSF. These DCs evidence similar expression of MHC-II, co-stimulatory and adhesion molecules than control DCs, but distinct lineage markers defining a CD11c⁺/CD14⁺/CD123⁺ DC subset. This phenotype is common to DCs from RLEC and human liver environment and differs from that obtained with skin or intestine environments. Functionally, they produce IL-10 but not IL-12p70 and favor IL-4/IL-10 secretion by T-cells rather than IFN- γ .

Conclusions: Our results confirm that tissue niches modulate DC differentiation and demonstrate that hepatic environment influences monocyte differentiation into a DC subset directing Th2 response, a key data for understanding the specialized immune response in liver. They also make RLEC co-culture system useful for studying liver DC functions.

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Keywords: Dendritic cells; Hepatic microenvironment; Stromal cells; Monocytes; Cell differentiation; Th2 response

1. Introduction

Because of its location and function, liver is continuously exposed to a wide range of antigens. Pathogenic microorganisms must be eliminated while large number of dietary or commensal organism antigens as well as hepatic metabolites must be tolerized. Therefore, liver has developed a specialized immune system that favors tolerance rather than immunity [1,2]. Liver contains

multiple cells that can act as antigen presenting cells (APCs) such as dendritic cells (DCs), Kupffer cells, sinusoidal endothelial cells, and even hepatocytes [3–6]. Hepatic DCs, considered as the most potent APCs, display high capacity to stimulate naïve T-cells and to initiate primary immune response. However, evidences are also accumulated about their role in tolerance, for example, in survival of liver allograft [3,7,8]. Thus, hepatic DCs might play a critical role in controlling the specialized immune response in liver.

DCs are a heterogeneous cell population and their lineage development is still under investigation. They have been shown to derive from bone marrow hematopoietic stem cells or blood precursors, i.e. monocytes and interferon producing cells [9–11]. They play a pivotal role in

Received 18 March 2005; received in revised form 5 July 2005; accepted 2 August 2005; available online 15 September 2005

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the control of immune response by directing T-cell polarization and cytokine production. It is becoming evident that their capacity to modulate the immune response greatly depends on their lineage development, stage of maturation, processed antigens and also tissue localization [10,12–15].

DCs are present in many tissues but their paucity hinders their characterization. Most of knowledge's about DCs comes from in vitro systems in which cytokines are added. These studies suggest that cytokine combinations can modulate monocyte differentiation into different subsets of DCs [16–21]. Today, questions arise about influence of tissue environment on control of DC differentiation. Recently, dermal, thymic and spleen stromal environments have been reported to support in vitro development of hematopoietic stem cells or monocytes into various DC subsets which exhibit different phenotypical and functional properties [14,22–27].

In this study, we have analyzed monocyte differentiation into DC in a hepatic environment. For this purpose, we have developed a co-culture system using rat liver biliary epithelial cells (RLEC) as feeder layer. Among stromal liver cells, specific interest in RLEC arises from its well-defined hepatic properties. RLEC, a precursor biliary cell line obtained from neonatal rat liver, is able to support, without exogenous cytokine addition, human hematopoiesis and human hepatocyte differentiation, two functions sequentially taking place in liver during development. Despite of their xenogeneic origin, RLEC favor liver-specific gene expression of human hepatocytes [28] and long-term multi-lineage differentiation of human CD34⁺/CD38⁻ hematopoietic cells [29]. Moreover, RLEC derive from the hepatic stem cells compartment localized in the terminal biliary ductules, i.e. Hering canal [30], close to bile ducts in the portal area where most DCs reside in human liver [31].

Here, we report that in RLEC co-culture system, monocyte differentiation into DCs occurs in the presence of IL-4 without GM-CSF addition. DCs generated in this model as well as in co-culture with human hepatic cells exhibit a CD11c⁺/CD14⁺/CD123⁺ phenotype and a cytokine secretion profile that favors Th2 polarization. This feature could be a key data for understanding the specialized immune response in liver.

2. Materials and methods

2.1. Cultures of feeder cells

RLEC were isolated as described by Williams et al. [32] and cultured in William's E medium with 10% FCS (Myoclon-Superplus, Invitrogen). Caco-2 cells, originating from human colorectal adenocarcinoma, were maintained according to American Type Culture Collection recommendations. Human hepatic cells, isolated from liver biopsies by collagenase digestion [33], were centrifuged at 70×g for 1 min. Hepatocytes were recovered from the pellet and enriched hepatic nonparenchymal cell population from the supernatant. They were cultured in Williams' E medium with 10% FCS, 2 mM L-glutamine, 5 µg/mL bovine insulin, 1 g/L BSA, 5×10⁻⁵ M hydrocortisone hemisuccinate. Primary cultures of human skin and renal epithelial cells were grown in culture medium provided by Biopredic International (France) and RPMI 1640 Glutamax-I medium (Gibco) with 10% FCS, respectively. Access to the biopsy material

was in agreement with French laws and satisfied the requirements of the Ethics Committee of the institution.

2.2. Culture of monocytes

Cryopreserved monocytes, isolated from healthy blood donor's buffy-coats by centrifugation on Ficoll-Hypaque gradient (Pharmacia) and purified by elutriation [34], were thawed and plated (2×10⁶ cells in 3 mL) in 6-well culture plates alone or on various stromal cell monolayers in RPMI 1640 medium containing 25 mM HEPES, 10% heat-inactivated FCS and 5 µM 2-mercapto-ethanol. Recombinant human GM-CSF (800 U/mL, Schering-Plough) and/or recombinant human IL-4 (1000 U/mL, PromoCell) were added at seeding. Cultures were fed every 2 days by removing 1 mL of spent medium and adding 1.5 mL of fresh medium containing cytokines in amount for 3 mL of medium. RLEC-conditioned medium (RLEC-CM) was produced by maintaining RLEC 24 h in complete RPMI medium. Cells were harvested by gentle pipetting on day 5 or 7. For flow cytometry analysis, maturation assay of DCs was performed by maintaining cells for two additional days in the presence of *Escherichia Coli* LPS (10 ng/mL, Sigma), Ribomumyl[®] (Pierre Fabre Médicaments) or Poly I:C (Sigma). For cytokine analysis, 5-day-old DCs were harvested and replated at 5×10⁵ per mL for 2 days in the same conditions, in the presence of LPS plus recombinant human TNFα (200 U/mL, PromoCell) or recombinant human CD40L (1 µg/mL, R&D Systems) plus recombinant human IFN-γ (1000 U/mL, Boehringer). IL-10 and IL-12p70 secretions were measured after 48 h of maturation by ELISA kits (Beckman Coulter).

2.3. Cell surface molecule expression analysis and endocytosis assays

Cells were stained by monoclonal antibodies (mAb) against CD1a, CD11b, CD40, CD45, CD54, CD58, CD80, HLA-DR, HLA-DQ (Immuno-tech), CD1d, CD11c, CD14, CD64, CD83, CD86, CD116, CD123 (Pharmingen) and DC-SIGN (e-Bioscience). For endocytosis assay, 1×10⁵ cells were incubated 20 or 60 min at 37 or 0 °C with 1 mg/mL of dextran-FITC (Molecular Probes). Analyses were performed using a FACSCalibur™ flow cytometer and CellQuest software (Becton-Dickinson).

2.4. T-cell stimulation assays

T lymphocytes were isolated from healthy blood donor's buffy-coats by centrifugation on Ficoll-Hypaque gradient and T-cell negative isolation kit allowing monocytes, granulocytes, B cells, NK cells and activated T-cells depletion (Dyna). All experiments were performed in 96-well culture plates with 200 µL of medium.

2.4.1. Allogeneic stimulation assay

T-cells (1×10⁵) were cultured in complete RPMI medium with 5-day-old immature DCs or DCs matured 2 days with CD40L plus IFN-γ (5×10³). T-cell proliferation was measured on days 5–6 by 16 h pulse with [³H]-methyl-thymidine (1 µCi/well). To analyze cytokine production by T-cells, 1×10⁵ T-cells, from 5-day-old MLR cultures, were re-stimulated with anti-CD3 (1 µg/mL) and anti-CD28 (1 µg/mL) antibodies. Cytokine secretion was measured 24 h after re-stimulation by ELISA kits for IFN-γ (Biosource), IL-4 (Becton-Dickinson) and IL-10.

2.4.2. Antigen-specific presentation assay

CD4⁺ T-cell clones (5×10⁴) recognizing hemagglutinin residue (NH₂-PKYVKQNTLKLATGM-COOH) from influenza virus (A/Texas/1/77 strain) in four different HLA-DR contexts were generated as described by Lamb et al. [35] and stimulated by 5-day-old DCs (2.5×10³) pulsed for 2 h with 30 µg/mL of peptide. M77-84, CD8⁺ T-cell clone (5×10⁵) recognizing melan-A/MART-1 derived peptide in HLA-A*0201 context was stimulated by 5-day-old DCs (3×10⁴) pulsed for 2 h with 50 µM of peptide. IFN-γ and IL-4 secretions by T-cell clones were measured by ELISA 24 h after the stimulation.

2.5. Statistical analysis

Statistical analyses were performed using non-parametric Mann–Whitney test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Monocytes maintained in RLEC environment differentiate into DCs in the presence of IL-4

To evaluate the capacity of RLEC to influence human monocyte differentiation, we analyzed morphology, survival and phenotype of cells differentiated within 7 days onto RLEC monolayers, in the absence or presence of IL-4, GM-CSF or GM-CSF/IL-4. For comparative purpose, we generated control macrophages and DCs by culturing monocytes on plastic in the presence of GM-CSF and GM-CSF/IL-4, respectively.

We observed that monocytes cultured onto RLEC were non-adherent and easy to harvest (Fig. 1A). Addition of

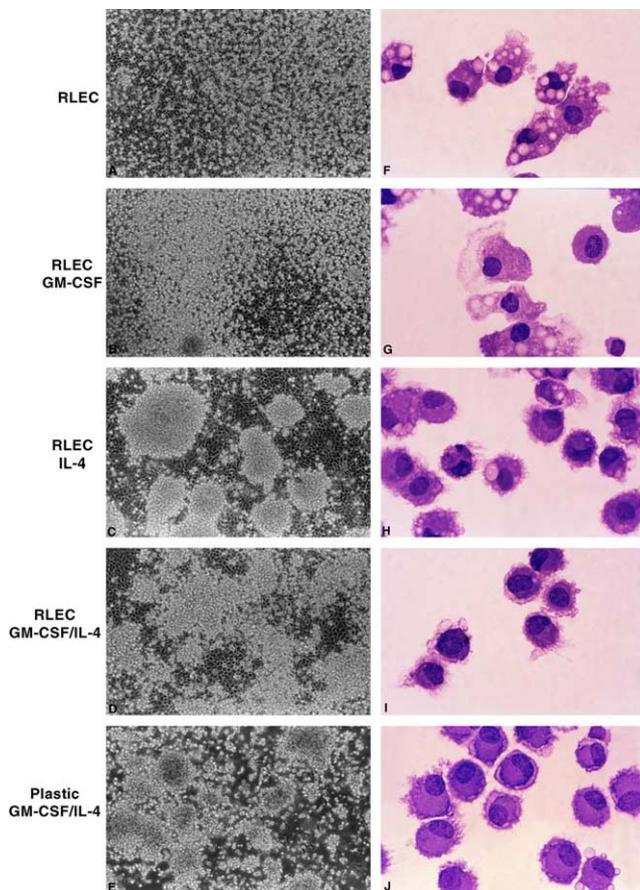


Fig. 1. Morphology and cytology of monocyte-derived cells from RLEC co-cultures. Phase-contrast micrographs (A–E) and photographs of May–Grünwald–Giemsa cytopins (F–J). Monocytes were cultured for 7 days with rat liver epithelial cell (RLEC) in the absence of cytokine (A, F), or in the presence of GM-CSF (B, G), IL-4 (C, H), GM-CSF/IL-4 (D, I). As control, DCs from cultures of monocytes onto plastic with GM-CSF/IL-4 are shown (E, J). Magnification: $\times 56$ (A–E); $\times 420$ (F–J). [This figure appears in colour on the web.]

GM-CSF did not induce cell adhesion and spreading as observed in control macrophage cultures (Fig. 1B). In the presence of IL-4 or GM-CSF/IL-4, cells aggregated in clusters as control DCs (Fig. 1C–E). Cell recovery from RLEC co-cultures, even without cytokine addition, was similar to macrophage and control DC cultures (Fig. 2A). Addition of cytokines to co-cultures did not significantly modify the cell yield. Monocyte-derived cells, from all culture conditions, showed endocytosis ability (Fig. 2B). However, two cell types could be distinguished according to the presence or absence of IL-4. Monocytes in co-culture with RLEC without IL-4 committed to the macrophage differentiation pathway as evidenced by their typical morphology (Fig. 1F and G) and expression of cell surface molecules (Fig. 3). Cells were $CD14^+$, $CD64^+$, $CD1a^-$ and $DC-SIGN^-$. Expression of adhesion, co-stimulatory and MHC-class II molecules was almost unchanged compared to monocytes. Moreover, addition of LPS did not induce expression of the mature DC marker, CD83. In fact, the phenotype of cells in co-culture without IL-4 was similar to that of control macrophages.

In contrast, IL-4 addition to RLEC co-cultures committed monocytes to the DC differentiation pathway as evidenced by the presence of veils and dendrites distributed over the cell surface (Fig. 1H–J). This morphological change was associated with disappearance of the macrophage marker CD64 and high expression of DC-SIGN (Fig. 3). Expression levels of adhesion, co-stimulatory and MHC-class II molecules were up-regulated compared to monocytes and close to those of control DCs (Fig. 3 and data

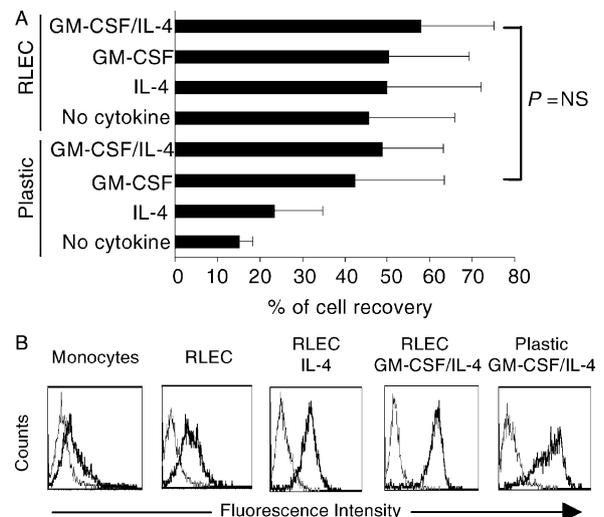


Fig. 2. Viability and endocytosis ability of monocyte-derived cells from RLEC co-cultures. Monocytes were plated on plastic or onto RLEC with the indicated cytokine combinations. (A) Percentage of cell recovery represents the ratio between number of viable recovered cells at day 7 and number of plated cells at day 0. Bars represent means \pm SD of 10 experiments. NS, no statistical significance. (B) At day 7, cells were incubated 20 min at 0 or 37 °C with Dextran-FITC (1 mg/mL) and analyzed by flow cytometry. Thin line profiles show control fluorescence values obtained at 0 °C ($n = 3$).

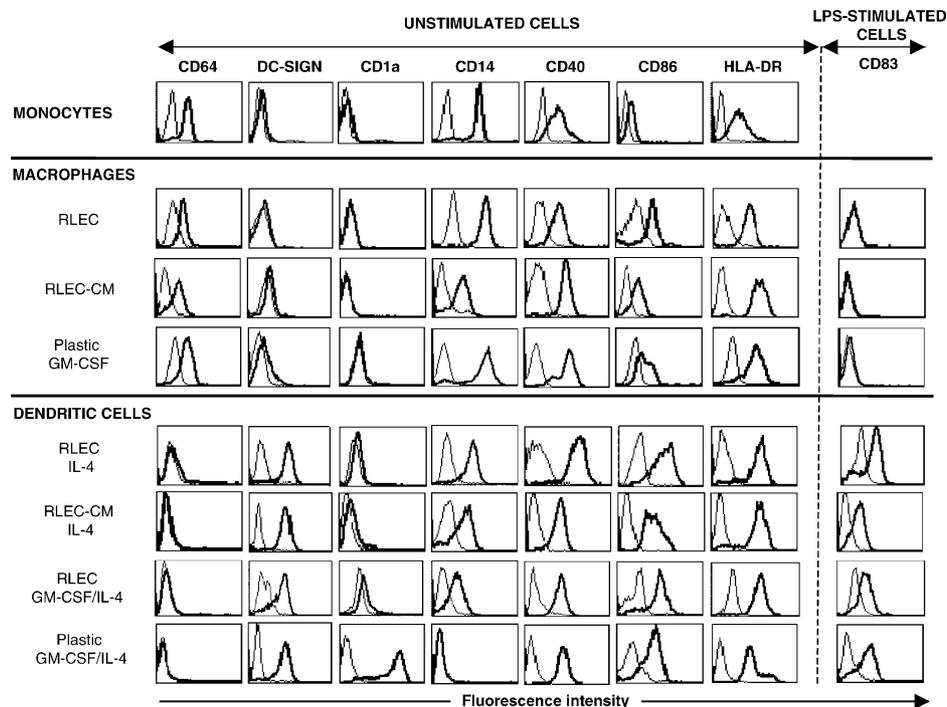


Fig. 3. Immunophenotype of monocyte-derived cells from RLEC co-cultures. Monocytes were plated onto plastic, onto RLEC or in RLEC-conditioned medium (RLEC-CM) with the indicated cytokine combinations. At day 7, cells were stained with specific antibodies (thick line histograms) or isotype controls (thin line histograms). For CD83 expression, 5-day-old cells were exposed to LPS for two additional days ($n=5$).

not shown for CD11b, CD58, CD80, CD116 and HLA-DQ). Moreover, exposition to LPS, ribomunyl[®] or Poly I:C induced expression of CD83, the mature DC marker. From extensive cell surface molecule analyze, DCs differentiated in RLEC co-cultures with IL-4 shared major characteristics with control DCs, but mainly differed in their CD1a/CD14 expression. CD14 remained present while CD1a expression was slightly induced (Fig. 3). Note that addition of GM-CSF to co-culture with IL-4 did not significantly change cell recovery, morphology and phenotype.

3.2. Hepatic environment favors differentiation of monocytes into a CD11c⁺/CD14⁺/CD123⁺ DC subset producing IL-10 but not IL-12p70

Co-cultures with RLEC, human hepatocytes or enriched human hepatic nonparenchymal cell population were compared with co-cultures with human skin, kidney or intestine epithelial cells, all in the presence of IL-4. Attention was focused on lineage marker expression and on cytokine secretion directing the immune response. Cells generated in all co-culture conditions displayed typical DC morphology (not shown), strongly exhibited the DC marker DC-SIGN while they did not express CD64 (Fig. 4). As previously observed in RLEC co-cultures, DCs from human hepatic co-cultures strongly expressed CD14. In addition, they simultaneously expressed high levels of CD11c and CD123, described as markers of myeloid and plasmacytoid DCs, respectively. This phenotype contrasted with those of control DCs and DCs from skin and intestine co-cultures,

which were CD123^{low} and CD14⁻. DCs produced in kidney environment expressed CD14 and CD123 but with lower intensity than DCs from hepatic environments (Fig. 4). The phenotype exhibited by hepatic DC subset was independent of cell contacts since monocyte differentiation into DCs with this phenotype occurred using transwells (not shown) and RLEC-conditioned media (RLEC-CM) in the presence of IL-4 (Figs. 3 and 4).

Because DC subsets can differ in their cytokine profiles, we sought by ELISA the production of IL-10 and IL-12p70 by 5-day-old DCs stimulated for one or two additional days by TNF α plus LPS or CD40L plus IFN- γ . Secretion levels were maximal at 48 h. At this time, DCs from all cultures performed produced IL-10 (Fig. 5). Control DCs secreted IL-12p70 in the presence of both maturing agents and DCs from skin or intestine environments secreted IL-12 with IFN- γ /CD40L. In contrast, DCs from hepatic co-cultures failed to produce IL-12 whatever the stimulating agents used. As well, DCs from kidney co-cultures did not produce IL-12p70.

3.3. DCs from RLEC microenvironment are efficient to specifically activate T-cell clones

We tested the ability of DCs from RLEC co-cultures to specifically present peptides on MHC-class II or -class I restricted contexts. We used CD4⁺ T-cell clones specific for a hemagglutinin peptide from influenza virus [35] and a CD8⁺ T-cell clone specific for a peptide derived from Melan-A/MART-1. Stimulation of T-cell clones by

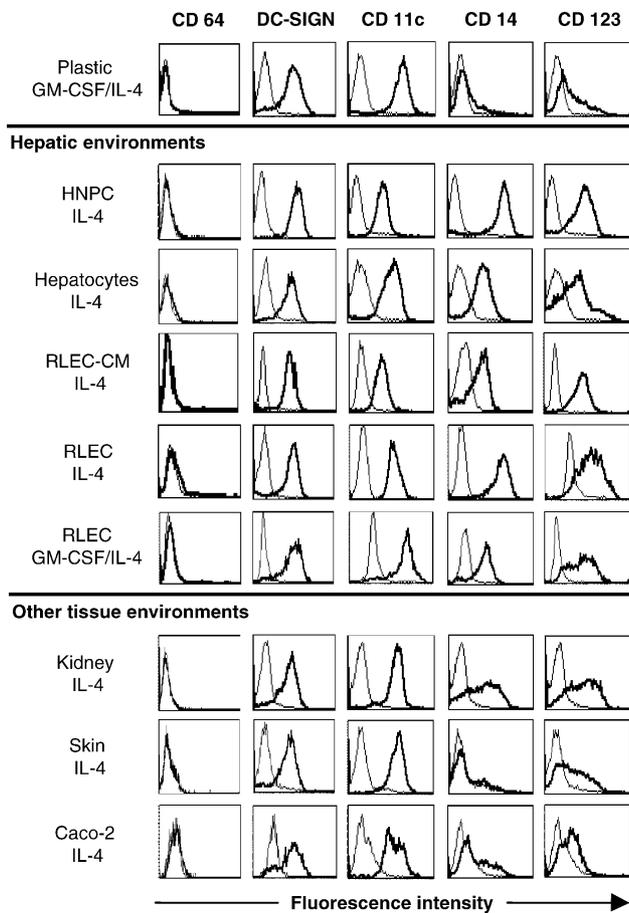


Fig. 4. Immunophenotype of DCs generated in different tissue environments. Monocytes were cultured for 7 days onto RLEC, in RLEC-conditioned medium (RLEC-CM) or onto various human tissue environments, all in the presence of IL-4. HNPC: hepatic non-parenchymal cells; caco-2: epithelial intestinal cells; skin: keratinocytes; kidney: epithelial renal cells. DCs were stained with specific antibodies (thick line histograms) or with isotype controls (thin line histograms). Analysis was performed on CD45⁺ cell population. Culture on plastic and onto RLEC in the presence of GM-CSF and IL-4 are shown as controls ($n=2$).

5-day-old DCs, in the presence or absence of peptide, was determined by measuring the secretion level of IFN- γ and/or IL-4, 24 h after stimulation. We observed that DCs from RLEC co-cultures, like control DCs, stimulated CD4⁺ T-cell production of IFN- γ and IL-4 (Fig. 6A) and CD8⁺ T-cell production of IFN- γ (Fig. 6B). As expected, monocytes were less potent stimulator cells and undetectable cytokine level was obtained in culture without peptide or with T-cell clone alone (not shown).

3.4. DCs from RLEC co-cultures direct Th2 response

We then realized mixed leucocyte reaction (MLR) in order to determine the influence of DCs on immune response orientation. Allogeneic T-cells were cultured for 5 days with 5-day-old immature DCs or DCs matured 2 days

with CD40L plus IFN- γ . DCs produced onto plastic with GM-CSF/IL-4 were used as controls. Thymidine incorporation assays demonstrated the efficiency of all DC subsets to induce allogeneic T-cell proliferation (not shown). Then, allogeneic T-cells, stimulated by DCs, were collected and re-stimulated for 24 h with anti-CD3 and anti-CD28 mAb and assessed for IFN- γ , IL-4 and IL-10 secretion. Interestingly, T-cells stimulated with immature and mature DCs from RLEC co-cultures produced less IFN- γ and more IL-10 than those primed with control DCs (Fig. 7A). The low ratios IFN- γ /IL-10 and IFN- γ /IL-4 (Fig. 7B) confirmed that DCs from RLEC co-cultures favored Th2 differentiation whereas control DCs directed T-cells toward Th1 response.

4. Discussion

In this work, we show that tissue microenvironment plays a major role in regulating DC development from monocytes. To mimic a microenvironment close to liver, we chose to use RLEC cell line as feeder layer. RLEC support human hematopoiesis, human hepatocyte differentiation and are easy to obtain compared to human hepatic cells [28,29]. Using RLEC co-culture system, we demonstrate that, in the absence of IL-4, monocytes undergo differentiation into macrophages whereas IL-4 addition commits monocytes toward DC differentiation pathway. These DCs share features with control DCs generated onto plastic with GM-CSF/IL-4, including typical morphology and expression of main cell surface markers. Despite their myeloid origin, DCs from RLEC co-cultures clearly express the usual plasmacytoid DC marker, CD123. Moreover, they are distinguishable from control DCs by a CD14^{bright}/CD1a^{low} phenotype. Previous studies have demonstrated plasticity of monocytes and diversity of monocyte-derived DC phenotypes using various cytokine cocktails [16,17,19,21]. Especially, differentiation of DCs expressing CD14 and CD123 occurred when monocytes are cultured in the presence of IL-3 plus IFN- β or GM-CSF plus IFN- α [18,20] and CD1a expression was linked to the conventional GM-CSF/IL-4 cocktail. In our experiments, peculiar phenotype of DCs differentiated in RLEC co-culture with IL-4 could be related to the influence of environment rather than GM-CSF deprivation since addition of GM-CSF to RLEC co-cultures do not significantly modifies phenotype. The role of hepatic environment on DC differentiation is strengthened by our comparative studies with hepatic, intestine, skin and kidney cells which associate high CD14 and CD123 expression with human and rat hepatic environments. Interestingly, in vivo, hepatic DCs described by others [36,37] share these characteristics. This leads us to define a hepatic DC subset characterized by a CD11c⁺/CD14⁺/CD123⁺ expression. Since similar monocyte differentiation occurs in the presence of

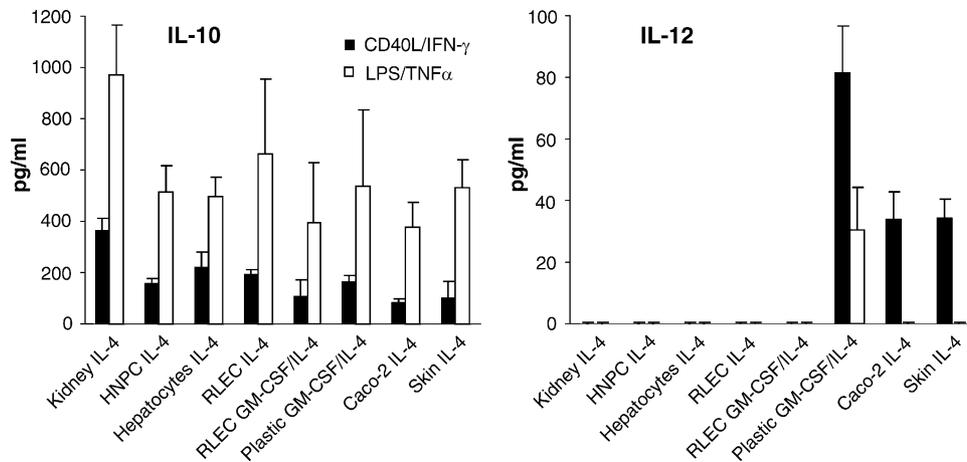


Fig. 5. Cytokine production profile of DCs generated in different tissue environments. Monocytes were cultured for 5 days on RLEC or onto different tissue environments with IL-4. HNPC: hepatic non-parenchymal cells; caco-2: epithelial intestinal cells; skin: keratinocytes; kidney: epithelial renal cells. Culture on plastic and onto RLEC in the presence of GM-CSF and IL-4 are shown as controls. DCs were harvested and recultured (5×10^5 cells/mL) for 2 days with CD40L (1 μ g/mL) plus IFN- γ (1000 UI/mL) or LPS (10 ng/mL) plus TNF α (200 U/mL). Production of IL-10 and IL-12p70 was measured by ELISA after 48 h of maturation. Note that feeder cells do not produce IL-10 or IL-12p70. Results are expressed as means \pm SD of three experiments.

RLEC-conditioned medium, phenotype could be related to cytokines and growth factors produced by local environment. Numerous soluble factors produced by RLECs [38] and also present in human liver [1,3] intricately interact to favor cell viability and specific DC differentiation.

Functionally, this DC subset has the capacity to specifically present peptides in MHC-class II and -class I contexts and to stimulate autologous T-cells in the presence of unknown antigens as keyhole limpet hemocyanin (unpublished data). Previous data demonstrate that DCs provide distinct signals that guide T-cells toward Th1 and Th2 differentiation [10,39]. This functional diversity results from distinct lineage origin, stage of maturation, nature of processed antigens and influence of tissue microenvironment on DC differentiation [12,13,27]. From our analyses, two profiles of DC cytokine secretion are distinguished according to tissue environments. DCs differentiated in intestinal and skin environments secrete IL-10 and IL-12p70 while DCs produced in hepatic and renal environments fail to produce IL-12p70. This deficiency in IL-12 secretion leads to Th2 polarization. Indeed, allogeneic T-cells stimulated by immature or mature DCs generated in RLEC co-cultures secrete IL-4 and IL-10 rather than IFN- γ . This modulation of Th2 cytokine production, also found in hepatic DCs in vivo [37,40,41], could contribute to the specialized immune response in liver. This feature is not liver-specific since DCs produced in renal environment are also found to produce IL-10 but not IL-12. Thus, our results strengthen the notion that tissue environment influences DC precursor differentiation into DC subsets with properties linked to physiological functions of organs. Visceral organs such as liver, kidney

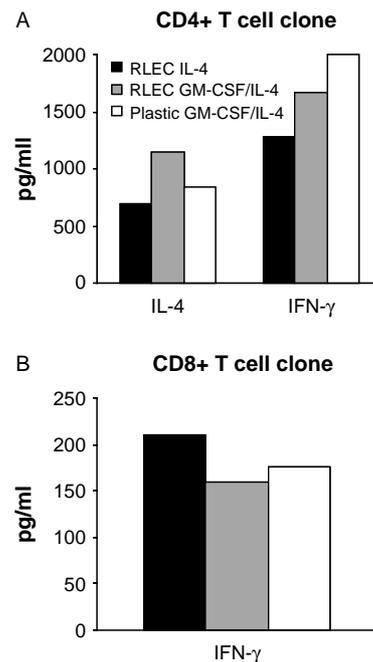


Fig. 6. T-cell clones activation by DC generated in RLEC co-cultures. Monocytes were cultured for 5 days on plastic or onto RLEC with the indicated cytokine combinations. (A) Hemagglutinin-specific CD4+ T-cell clone (5×10^4) was cultured in the presence of DCs (2.5×10^3) with or without hemagglutinin peptide. T-cell response was evaluated 24 h after stimulation by measuring IL-4 and IFN- γ secretions by ELISA. One experiment representative of four experiments made with different clones is shown. (B) M77-84, CD8+ T-cell clone (5×10^3) was cultured in the presence of DCs (3×10^4) with or without Melan-A/MART-1 peptide. T-cell response was evaluated 18 h after stimulation by measuring IFN- γ secretion by ELISA. One experiment representative of four experiments is shown. All results are expressed as the difference between secretions from cultures with and without peptide and values are the mean of triplicate.

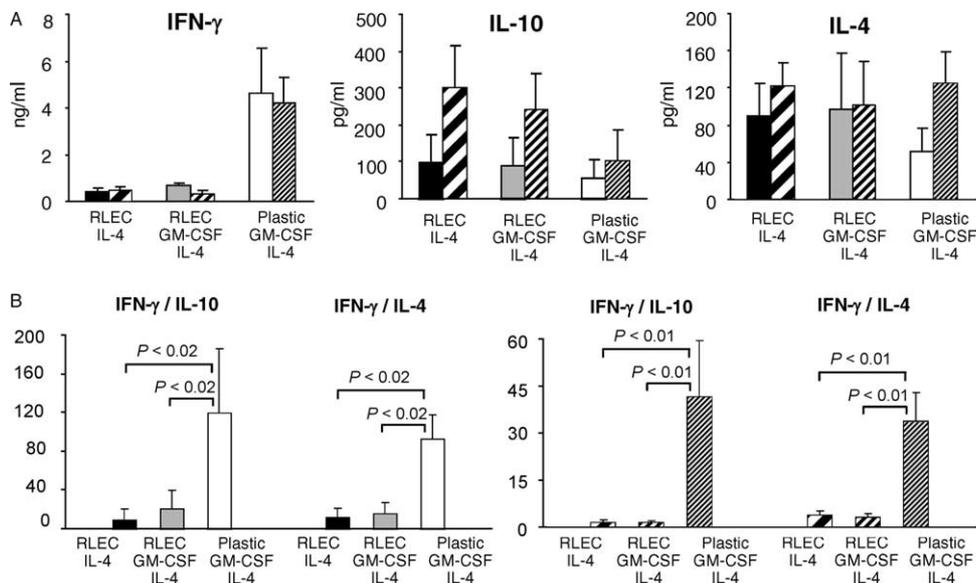


Fig. 7. Cytokine secretion by allogeneic T-cells after stimulation with DCs. Allogeneic T-cells (1×10^5) were cultured with DCs (5×10^3) differentiated in RLEC environment with the indicated cytokine combinations and with control DCs generated onto plastic in the presence of GM-CSF and IL-4. Five-day-old immature DCs (plain bars) as well as DCs stimulated two additional days by CD40L ($1 \mu\text{g/mL}$) and IFN- γ (1000 UI/mL) (hatched bars) were used as stimulator cells. T-cells (1×10^5) were collected after 5 days of stimulation, washed and re-stimulated with anti-CD3 and anti-CD28 mAbs, mAbs is lacking. Supernatants were harvested and analyzed by ELISA for IFN- γ , IL-10 and IL-4 production. $n = 6$ for immature DCs and $n = 3$ for CD40L/IFN- γ stimulated DCs.

and heart are more likely involved in peripheral tolerance whereas skin and gut represent crucial barriers against the external environment and require strong immune response to eliminate invading pathogens [42].

In conclusion, our data demonstrate that local tissue environment drives DC differentiation and function. They support the possibility that multiple subsets of DCs could be elicited according to tissue niches within organs, in physiological and pathological situations [15]. Culture systems with stromal cells mimicking local tissue environment could allow better understanding of DC hematopoiesis. In this context, RLEC co-culture could be a useful model for studying hepatic DC development and for screening molecules susceptible to modulate DC functions.

Acknowledgements

We thank Profs. F. Jotereau and V. Catros for providing us M77-84 T-cell clone and human renal cells, respectively, Prof. O. Fardel for critical reading of the manuscript, Annette Gaudin for assistance with elutriation and the Biological Resource Center of Rennes for supply of human hepatic cells.

This study was supported by Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Association pour la Recherche sur le Cancer and Ligue Nationale contre le Cancer.

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