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Inflammation Research

Human monocyte-derived dendritic cells are a source of several complement proteins

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Abstract. *Objective:* Little is known about the role of local production of complement components by dendritic cells (DCs) during the generation of specific immune responses. In this study, we demonstrate that human DCs are an extra-hepatic source of several soluble complement proteins.
Methods: Reverse transcriptase polymerase chain reaction (RT-PCR) and Western blot were used to evaluate the expression and production of several complement proteins.
Results: We show that DCs produce C3, C5, C9, Factor (F)I, FH, FB, FD and properdin at levels similar to macrophages. Treatment of DCs with lipopolysaccharide (LPS) promoted an increase in the expression of C3 and FI mRNAs and a decrease in C5 mRNA, while C9, FH, FB, FD and properdin mRNA levels were not affected. Treatment with interleukin (IL) -1 or dexamethasone induced a modest increase in C3 mRNA levels and did not affect the expression of other complement components.
Conclusion: DCs are a source of complement proteins whose synthesis may be regulated in response to different inflammatory stimuli.

Key words: Dendritic cells – Complement – Innate immunity

Introduction

The alternative pathway is the most important route for complement system activation in innate immunity. It forms C3- and C5-convertases that generate active products related to complement biological functions. C3b attaches covalently to antigen surfaces and other active C3-derived fragments bind to specific receptors present on a variety of inflammatory cells. These polypeptides mediate phagocytosis, activate the inflammatory response via release of C3a and C5a anaphylatoxins and solubilize and clear immune-complexes [1]. Moreover, C3d also acts as an adjuvant enhancing acquired immunity and mediates the activation of B cells, thereby increasing antibody production against T-dependent antigens [1].

The primary source for the majority of complement proteins is the liver but other sources include monocytes and macrophages, polymorphonuclear leukocytes, fibroblasts and endothelial cells [2]. In the kidney [3] and brain [4], local complement synthesis by resident or infiltrating cells is responsible for the maintenance of inflammatory processes and the importance of locally produced C3 has been demonstrated by several groups [3, 5]. The importance of complement proteins produced outside the liver was clearly demonstrated when $C3^{(-/-)}$ mice were reconstituted with wild-type bone marrow and local C3 synthesis was restored in the spleen and humoral response was rescued [5]. Local C3 synthesis has been implicated as a factor in experimental acute renal allograft rejection [3]. When kidneys from $C3^{(-/-)}$ animals were transplanted into $C3^{(+/+)}$ recipients, these mice survived for at least 100 days. However, if the recipient was $C3^{(-/-)}$, a kidney from $C3^{(+/+)}$ donor was rapidly rejected in 14 days. This observation implies that the local production of C3 may represent a significant determining factor in rejection [3].

Among inflammatory cells, DCs are considered essential for innate and acquired immunity. They act as sentinels of the immune system presenting antigens captured in the periphery to T lymphocytes. Though C1q is secreted by DCs [6] it has not been determined if these cells are capable of producing the complete repertoire of soluble complement proteins. This capacity, if confirmed, would point to DCs as a relevant source of complement proteins at the main entrance of antigens such as natural barriers and in the lymphoid structures. In addition, a local production of complement proteins by DCs could contribute significantly to the evolution of the immune responses.

We therefore decided to analyze the in vitro production of complement proteins by DCs. This production was compared to that of monocyte-derived macrophages, a known local source of complement. In addition, we investigated whether the production of complement by DCs was depend-

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ent upon the maturation of DCs or the nature of the inflammatory stimulus.

Material and methods

Generation of monocyte-derived DCs and macrophages

Monocytes were isolated from buffy coats obtained from healthy donors using Ficoll density gradient centrifugation. After 2 h of culture in 6-well plates (2×10^7 cell/well) the non-adherent cells were removed by washing with phosphate-buffered saline (PBS).

DCs and macrophages were generated in vitro from the adherent fraction of human mononuclear cells cultured in RPMI supplemented with 10 % fetal calf serum. Granulocyte macrophage-colony stimulating factor (50 ng/ml, R&D) and IL-4 (50 ng/ml, R&D) were added to DCs cultures at days 1, 3 and 5. The purity of the DCs cultures was considered adequate when 98 % of cells were $CD14^-/CD1a^+$, a well known phenotype for DCs while macrophages are $CD14^+$ and $CD1a^{low}$. In some experiments LPS (1 µg/ml) was used to induce DC maturation and macrophage activation. In other experiments, IL-1 (5 ng/ml) or dexamethasone (25 ng/ml) were used in order to block DC maturation. Where indicated, LPS, IL-1 or dexamethasone was added at day 5 for 48 h.

Flow cytometry analysis

Cells were harvested and washed in PBS containing 0.5 % BSA and 0.02 % NaN_3. The presence of DCs and macrophages surface markers was evaluated by flow cytometry using a panel of phycoerythrin- or fluorescein isothiocyanate-conjugated monoclonal antibodies specific for CD14 (TüK4), CD1a (VIT6B), HLA-DR (TÜ36), CD86 (BU63) and isotype-controls (Caltag Laboratories). Fluorescence intensity was monitored with a FACScalibur flow cytometer using the CellQuest software.

RT-PCR

At day 7 total RNA was extracted from DCs and macrophages cultures using "Total RNA Isolation System" (Promega) according to manufacturer's instructions. RT-PCR was performed in two steps: 1) 200 ng of total RNA was incubated with 500 mM oligo dT for 5 min at 70 °C followed by addition of 100U of Moloney Murine Leukemia Virus Reverse Transcriptase (Promega), 0.4 mM dNTPs and buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT). The reaction was incubated at 42 °C for 1 h; 2) cDNA (see Table 1) was amplified by PCR (40 cycles) in the presence of 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 0.4 mM dNTPs and 0.8 pmol of each specific primer (see Table 1). No amplification was observed in reactions without cDNA. Levels of human mRNA were evaluated after amplification of the constitutively expressed *human glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene. The sizes of RT-PCR products agree with amplification of cDNA sequences and they span more than one exon (except for C9 cDNA that was amplified with primers within exon 4). No amplification of genomic DNA was observed in the reactions. RT-PCR products were quantified by densitometric analysis using an Alpha Scan Imaging Densitometer (Alpha-Innotech Corporation) and normalized with respect to the GAPDH cDNA signals.

Western blot

Supernatants from DC and macrophages cultures were analyzed by SDS-PAGE electrophoresis (10 % acrylamide gel) before transferring to nitrocellulose membranes. Membranes were blocked by incubation for 12 h with TBST buffer (5 mM Tris, 75 mM NaCl, 0.028 % tween 20) containing 5 % non-fat milk. Primary antibodies specific to human C3, FI and FB (Calbiochem-Novabiochem) were diluted 1: 1000 in TBST containing non-fat milk and incubated with the membranes at 4 °C for 12 h. After washing, the blots were incubated with alkaline phosphatase-conjugated rabbit-antigoat IgG (dilution 1: 10000) for 2 h at room temperature. Immunoreactive bands were visualized after incubating membranes with 5-bromo,4-chloro,3-indolylphosphate (BCIP) and

Table 1. Primers used in RT-PCR.

cDNA	cDNA (µL)	primers*	sequence (5'-3')	annealing temperature (°C)	amplified fragment (bp)
C3	2	1338F 2887R	GAA GAA GCA GGA GCT CTC GG CCA CAG TTT TGT TGA TTC TGA TTC	56	1550
C5	4	1321 F 2710R	ACT GAT GCT CCA GAT CTT CC CAG TGA ATG TCA CCA AGT GA	54	1390
C9	5	333F 417R	GAT GCA TAA AGA TGC GAC TTC GGC TCA CTT TCA CAA TCA TCC	50	85
Factor I	5	699F 1443R	GAT GAC TTC TTT CAG TGT AGC CAG AAA CGA TGC ATG	50	744
Factor H	5	1F 1483R	AAT TCT TGG AAG AGG AGA AC TTT TAA GGC ATA TGT ATA CTG	50	1483
Factor B	2,5	201F 422R	CAC CAC TCC ATG GTC TTT GGC CAC TCT GCC TTC CTG ACA GTC	54	222
Factor D	2	97F 503R	ATG CGC TCG GTG CAG CTG AA TCC AGC ACT GGC AAG AGC AC	56	407
Properdin	2	231F 725R	TGC TGT CTC AAC ACT GCC TT TCG TGT CTC CTT AGG TTC GT	55	495
CR1	2,5	301F 800R	CTA CCA ACC TAA CTG ATG AG ATC GTC ATT GCT GGT GCA GT	56	500
GAPDH	0,5	2F 320R	TCT CTG CTC CTC CTG TTC GAC GGA TCT CGC TCC TGG AAG ATG	50	319

* Numbers refer to nucleotide position in cDNA, F – forward, R – reverse.

nitroblue tetrazolium (NBT) (Promega) in revelation buffer (100 mM NaCl, 100 mM tris pH 9,5 and 5 mM $MgCl_2$). In these analyses, and others performed in our laboratory, anti-human FI (Calbiochem-Novabiochem) preferentially detected FI heavy chain (50 kDa).

Results

DC and macrophage phenotype

Dendritic cells were phenotypically characterized by flow cytometry and exhibited the phenotype $CD14^-/CD1a^+$, clearly distinguishing them from macrophage which are $CD14^+/CD1a^{low}$. As expected, immature DCs presented lower expression levels of CD86 and HLA-DR when compared to mature DCs (Fig. 1).

Complement mRNA expression

Analysis of mRNAs levels of complement proteins from immature and mature DCs and macrophages by RT-PCR showed that C3, C5, C9, FI, FH, FB, FD and properdin mRNAs were expressed by all four types of cells (Fig. 2). Upon LPS-in-

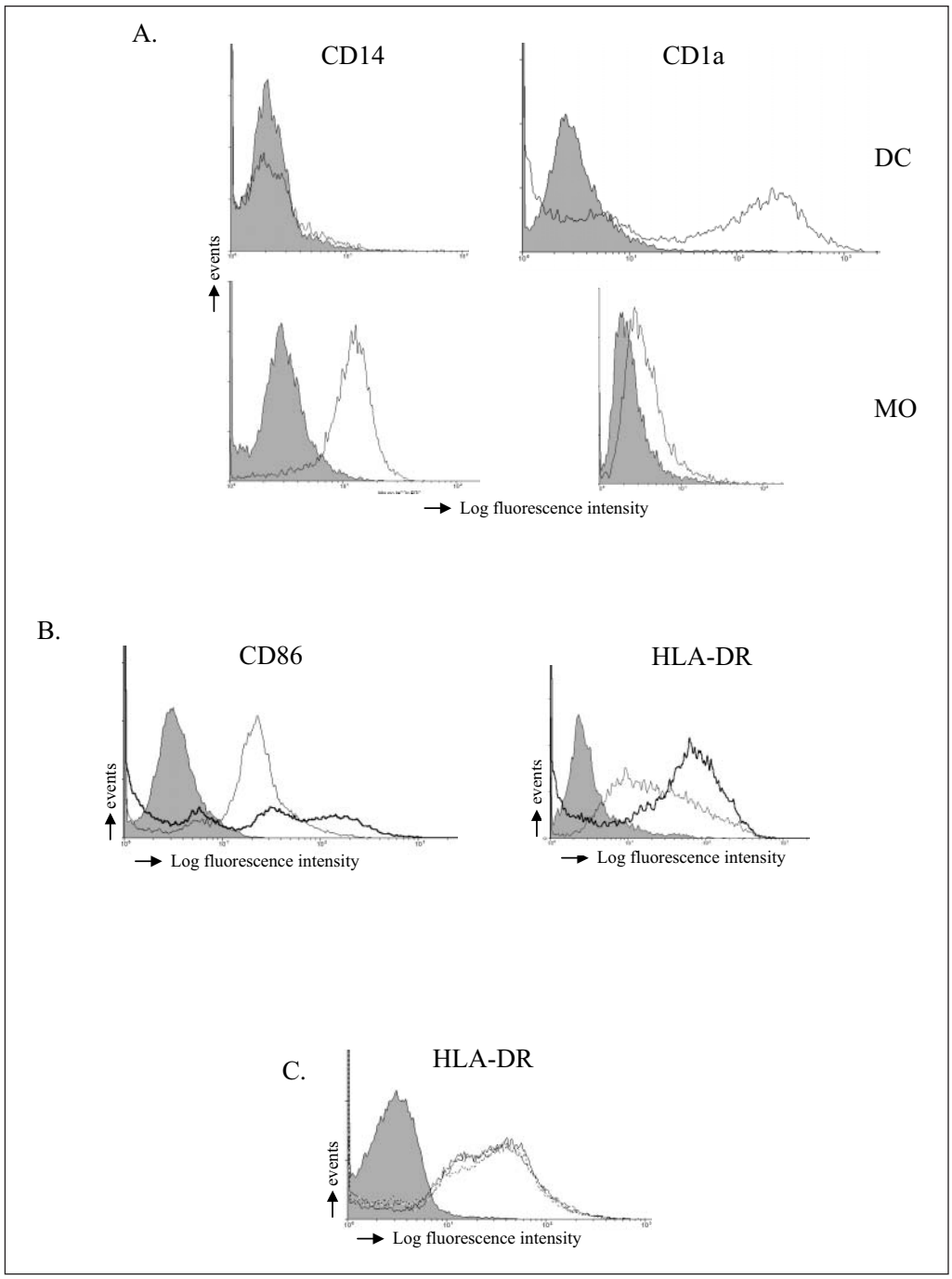

Fig. 1. Dendritic cells (DC) and macrophages (MO) were cultured for 7 days and analyzed by FACS. (A) CD14 and CD1a expression by DC and MO. (B) CD86 and HLA-DR expression by immature DC (thin line) and mature DC (bold line). (C) HLA-DR expression by immature DC (thin line), DC cultured for 48 h with IL-1β (dotted line) and dexamethasone (dashed line). Similar results were obtained in three independent experiments using blood cells from three different healthy adult donors.

duced maturation of DCs, we observed increased expression of C3 (5.5 fold) and FI (4.5 fold) mRNAs and decreased expression of C5 mRNA (2.0 fold). On the other hand, the expression of C9, FH, FB, FD and properdin mRNAs was not affected by LPS-induced maturation of DCs. Complement mRNA expression was very similar for DCs and macrophages, with the exception of FB and FD. While FB expression in macrophages increased (2.0 fold) after LPS treatment, we did not observe an increase of FB expression in mature DCs. The expression of FD was always lower (3.0 fold) in immature or mature DCs than in macrophages (Fig. 2).

We asked whether the regulation of levels of complement mRNAs was a result of DC maturation itself or a consequence of the treatment with LPS. We therefore blocked DC maturation on the 5th day of culture with IL-1 (5 ng/ml) or dexamethasone (25 ng/ml) to inhibit DC maturation. IL-1 is a pro-inflammatory cytokine that regulates the expression of several complement genes. This cytokine inhibits the expression of costimulatory molecules that are important markers of mature DCs. Dexamethasone is a corticosteroid with anti-inflammatory activity and regulates some complement genes. Thus the treatment of DCs either with IL-1 or dexamethasone produced cells with an immature phenotype (Fig. 1).

We observed that DCs incubated with IL-1 or dexamethasone present slightly increased C3 mRNA levels (2.0 fold, Fig. 3). The expression of C5 and FI in LPS-treated DCs was not affected by incubation with either IL-1 or dexamethasone. Since the basal expression of FI mRNA from untreated DC and macrophage cultures was very low (Fig. 2) we used double the amount of cDNA to FI mRNA amplification (Fig. 3). The expression of C9, FH, FB and FD mRNAs in DCs and macrophages was also not affected by these treatments. Therefore, the expression of complement mRNAs is not a consequence of DC maturation per se. Rather, the response

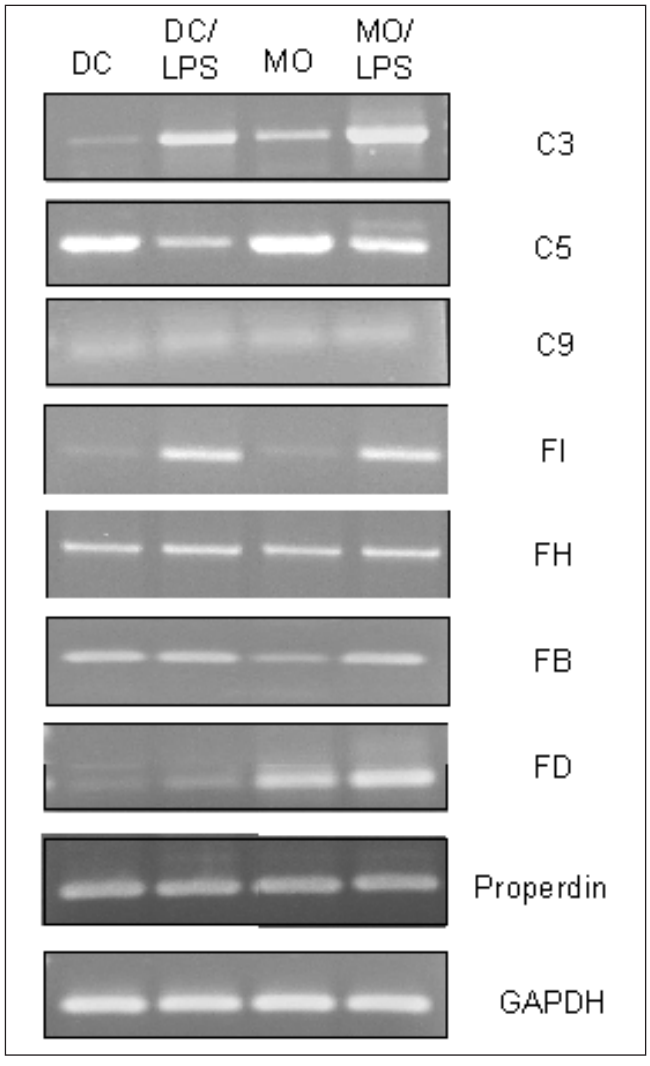

Fig. 2. Complement mRNA expression by immature and mature dendritic cells. The dendritic cells (DCs) maturation was obtained after LPS treatment. Total RNA was extracted from DC or macrophage (MO) cultures after incubation for 48 h with LPS (1 μg/ml). GAPDH was used as a control. Similar results were obtained in three independent experiments using blood cells from three different healthy adult donors.

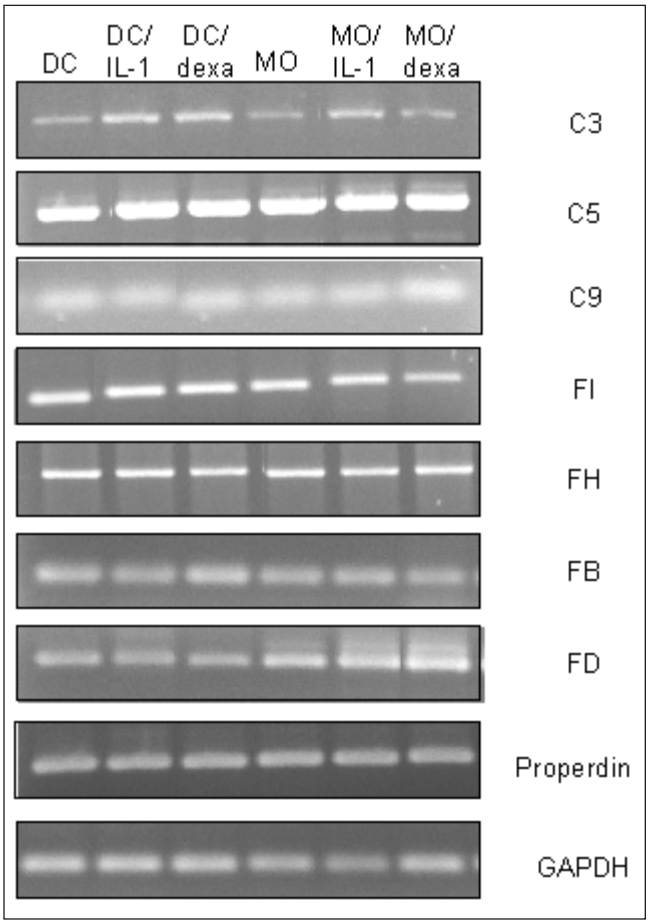

Fig. 3. Complement mRNA expression by dendritic cells after treatment with dexamethasone or IL-1. IL-1 or dexamethasone (dexa) block dendritic cell (DC) maturation. DC and macrophage (MO) cultures were treated with IL-1β (5 ng/ml) or dexamethasone (25 ng/ml) for 48 h. mRNA expression was evaluated by RT-PCR and compared to that of untreated cultures. Similar results were obtained in three independent experiments using blood cells from three different healthy adult donors.

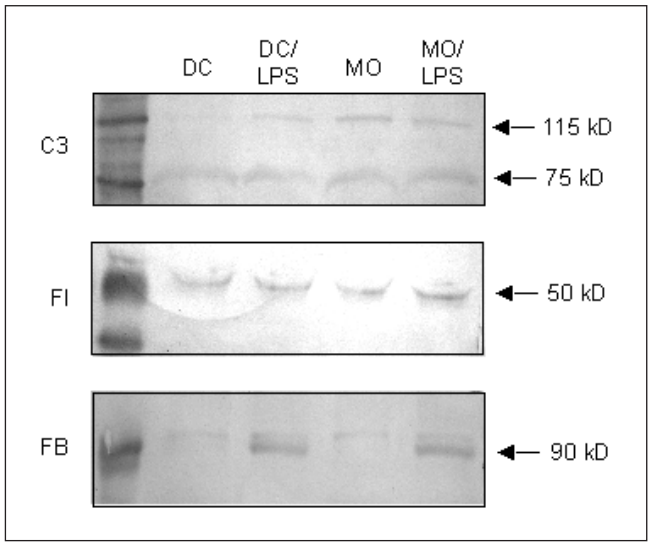

Fig. 4. C3, FI and FB secretion by immature and mature dendritic cells (DC). Day-7 supernatants from macrophages (MO) and DC were analyzed by Western blot for complement protein secretion. The first lane was loaded with purified C3 protein (alpha chain: 115 kDa; beta chain: 75 kDa), FI (heavy chain: 50 kDa; light chain: 38 kDa) or FB (90 kDa). All data refer to a single supernatant. Similar results were obtained in three independent experiments using blood cells from three different healthy adult donors.

of complement gene expression in DCs seems to be both gene and stimuli-specific.

Complement protein production

We evaluated the production of some of the alternative pathway proteins in DC and macrophage supernatants by Western blot. The presence of C3, FI, and FB was analyzed independently on the same cell supernatant using specific antibodies. In each experiment we used blood cells from different healthy adult donors to cultivate DCs and macrophages. In all experiments the results were similar and Figure 4 illustrates one of three independent experiments. Culture medium was included as a negative control and no specific bands were visualized.

C3 [alpha (115 kDa) and beta (75 kDa) chains] and FI (heavy chain 50 kDa) were detected in the supernatant cultures of both mature and immature DCs as well as in macrophage supernatants (Fig. 4). However, in contrast to our findings in RT-PCR experiments (see above), no significant differences in C3 secretion was observed upon LPS-induced DC maturation. A 90 kDa band corresponding to FB single-chain molecule was visualized only in supernatant cultures of mature DCs and activated macrophages (Fig. 4).

Discussion

Here, we have shown for the first time that DCs are able to express C3, C5, C9, FI, FH, FB, FD and properdin mRNAs. We also show that all three proteins tested (C3, FI and FB) are secreted by DC cultures. These latter three proteins are essential for alternative pathway activation (C3 and FB) and regulation (FI). We speculate that the maturation of DCs affects the expression of mRNA and secretion of complement proteins to different degrees. Our results show that LPS treatment did not affect the expression of FB mRNA by DCs. However, LPS-treated cells secreted more FB than untreated DCs. Nevertheless, no difference in the secretion of C3 and FI was observed in DC supernatants before or after maturation.

Analyzing RT-PCR and Western blot results together we did not observe a strict correlation between levels of mRNA expression and protein secretion. This is probably due to the activity of unknown factors essential for the translation, synthesis or secretion of complement proteins.

The primary site of synthesis for most complement proteins is the liver. A local secondary production of complement proteins in different tissues has been attributed to the monocytic macrophage lineage [2]. Considering this, one may argue that part of the expression and/or production of complement proteins observed in the present study may be derived from adherent cells other than DCs in the cultures. However, only 2 % of the cells in our DC cultures did not express the DC phenotype ($CD14^-/CD1a^+$). Since in all evaluations, highly enriched DC cultures expressed complement proteins at levels similar to or only slightly less than that observed in macrophage cultures (100 % $CD14^+/CD1a^{low}$), the products here analyzed must be derived principally from DCs.

Local synthesis of these proteins can be critical at sites where circulating complement is not promptly available for activation and binding to foreign antigens. This hypothesis is consistent with the fact that several complement proteins such as C3, FI and FB are considered acute-phase proteins that can be up-regulated by several stimuli, such as LPS, often present at the inflammatory site during infection. The synthesis of complement by DCs probably does not contribute significantly to overall serum levels of theses proteins. However, DC complement production could significantly affect the local concentrations of these proteins in the DC microenvironments thereby contributing to the regulation of the immune response. Furthermore, it is noteworthy that in some tissues, such as skin and mucosa, DCs can represent up to 2 % of total cells [7, 8], not an irrelevant proportion of the population. Moreover inflammatory signals like anaphylatoxins (e. g. C5a), chemokines (e. g. MIP-1α) and cytokines (e. g. TNF-α) are key microenvironmental factors that determine migration, maturation and functional activity of DCs [9–12]. An increase in DCs number at inflammatory sites could be followed by an increase in complement production and activation and consequent release of anaphylatoxins contributing to the maintenance of inflammatory process.

Complement activation releases C3 and C5 fragments whose receptors are expressed by DCs [9, 13]. It has been shown that C3a [10, 14] and C5a [11, 15] are able to attract immature DCs to the peritoneal cavity of severe combined immunodeficiency (SCID) mice and that iC3b-covered apoptotic cells are more efficiently engulfed by immature DCs [16, 17], resulting in reduced expression of costimulatory molecules and impaired DC maturation. Castellano et al. [6]

have demonstrated that opsonization of apoptotic cells by C1q (produced by DCs) and mannose binding lectin facilitates apoptotic cell uptake by DCs that respond by increasing secretion of IL-6, IL-10 and TNF-alpha but not IL-12 p70 [18]. All these studies point to complement as a local modulator of DC function.

The present study provides evidence for local complement production by DCs. These cells may be a relevant source of complement proteins at the natural barriers or in the lymphoid structures. Moreover, we showed that the expression of complement mRNAs is regulated in a stimuli-specific manner and is independent of DC maturation. Since the alternative pathway is the principal means of complement activation, DCs may contribute, at least partially, to relevant biological functions of this system, including elimination of parasites.

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References

[1] Walport MJ. Complement – First of two parts. N Engl J Med 2001; 344: 1058–66.
[2] Morley BJ, Walport MJ. The Complement Facts Book. 2000. Academic Press. London. 228 p.
[3] Sacks S, Zhou W. The effect of locally synthesized complement on acute renal allograft rejection. J Mol Med 2003; 81: 404–10.
[4] Gasque P, Chan P, Mauger C, Schouft MT, Singhrao S, Dierich MP et al. Identification and characterization of complement C3 receptors on human astrocytes. J Immunol 1996; 156: 2247–55.
[5] Fischer MB, Ma M, Hsu NC, Carroll MC. Local synthesis of C3 within the splenic lymphoid compartment can reconstitute the impaired immune response in C3-deficient mice. J Immunol 1998; 160: 2619–25.
[6] Castellano G, Wolman AM, Nauta AJ, Roos A, Trouw LA, Seelen MA et al. Maturation of dendritic cell abrogates C1q production in vivo and in vitro. Blood 2004; 103: 3813–20.
[7] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392: 245–52.
[8] Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. Annu Rev Immunol 2000; 18: 767–11.
[9] Sozzani S, Sallusto F, Luini W, Zhou D, Piemonti L, Allavena P et al. Migration of dendritic cells in response to formyl peptides, C5a and a distinct set of chemokines. J Immunol 1995; 155: 3292–95.
[10] Soruri A, Kiafard Z, Dettmer C, Riggert J, Kohl J, Zwirner J. IL-4 down-regulates anaphylatoxin receptors in monocytes and dendritic cells and impairs anaphylatoxin-induced migration in vivo. J Immunol 2003; 170: 3306–14.
[11] Soruri A, Riggert J, Schlott T, Kiafard Z, Dettmer C, Zwirner J. Anaphylatoxin C5a induces monocyte recruitment and differentiation into dendritic cells by TNF-alpha and prostaglandin E2-dependent mechanisms. J Immunol 2003; 171: 2631–6.
[12] Sallusto F, Lanzavecchia A. Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. Immunol Rev 2000; 177: 134–40.
[13] Kirchhoff K, Weinmann O, Zwirner J, Begemann G, Gotze O, Kapp A et al. Detection of anaphylatoxin receptors on CD83+ dendritic cells derived from human skin. Immunology 2001; 103: 210–7.
[14] Gutzmer R, Lisewski M, Zwirner J, Mommert S, Diesel C, Wittmann M et al. Human monocyte-derived dendritic cells are chemoattracted to C3a after up-regulation of the C3a receptor with interferons. Immunology 2004; 111: 435–43.
[15] Weinmann O, Gutzmer R, Zwirner J, Wittmann M, Langer K, LIsewski M et al. Up-regulation of C5a receptor expression and function on human monocyte derived dendritic cells by prostanglandin E_2. Immunology 2003; 110: 458–65.
[16] Verbovetski I, Bychkov H, Trahtemberg U, Shapira I, Hareuveni M, Bem-Tal O et al. Opsonization of apoptotic cells by autologous iC3b facilitates clearance by immature dendritic cells, down-regulates DR and Cd86, and up-regulates CC chemokine receptor 7. J Exp Med 2002; 196: 1553–61.
[17] Morelli AE, Larregina AT, Shufesky WJ, Zahorchak AF, Logar AJ, Papworth GD et al. Internalization of circulating apoptotic cells by splenic marginal zone dendritic cells: dependence on complement receptors and effect on cytokine production. Blood 2003; 101: 611–20.
[18] Nauta AJ, Castellano G, Xu W, Woltman AM, Borrias MC, Daha MR et al. Opsonization with C1q and mannose-binding lectin targets apoptotic cells to dendritic cells. J Immunol 2004; 173: 3044–50.