Modulation of Dendritic Cell Activation by Chemokines and Cellular Injury

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Dendritic cells (DC) are professional antigen presenting cells expressing MHC class II, derived from a common marrow precursor. They are motile, diffused and have a spidery shape with many long cytoplasmic processes. The aim of this project was to test the hypothesis that cellular injury induces the activation and functional maturation of DC. To test the effects of injury on DC activation, immature DCs were used as substrate for DC activation assays. They were obtained from their precursor in peripheral blood mononuclear cells (PBMNCs) by culturing them GM-CSF and IL-4. Expression of surface B7 was measured by immunofluorescence and flowcytometry. β- chemokines were used as potential injury mediators, including: RANTES, MIP-1α, MIP-1β, MCP-1,-2,-3 and -4, as well as other inflammatory cytokines such as TNF-α and IL-1. They were screened on immature DCs to examine whether or not they modulate B7-1 and B7-2. A model of cellular injury was established to investigate whether the injured parenchymal cells deliver signals to initiate DC activation or upregulation of B7-1/B7-2 by release of soluble mediators. H_2O_2 was used as an injury mediator to injure renal tubular epithelial cells (RTECs). RANTES, MIP-1α and MIP-1β upregulated B7-1. MCP-1,-2,-3 and -4 downregulated the expression of HLA-DR greatly. Furthermore, MCP-1,-2,-3 and -4 upregulated B7.2, while and -4 and MCP2 upregulated B7.1. We observed that immature DCs could not be readily stimulated with chemokines and pro-inflammatory cytokines IL-1 and TNF-α unless GM-CSF and IL-4 were used continuously. The supernatant of injured renal epithelial cells had an effect on DC activation. These findings may explain the role of DCs as a link between the innate and the adaptive immune response, as well as being an active participant in determining the outcome of an antigen encounter.

In humans, there are 2 major dendritic cells (DCs) subsets (Ueno H *et al* 2008); CD11c+ myeloid DCs (DC1) and CD11c- lymphoid DCs (DC2). Myeloid DCs are potent antigen presenting cells (APC) of the adaptive immune system, while lymphoid DCs play an important role in innate immunity by producing large amounts of IFNα and-β. All myeloid DCs secrete IL-12 upon activation (Gatti *et al.,* 2000;

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Langenkamp *et al.,* 2000; Steinman *et al.,* 2006). In most tissues, the majority of DCs are present as immature cells that are unable to present antigens to T cells. They have low levels of CD80, CD86 and CD40 (Granucci *et al*., 2001). However, they express MHC class-II at high levels (Stern *et al*., 2006). DC activation leads to the increase of cell surface expression of MHC class-I and II, CD80, CD86, CD40 and CCR7 (maturation markers) (Granucci *et al.,* 2001; Lanzavecchia *et al.,* 2001; Sallusto *et al.,* 2000; Caux *et al.,* 2002; Tan *et al.,* 2005). Of particular interest, the β-chemokines CCL20/MIP-3α and its receptor CCR6 control the migration of different cell types to the rheumatoid arthritis (RA) synovium.

Both immature and mature DCs have a considerable role in RA synovium (Dieu *et al*., 1998; Lutzky *et al.,* 2007). DCs are vital in regulating the immune responses. As professional APC, they have an unique ability to induce the immune response and to transfer information from the outside world to the cells of the adaptive immune system. Additionally, DCs are able to induce tolerance (Menges *et al*., 2002). As immature DCs, they are seeded via the blood into the tissue where they take up antigens and respond to changes in the cellular environment. Invading pathogens induce the activation and maturation of DCs. Mature DCs reach local lymph nodes where they prime and activate T cells (Miossec *et al*., 2008; Joffre *et al.,* 2009). Both CD40L and IL-1 play an important role in regulating DC activation, enable the production of pro-inflammatory cytokines by DCs, and enhance the differentiation of naïve T cells into effector cells (Wesa *et al.,* 2002). T-cell interaction with DCs (in the presence or absence of antigen) can result in dynamic contacts that last for minutes or hours (Bousso, 2008).

The purpose of this study was to test the effects of these mediators on DCs activation in the presence of GM-CSF and IL-4, and to investigate whether injured parenchymal cells deliver signals to a DC precursor to initiate DC activation or upregulation of B7-1/B7-2 by release of soluble mediators. Therefore, renal tubular epithelial cells (RTECs) were exposed to injury using H_2O_2 (Hernandez *et al.,* 1987; Jaeschke, 1991; Miller *et al.,* 1992; Rugtveit *et al.,* 1995; Bender *et al.,* 1996).

We chose to select Chemokines for testing the injury hypothesis because they are secreted by a wide variety of parenchymal cells, and they are also produced in response to a wide array of injurious stimuli (Sallusto *et al.,* 1995). The group of β-chemokines are more attractive because they act preferentially on monocytes which are the closest lineage to DCs (Rugtveit *et al.,* 1995).

Materials and Methods

Culture Medium

RPMI (GibcoBRL) supplemented with 10% FCS (Globe Pharm), L- glutamine (Gibco BRL), and penicillin/streptomycin (GibcoBRL), and was used throughout for culture of blood derived DCs and dendritic cell lines. Medium 199 (GibcoBRL) supplemented with 20% FCS (Globe Pharm), insulin, triiodothyronine, and hydrocortisone (Sigma), and was maintained regularly and used for culture of RTECs. Dulbecco's Modified Eagle Medium (DMEM) containing sodium pyruvate and glucose (GibcoBRL), was supplemented with 5% FCS (Globe Pharm), L-glutamine.

Cytokines & Chemokines

Recombinant human cytokines including:- IFN-γ, TNF-α , IL-1β, IL-4, GM-CSF were obtained from (First Link, UK). RANTES, MIP-1α, MIP-1β, MCP-1,-2,-3 and -4 were purchased from Genzyme (MA, USA). The concentrations were followed, without modification, as recommended by the manufacturer.

Reagents

LPS was obtained from (Dipco), Lymphoprep^{TM} from (Nycomed Pharma), Phorbol dibutyrate (PDBU), Ionomycin, insulin triiodothyronine, hydrocortisone and H_2O_2 from (Sigma).

Antibodies

Primary antibodies used for immunofluorescence and flowcytometry were as follows: CD3, CD19, CD14,

CD40, CD54, CD58, CD57 and HLA-DR (Dako), Human Class-I and -II (Becton-Dickinson), anti B7-1/B7-2 (Becton-Dickinson).

Counting Viable Cells on a Haemocytometer

A haemocytometer was used throughout to estimate the number of viable cells in a given suspension. To count the cells, 10µl of cell suspension was taken and mixed with an equal volume of trypan blue (0.4%, Sigma). Ten microliters of the mixture was drawn up and applied to a haemocytometer.

Culture of DCs from Human Peripheral Blood

Peripheral blood was obtained from healthy volunteers. It was diluted 1:1 with complete medium (RPMI+10% FCS). The diluted blood was then layered onto the surface of an equal volume of Lymphoprep™ (Nycomed Pharma), and the gradient was centrifuged at 447 xg room temperature for 20 min at. Peripheral blood mononuclear cells (PBMCs) were obtained from the interface cell layer and washed three times in complete medium by centrifugation at 252 xg at 4°C for 5 min. PBMCs were counted using trypan blue (0.4%; Sigma) to enumerate viable cells using a hemocytometer. PBMCs were cultured in RPMI-10% FCS at a concentration of 10^6 cells/ml in the tissue flasks (Costar), and allowed to adhere to the flask for 2 hours in a 5% CO2 incubator at 37°C. After 2 hours, the non-adherent cells were removed by pipetting. Cells were washed gently with warm medium (37°C), and adherent cells were cultured in complete medium (RPMI- 10% FCS) supplemented with 50ng/ml GM-CSF and 25ng/ml IL-4 at 37°C for 7 days. Cultures were fed on day 4 with fresh medium with cytokines (50ng/ml GM-CSF and 25ng/ml IL-4). On day 7, non-adherent DCs were harvested by vigorous washing of the flask with cold medium, and resuspended in fresh complete medium after washing once by centrifugation at 252 xg at 4°C for 5 min. They were counted and transferred to a 24 well culture plate (Nunclon) at a concentration of $5x10^5$ cells/well. They were either further cultured in the absence of any stimuli for 2 days (resting phase) and then in the presence of stimuli for activation for another 2 days (stimulation phase in the absence of cytokines). Alternatively, they were cultured in the presence of stimuli and GM-CSF (50ng/ml) and IL-4 (25 ng/ml) for 3 days at 37°C.

Identification and Purification of DCs

Since there was no human DC-specific lineage marker, DCs were identified according to the following criteria**:-** (i) DC morphology by inverted phase contrast microscopy; ii) lack of expression of the markers specified to other lineages, namely CD3 (pan T cell marker), CD19 (pan B cell marker), CD57 (NK cell marker), and CD14 (macrophage lineage marker).

Lymphocyte depleted PBMCs were cultured for 7 days in FCS-containing RPMI in the presence of GM-CSF and IL-4. Cells were then harvested on day 7 and plated in fresh medium containing GM-CSF and IL-4 for 3 additional days. Phase contrast microphotographs were taken to illustrate the morphology of DCs**.** Cells appeared as clumps or isolated floating cells. Clumps of cells displayed sheet-like processes (veils), and cells appeared to adhere loosely to each other through their dendrites. These clumps of cells were non-adherent and floated in the culture. Isolated floating cells also displayed veils at their edges and were large. To test the purity of DCs, they were harvested after culturing them for 7 days in the presence of IL-4 and GM-CSF and the expression of different lineage specific markers, as well as expression of MHC class II molecules, were measured by direct immunofluoresence and flowcytometry.

- DC Activation by β-Chemokines

Immature DCs were generated from PBMNCs of healthy individuals by a 6 day culture in the presence of IL-4 and GM-CSF. Mature DCs were obtained by a further incubation with 1µg/ml LPS for 24 days.

Having confirmed that B7-1, B7-2 and MHC class II can be upregulated by TNF-α, IL-1 and LPS in the presence of GM-CSF and IL-4, an in vitro culture assay was then set up to test the effect of β-chemokines on DC activation. Stimulated DCs were harvested after propagation in the presence of GM-CSF and IL-4 for 7 days with a group of β-chemokines including RANTES, MIP-1α, MIP-1β, MCP-1, 2, -3 and -4 for various durations (1-3 days) and concentrations (10-100ng/ml).

Cytokine Determination

An ELISA kit was purchased from Genzyme (Leuven, Belgium) for the quantification of IL-12p70. IL-10 concentration was measured by using 2-site sandwich ELISA with antibody from Pharmingen (San Diego, CA, USA). DC cells were incubated in fresh medium for 3 days before their overnight stimulation with LPS, TNF and IL-1 in the presence of IL-4 and GM-CSF.

Culture of renal tubular epithelial cells (RTECs)

A vial of frozen renal tubular epithelial cells (RTEC) was removed from liquid nitrogen and thawed at 37°C. The cells were then washed once in complete medium (M199-20%FCS) at 4°C for 5 min-then centrifuged at 252 xg. The pellet was resuspended in fresh complete medium, and cells were cultured in T25 (Costar) culture flasks at 37°C in 5% CO_2. Cells were split 1:2

when they became confluent. To spilt RTEC, the medium was removed, and the adherent cells were washed 3 times with 2ml of PBS. Two ml of trypsin+EDTA (GibcoBRL) was added to release the adherent cells. Serum containing medium was added to reduce the action of trypsin. The medium were then split in two halves, each of which was made up to 5mls.

Injury Experiment

-Determination of the Range of Dosage and the Time Required for H_2O_2 to Achieve Sublethal Injury to RTEC

The RTECs were plated in a 24 wells culture plate (Nunclon) and were incubated until they became confluent. Confluent cells were then exposed to H_2O_2 at final concentrations of 100mM, 500mM, and 1000mM, for periods 15, 60, and 120 min. For each time point, the medium of wells containing injured epithelial cells was rinsed off and trypan blue diluted 1:1 with complete medium was added to each well. The number of dead cells was counted using converted phase contrast microscopy.

-Blood Derived DCs Injured Epithelial Cell Interaction

In preliminary experiments the sublethal dose of H_2O_2 was determined. The RTECs were injured with H_2O_2 at a concentration of 500μM for 15 min. These cells were then washed once with complete medium, and incubated with fresh medium at 37°C. The supernatant of injured cells (one day after being injured) was used to stimulate DCs at a concentration of $5x10^5$ cells/well. The DCs were also stimulated with the supernatant of injured epithelial cells for 3-5 days in the presence of cytokines (50ng/ml GM-CSF and 25ng/ml IL-4).

Flowcytometry

One million cells were washed in PBS and plated in 10ml of MEM (Sigma-Aldrich) with 1% Engelbreth-Holm-Swarm-extracellular matrix (EHS-ECM). After a further 24 hours, the cells were removed by trypsin, washed in PBS, fixed with 2% paraformaldehyde for 20 minutes and then permeabilised with 0.1% saponin in PBS/10% FCS. Cells were then digested with DNase (Invitrogen) for 1 hour at 37°C. Subsequent to incubation with FITC conjugated appropriate antibodies, the percentage of positive cells was measured on a FACS machine.

Immunofluorescence

Double colour fluorescence was carried out by incubating cells (10^5 cells) with directly conjugated monoclonal antibody (mAb) either with FITC (green) or PE (orange). mAb were diluted 1:100 with PBS. Isotype matched antibodies were run in parallel. Samples were viewed using a Zeiss microscope.

Statistical Analysis

Statistical analysis was carried out using Microsoft Excel spreadsheet and the StatView SE +graphics software. The probability of a significant difference between groups was determined by Mann-Whitney U test and Wilcoxon Signed rank test. All software was run on a Macintosh computer.

Results

Activation of Blood derived DCs

Having established that DCs derived by the protocol described in the methods section were typical immature DC, next, an *in vitro* culture assay was set up to measure the activation of DCs. In the first experiment, DCs were stimulated by TNF-α, IL-l and LPS, which are known to upregulate B7-1 and B7-2 on DC and other cells. Additionally, variable periods (1-3 days), and TNF-α (10-50 ng/ml), IL-1 (10-50 ng/ml) were titrated. It was observed that expression of MHC class-II and HLA-DR was upregulated by IL-l and LPS and, to a lesser extent, by TNF-α. Similarly, the expression of B7-1 was upregulated by IL-l and LPS, and to a lesser extent by TNF-α. The expressed B7-2 was upregulated by LPS, but not by IL-l and TNF-α (Table 1).

Furthermore, the cell density was also titrated in DC activation assays to see whether or not DC activated can be affected by cell density. Therefore, DCs were activated at different density with the same concentration of LPS (5ug/ml) for 2 days. However, DC activation was not affected by cell density, and the levels of MHC class II and B7-1 upregulation were the same for DCs activated by LPS at different densities (Table 2).

Presence of GM-CSF and IL-4 facilitated upregulation of B7-1 B7-2 and MHC class II molecules by pro-inflammatory cytokines and LPS when compared to untreated cells. Cells treated with TNF-α or IL-l showed an

increased expression of class II molecules and B7-1, and those treated with LPS showed an expression of MHC class II, B7-1 and B7-2. However, in the continual presence of IL-4 and GM-CSF, the upregulation of these molecules increase further. Expression of B7-2 molecules was also observed in cells that were treated with TNF α and IL-1 (Table-1).

Table-1. Cell Surface Markers of DC Stimulated with TNF-α, IL-1 and LPS in the Presence or Absence of GM-CSF and IL-4.

Stimulator	B7-1 (CD80)	B7-2 (CD86)	MHC class-II	HLA-DR
TNF-α	+	-	+	-
TNF-α+ GM-CSF+IL-4	+++	+	+++	+++
IL-1	+	-	+	-
IL-1+ GM-CSF+IL-4	+++	+	+++	+++
LPS	+	+	+	-
LPS+ GM-CSF+IL-4	+++	+++	+++	+++

Table 2. The Effect of Cell Density on the DC Activation

Cell Density	Stimulator	B7-1	B7-2	MHC class-II	HLA-DR
$5x10^5$	No LPS	-	-	-	
$5x10^5$	LPS	-	+	+	+
$2x10^5$	LPS	-	+	+	+
$1x10^5$	LPS	-	+	+	+
$5x10^4$	LPS	-	+	+	+

DC Activation by β-Chemokines

β-chemokines could not facilitate the upregulation of MHC class-II, B7-1, B7-2 CD40, CD54 and CD58 molecules on DCs that had been stimulated in the absence or GM-CSF and IL-4. In the continual presence of GM-CSF and IL-4, β-chemokines could not mediate upregulation of MHC class II, B7-1 and B7-2 molecules when used at low concentrations in the DC activation assay. RANTES, MIP-1α MIP-1β, MCP-1, MCP-2, MCP-3 and MCP-4 did not facilitate DC activation when they were used at 50 ng/ml. However, it was observed that RANTES and MIP -1β facilitated the upregulation of MHC class II, B7 -1, CD40, CD54 and CD58 molecules, but not B7-2, when they were used at 500-1000 ng/ml. The upregulation of the B7-1 molecules was only observed when MIP -1α was used at 500 ng/ml (Table-3).

The expression of B7-1 was upregulated on DCs when they were activated by MCP-2 and MCP-4 (1000 ng/ml), and to a lesser extent by MCP-3 (1000 ng/ml), but not by MCP-1 (at 1000 ng/ml). On the other hand, the expression of B7-2 was upregulated by treating cells with MCP-1 and MCP-4 and to a lesser extent by MCP-3 and MCP-2. However, the expression of MHC class II molecules was downregulated significantly on

DCs that were activated by MCP-1, MCP-2, MCP-3 and MCP-4 (Table-3), indicating that, even in the continuous presence of IL-4 and GM-CSF, β-chemokines have different effects on DC activation.

Table 3. Cell Surface Markers of DC Stimulated with β-Chemokines in the Presence or Absence of GM-CSF and IL-4.

Stimulator	B7-1 (CD80)	B7-2 (CD86)	MHC class-II	CD40	CD54	CD58
RANTES	-	-	-	-	-	-
RANTES + GM-CSF + IL-4	-	+	-	+	+	+
MIP-1α	-	-	-	-	-	-
MIP-1α + GM-CSF + IL-4	-	-	+	+	+	+
MIP-1β	-	-	-	-	-	-
MIP-1β + GM-CSF + IL-4	-	+	+	+	+	+
MCP-1	-	-	-	-	-	-
MCP-1 + GM-CSF + IL-4	+	-	-	+	+	+
MCP-2	-	-	-	-	-	-
MCP-2 + GM-CSF + IL-4	+	+	-	+	+	+
MCP-3	-	-	-	-	-	-
MCP-3 + GM-CSF + IL-4	+	+	-	+	+	+
MCP-4	-	-	-	-	-	-
MCP-4 + GM-CSF + IL-4	+	+	-	+	+	+

DC Ativation by Supernatant of Injured Renal Tubular Epithelial Cells

In RTECs exposed to various concentrations of H_2O_2 for 15min, 60 min and 120 min, the number of dead cells was directly proportional to the dose of H_2O_2 in the culture medium. Maximum cell death was observed at a concentration of 1000mM H_2O_2. The number of dead cells was directly linked to the length of time for which epithelial cells were directly exposed to H_2O_2 (Table 4). It was also observed that the expression of B7-1, B7-2 and MHC class II molecules were upregulated by the supernatant of injured renal tubular epithelial cells.

Cytokines Production by DCs

Significant levels of IL-12p70 were only detected in supernatant of mature DCs obtained after LPS exposure (Table 5). Interestingly, IL-10 was significantly produced after activation compared to non primed DCs.

Table 4. The Range of Dosage of H_2O_2 Required to Achieve Lethal Injury to Epithelial Cells.

H_2O_2 dose	Number of dead cells/4 fields	Number of dead cells/4 fields	Number of dead cells/4 fields
	15min	60min	120min
100mM	55	77	104
500mM	76	85	143
1000mM	106	125	255

Table 5. Cytokines Produced by DC Stimulated with TNF-α, IL-1 and LPS in the Presence GM-CSF and IL-4.

Serial No.	IL-12p70 (pg/ml) Without LPS	IL-12p70 (pg/ml) With LPS	IL-10 (pg/ml) Without LPS	IL-10 (pg/ml) With LPS
n=4	3.4	213	26.4	180

Discussion

It has been previously proposed, based on Janeway's hypothesis (Janeway, 1992), that the innate immune system is activated not only by conserved microbial structures, but also by receiving a signal through microenvironmental cell injury (Ibrahim, 1995). According to this hypothesis, DCs play a role as cellular sentinels which have evolved to respond to injury by maturation and upregulation of the costimulatory molecule during their migration from the site of injury to the secondary lymphoid organs. DCs from non lymphoid peripheral organs migrate to secondary lymphoid organs reflecting the presence of two different functional states of DCs (Larsen *et al*., 1990). An immature non-lymphoid DC specialises in antigen capture/ processing which leads to the mature lymphoid DCs with potent immune-stimulation of T cells. Evidence for this clonal dichotomy of DCs included studies in which freshly isolated LCs, have been shown to acquire the properties of mature DCs when exposed to either GM-CSF alone, or in combination with either IL-l or TNF-α (Kock *et al.,* 1990; Heufler *et al*., 1992).

We felt it appropriate to test the effect of cellular injury on functional maturation of DCs, supernatant of injured renal epithelial cells, β-chemokines as well as microbial derivative (e.g., LPS) and inflammatory cytokines. They were chosen as surrogates to injury and were screened on immature DCs to examine whether or not they modulate the expression costimulatory molecules B7-1 and B7-2. DCs which propagated from progenitors by GM-CSF and IL-4 could not be readily stimulated with pro-inflammatory cytokines such as IL-1 and TNF-α and to a lesser extent LPS (Jaeschke 1991; Romani *et al.,* 1994). It was possible to demonstrate the induction of activation of DCs by pro-inflammatory cytokines and LPS as measured by the upregulation of HLA-DR, B7-1 and B7-2. This was consistent with previous publications suggesting that withdrawal of GM-CSF/IL-4 from DC culture would cause reversal to immature phenotype (Bender *et al*., 1996; Romani *et al*., 1996).

DCs that developed in GM-CSF and IL-4 for 7 days did not reach a mature phenotype when deprived from cytokines and stimulatory mediators. However, the significant upregulation of costimulatory

molecules as well as MHC class II by stimulating DCs with TNT-α and IL-1, and LPS (in presence of GM-CSF and IL-4) may indicate that the presence of these two cytokines in the culture along with stimulatory mediators is required for full maturation of DCs. This has been confirmed by results obtained from activation of DCs with β-chemokines including RANTES, MIP-1α and MIP-1β in the continuous presence of GM-CSF and IL-4. Therefore, GM-CSF and IL-4 are not only required for differentiation of DCs from their progenitor (Sallusto *et al*., 1994), but also they induce full maturation of DCs in synergy with other stimulatory mediators.

The β-chemokines were chosen to test the effect of injury on DC activation because they act preferentially on monocytes (which have the closest lineage to DCs) (Rossi *et al*., 1992). They are produced by an array of cells including keratinocytes, mesangial cells, epithelial cells, fibroblasts, smooth muscle cells and endothelial cells. These cells can produce chemokines in response to a wide variety of injurious stimuli, microbial or non microbial, including viruses, bacterial products, IL-1, TNF-α C5a, and IFN-γ (Strieter *et al.,* 1996). It has been shown that cardiac myocytes release chemotactic cytokines such as MCPs when they are stimulated with either IL-1, TNF-α or LPS (Massey *et al*., 1995). In a rat model of lung injury induced by hepatic ischemia/ reperfusion, a chemoattractant factor appeared to play an important role in lung pathology (Colletti *et al*., 1995). Human lung microvasculature endothelial cells secreted MCP-1 when these cells were activated with IL-1, TNF-α, IFN-γ or LPS (Brown *et al*., 1994). RANTES expression has been shown to be increased in cultured mouse mesangial cells when these cells were stimulated with TNF-α, IL-1 or LPS (Wolf *et al*., 1993).

Cultured human synovial fibroblasts express mRNA for IL-8, MCP-1 and RANTES, when stimulated with IL-1 and TNF-α (Jordan *et al*., 1995). As the name implies, the primary function of chemokines that are secreted in response to a variety of injurious stimuli is chemotaxis. However, it has become increasingly apparent that they may also regulate the function of the cells they recruit. For example, β–chemokines stimulate monocytes to modulate ionic Ca2+ (Bischoff *et al*., 1993), induce a respiratory burst (Sallusto *et al*., 1995), and express β2 integrins (Jiang *et al*., 1992).

The findings of this study may suggest that these β-chemokines are not only involved in the activation of DCs, but also in the recruiting them to sites of infection. This suggests that chemokines have a dual function which may be exploited in the design of novel immuno-therapies. This is also a link between innate and adaptive immunity, through which activation of the innate immune system leads to induction of the adaptive immune response. Results obtained in this study confirmed that RANTES, MIP-1α and MIB-1β can activate DCs by upregulation of B7-1 and MHC class II molecules.

These inflammation mediators and cytokines can also be produced by macrophages in response to microbial molecules. For example, when macrophages encounter LPS, they produce a variety of mediators such as IL-1, IL-6 and TNF-α, which contribute to both local and systemic effects that mediate immune response (Ibrahim *et al*., 1995). However, local production of TNF-α at sites of encounters with infectious antigens by macrophages may induce the maturation of DCs and their migration to the secondary lymphoid organs (Sallusto *et al*., 1994).

Additionally, it was observed that the supernatant of injured RTECs cause the upregulation of B7-1, B7-2 and MHC class II. RTECs were grown in medium 199 +20% FCS which is richer than RPMI containing many more vitamins and amino acids, as well

as other components such as Tween 80, (which is a detergent and might itself be injurious). However, it is likely that the effect of the mediators secreted by injured epithelial cells was inhibited by either some other component of medium 199, or by FCS. The latter is enriched by multiple growth factors that are present in the blood of a developing foetus. It is also likely that some of the constituents of medium 199 interfere with the activity of DCs that were stimulated with supernatant of injured epithelial cells. Since DCs were grown in 10% FCS containing RPMI, increasing the amount of FCS in the culture medium (from 10% to 20%) might therefore interfered with the activation of DCs that have been stimulated with the supernatant of injured cells.

DCs in the epidermis are essential for presenting antigens to previously primed Th-2 and crucial for the development of many skin diseases. Our results showed that DC generated from human peripheral monocytes were also potent to APC for CD4 T cells stimulated with LPS. High levels of IL-12 and IL-10 were measured in the culture supernatant when stimulated by LPS display a Th-2 phenotype whatever the type of DCs (ie., immature vs mature) used to present the antigen. Taken together, this may suggest, that immune deviation strategies with DCs and exogenous cytokines might be limited by the fixed phenotype of antigen-specific Th-2 cells.

In conclusion, since there might be differences between the expression of B7 molecules at both mRNA and surface protein levels, further characterization of the expression of B7 molecules at the mRNA level is required. Upregulation of B7 molecules on DCs after interaction with T cells should also be investigated.

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