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Anaphylatoxin C5a Induces Monocyte Recruitment and Differentiation into Dendritic Cells by TNF-α and Prostaglandin E_2-Dependent Mechanisms[1]

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Although monocytes can be directed to develop into dendritic cells (DC) in vitro, the molecular mechanisms that induce their transformation in vivo are largely unknown. In the present study we employed an in vivo SCID mouse model to investigate the impact of two proinflammatory chemotaxins, the anaphylatoxin C5a and the chemokine macrophage inflammatory protein-1α (CCL3), on the differentiation of human monocytes and immature DC generated from monocytes in the presence of GM-CSF and IL-4. Both C5a and macrophage inflammatory protein-1α recruited human monocytes and immature DC into the peritoneal cavity of SCID mice, but only C5a induced their differentiation into phenotypically mature DC by 48 h after injection. Macrophages derived from monocytes by in vitro culture were resistant to C5a-mediated transformation in vivo. The effect of C5a was indirect, since C5a-stimulated TNF-α and PGE_2 were found to be obligatory as well as sufficient to induce differentiation of monocytes. In contrast to monocytes, in vitro generated immature DC required TNF-α, but not PGE_2, for their C5a-mediated maturation in vivo. C5a-transformed monocytes represented an inflammatory type of DC, as they constitutively secreted high amounts of TNF-α, but also retained the capacity to release the Th1 cytokine IL-12 p70 upon stimulation with CD40 ligand. In summary, we identified for the first time a cascade of inflammatory signals that can induce the transformation of monocytes into DC in vivo. This novel function emphasizes the important immunoregulatory role of C5a at the interface of innate and adaptive immunity. ***The Journal of Immunology*****, 2003, 171: 2631–2636.**

Interleukin-4 is instrumental, in combination with GM-CSF, in the transition of monocytes into dendritic cells (DC)[3] in vitro (1, 2). These cells are widely used for experimental as well as clinical purposes (3). However, it is not clear whether a similar cytokine milieu may be encountered in vivo. Recently it was shown that monocytes can become DC under more physiologic conditions as well. Human monocytes were differentiated into DC in an in vitro model of transendothelial trafficking without exogenous cytokines (4). This finding was further corroborated in an in vivo model in which a subset of murine monocytes differentiated into DC after phagocytosis of particulate Ag (5). However, possible molecular mechanisms initiating the transformation process in these models remained unknown.

The main function of DC is to collect Ags in inflamed tissues and to migrate to the local lymph nodes, where specific immune responses are initiated (6). Migration of DC is critically governed by the differential expression of chemokine receptors (7). Immature DC are responsive to inflammatory chemokines (such as macrophage inflammatory protein-1α (MIP-1α)), which may guide them to sites of inflammation where Ag sampling can take place, and maturation can be induced (8, 9). The maturation process may be triggered by inflammatory stimuli such as IL-1β, TNF-α, or LPS and leads to down-regulation of receptors for inflammatory chemokines. On the other hand, CCR7, the receptor for the constitutive chemokines CCL19 and CCL21, is up-regulated and may induce migration of DC to lymphoid organs (10–12) Maturation of DC is reinforced during its interactions with T cells by membrane and soluble molecules such as CD40 ligand (CD40L) and IFN-γ provided by T cells themselves (13–15). Mature DC have the potential to produce high levels of IL-12, which promote the development of Th1 responses (16). However, IL-10, TGF-β, corticosteroids, vitamin D, and PGE_2 can interfere with DC maturation and convert DC from Th1- to Th2-skewing APCs (17).

The anaphylatoxin C5a has been shown to be a chemoattractant for immature DC (8). One of the main functions of anaphylatoxins is the recruitment and activation of leukocytes at sites of infection, inflammation, and trauma. The C5a (74 aa) peptide regulates its inflammatory functions by interacting with its receptor, C5aR, which belongs to the rhodopsin family of seven-transmembrane, G protein-coupled receptors (18, 19). C5a, which is generated by activation-induced cleavage of the fifth component of complement, is a potent inducer of proinflammatory activities (20, 21). Upon contact with C5a, mononuclear phagocytes release IL-1β, IL-6, TNF-α, and PGE_2 (22–26). A defined mixture of these stimuli has been shown to induce maturation of immature DC generated from monocytes in vitro in the presence of GM-CSF and IL-4 (27).

It was therefore of particular interest to investigate a possible role for C5a in the generation and maturation of DC in vivo. Our findings demonstrate that beyond its mobilizing effect on human monocytes, C5a stimulated the release of TNF-α and PGE_2 in vivo, which, in turn, induced transformation of monocytes into mature DC. Despite the pivotal role of PGE_2 in DC generation,

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[3] Abbreviations used in this paper: DC, dendritic cell; CD40L, CD40 ligand; MIP-1α, macrophage inflammatory protein-1α.

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these cells retained the capacity to release the Th1 cytokine IL-12 p70 upon stimulation with CD40L and secreted high amounts of TNF-α constitutively.

Materials and Methods

Reagents

Recombinant human anaphylatoxin C5a was generated as previously described (28). The LPS concentration, as determined by the *Limulus* assay (Coatest Endotoxin; Pharmacia, Freiburg, Germany), was <20 pg LPS/μg anaphylatoxin. Recombinant human and murine MIP-1α were obtained from PeproTech (Cell Concepts, Umkirch, Germany).

Secretion of human and murine TNF-α, murine IL-1β, murine IL-6, PGE_2, human IL-12 p40 (all from R&D Systems, Wiesbaden-Nordenstadt, Germany), and human IL-12 p70 (BD PharMingen, Heidelberg, Germany) in serum or supernatant was measured by ELISA according to the manufacturer's instructions. Latex beads (FluoSpheres, 0.5 μm) were obtained from Molecular Probes (Eugene, OR).

Human monocytes/macrophages and DC

Leukocytes were obtained by leukapheresis from volunteer blood donors at the Department of Transfusion Medicine, University Clinic Gottingen. PBMC were isolated by centrifugation on a Ficoll-Hypaque (Lymphoprep; Axis-Shield, Oslo, Norway) discontinuous gradient. PBMC were cultured for 1 h at 1×10^7 cells/ml in endotoxin-free RPMI 1640 (Biochrom, Berlin, Germany) supplemented with 5% heat-inactivated autologous serum in flat-bottomed plates. After washing off nonadherent cells, adherent mononuclear cells (>90% $CD14^+$ monocytes) were resuspended in PBS for injection into SCID mice or cultured in RPMI 1640 supplemented with 10% FCS (PAN Biotech, Aidenbach, Germany), penicillin/streptomycin, L-glutamine, GM-CSF (300 U/ml), and IL-4 (300 U/ml; both from R&D Systems). After 4–7 days, cultured DC expressed HLA-DR, but not CD14, and were characterized as immature due to their moderate expression of CD86 and low expression of CD83. To obtain macrophages, adherent PBMC were cultured in the presence of 5% pooled human serum for 4 days.

Migration of human cells in a SCID mouse model

All animal work was conducted in accordance with guidelines for the welfare of animals and was approved by the administration of Lower Saxony, Germany. SCID mice (strain CB-17 SCID of both sexes; 19–24 g) were obtained from the Department of Experimental Animal Research of University of Gottingen or from Charles River (Sulzfeld, Germany). Human cells (1 or 2×10^7 as indicated) were resuspended in 200 μl of PBS and injected into the tail vein of SCID mice. In parallel, 200 μl of PBS containing C5a or MIP-1α (10 or 20 μg) was injected i.p. After 24 h the mice were sacrificed. In selected experiments a second injection of C5a or MIP-1α 24 h after the first one was performed, and the mice were sacrificed 24 h later. Peritoneal cells were harvested by flushing with 7 ml of PBS and were counted using a hemocytometer. Migrated human cells were then analyzed by flow cytometry.

To inhibit C5a-induced murine TNF-α or PGE_2 production in vivo, SCID mice were treated with blocking anti-TNF-α Ab (500 μg; R&D Systems) or the cyclooxygenase inhibitor indomethacin (60 μg/injection; Sigma-Aldrich, Deisenhofen, Germany), which were injected i.p. together with C5a.

To investigate the impact of exogenous TNF-α and PGE_2 on monocyte transformation in vivo, human MIP-1α (10 μg) was injected i.p. together with 500 ng of TNF-α and 500 ng of PGE_2. After 24 h, the injections of MIP-1α, TNF-α, and PGE_2 were repeated. Another 24 h later, peritoneal cells were harvested and analyzed.

To measure cytokine secretion in supernatants of transformed monocytes, $HLA\text{-}DR^+$ human cells from the peritoneal cavities of SCID mice were cultured ex vivo at 2×10^6 cells/ml in RPMI 1640/10% FCS for 48 h. In selected experiments cells were also stimulated ex vivo with CD40L/enhancer (100 ng/ml; Alexis, San Diego, CA). Absolute numbers of migrated human cells were calculated from the percentage of $HLA\text{-}DR^+$ cells determined by flow cytometry, and the total peritoneal cell count was measured in a hemocytometer.

Human or murine B ($CD19^+$) and T ($CD3^+$) lymphocytes were never detectable in the peritoneal cavity of SCID mice.

Flow cytometric analysis

For analysis of human Ags, peritoneal cells were stained with anti-HLA-DR (Hölzl Diagnostics, Koln, Germany) alone or in combination with anti-CD86, anti-CD14 (both from Dakopatts, Hamburg, Germany), anti-CD83 (Beckman Coulter, Krefeld, Germany), or anti-CCR7 (R&D Systems). All mAb were either FITC- or PE-conjugated as indicated. Cells (2×10^5) were washed with PBS containing 1.5% FCS and 10 mM sodium azide and were then blocked with heat-aggregated human and murine IgG (10 μg each in 100 μl) for 20 min on ice. After washing three times with PBS containing 1.5% FCS and 10 mM sodium azide, cells were incubated with labeled mAb for 45 min. FITC-conjugated IgG1/PE-conjugated IgG2a murine isotype mix (Dakopatts) was used as a negative control. Finally, cells were washed as described above, resuspended in PBS containing 1% formaldehyde, and analyzed in a flow cytometer (EPICS XL; Beckman Coulter, Fullerton, CA). Gating was performed to exclude dead cells from the analyses.

Results

Transformation of human monocytes into DC in the SCID mouse

Recently we demonstrated that anaphylatoxin-induced accumulation of human monocytes in the peritoneal cavity of SCID mice is the result of chemotactic cell migration, which can be specifically inhibited by blockage of anaphylatoxin receptors on injected monocytes. Spontaneous, random influx of human monocytes into the peritoneal cavity in the absence of anaphylatoxin did not occur (29). To investigate the impact of C5a and MIP-1α on the differentiation of human monocytes and derivates in vivo, we employed the SCID mouse model in the present study.

For this purpose, freshly isolated human monocytes were injected i.v. together with C5a or MIP-1α i.p. Fig. 1 demonstrates that human monocytes were mobilized abundantly in the peritoneal cavity as early as 4 h after their i.v. injection. At this early time point, monocytes were positive for HLA-DR and CD14, but negative for CD86 and CD83. Twenty-four hours later, about half of the human monocytes had lost CD14, but gained CD86 and HLA-DR expression. After 48 h and two injections of C5a, the vast majority of migrated human cells was strongly positive for HLA-DR, CD86, and CD83, thus resembling phenotypically mature DC. In contrast, the phenotype of human monocytes whose i.p. accumulation was induced by human MIP-1α remained unchanged. It should also be noted that the degree of transformation

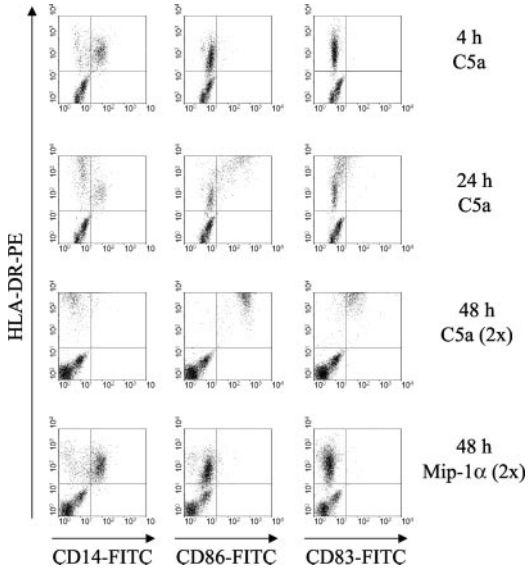

FIGURE 1. C5a, but not MIP-1α, induces transformation of human monocytes in vivo. Monocytes (1×10^7) were injected i.v. into SCID mice together with C5a or MIP-1α (10 μg each) i.p. Four, 24, or 48 h later, peritoneal cells were harvested, counted, stained, and analyzed by flow cytometry. Where indicated, a second injection of C5a or MIP-1α (10 μg each) was given 24 h after the first one. Monocytes injected in all four mice were from the same blood donor. One representative experiment of three is shown.

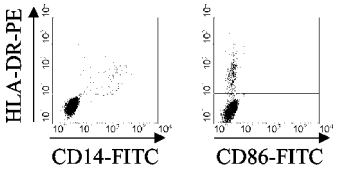

FIGURE 2. C5a does not induce transformation of human macrophages in vivo. Monocytes that had been cultured for 4 days in vitro in the presence of 5% human serum were injected i.v. into SCID mice together with C5a (10 μg) i.p. After 24 h, the C5a injection was repeated. Another 24 h later, peritoneal cells were harvested, counted, stained, and analyzed by flow cytometry. One representative experiment of three is shown.

of C5a-mobilized monocytes into mature DC was possibly donor-dependent, since in >30 independent experiments CD83 neoexpression on human cells varied between ~20–95% of cells (see also Figs. 4 and 5).

We also investigated whether monocytes that had been activated by in vitro culture for 4 days could be transformed into DC in vivo in a similar manner as freshly isolated monocytes. Fig. 2 demonstrates that cultured monocytes lost their potential to function as precursors of DC in the SCID mouse model. After 48 h and two C5a injections, human macrophages still expressed CD14 without signs of CD86 up-regulation.

Blockage of TNF-α and prostanoid synthesis prevented C5a-induced transformation of human monocytes

In search of factors that might be involved in the C5a-induced transformation of human monocytes into DC, we investigated the impact of C5a on murine TNF-α and prostanoid production in vivo. Fig. 3 demonstrates that C5a strongly increased serum levels of murine TNF-α and PGE_2 in SCID mice, whereas human MIP-1α was without effect. We also injected murine MIP-1α to exclude the possibility that human MIP-1α may be unable to stimulate the murine receptor equivalents. Again, no increase in blood levels of TNF-α and PGE_2 could be detected. It should be noted that human monocytes accumulated in the peritoneal cavity of SCID in response to murine MIP-1α, similar to human MIP-1α without signs of transformation (data not shown). In contrast to TNF-α and PGE_2, C5a did not stimulate measurable levels of murine IL-1β or IL-6 in vivo (data not shown).

Based on these results, we independently blocked murine TNF-α by specific Ab injection (Fig. 4*A*) or prostanoid synthesis by indomethacin treatment (Fig. 4*B*) in vivo. In both experiments C5a-induced transformation of human monocytes into DC was almost completely inhibited, as monocytes sustained their surface expression of CD14 and failed to up-regulate HLA-DR, CD86, and CD83.

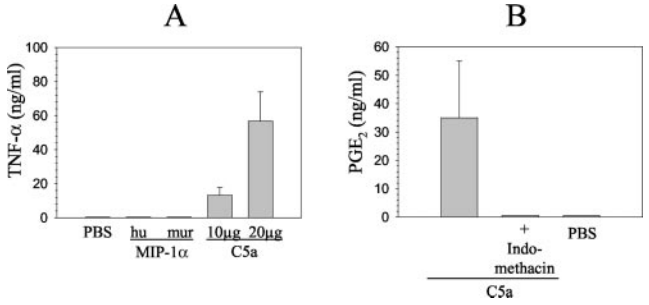

FIGURE 3. C5a stimulates murine TNF-α and PGE_2 release in vivo. SCID mice were injected with 10 μg of C5a (20 μg where indicated) and human or murine MIP-1α (10 μg each) i.p. together with human monocytes (1×10^7) i.v. Twenty-four hours later, serum was collected, and murine TNF-α (*A*) or PGE_2 (*B*) concentrations were determined by ELISA. Control mice received only PBS. Where indicated, indomethacin (60 μg) was injected i.p. together with C5a. At least four mice were investigated in each group. The mean ± SEM are shown.

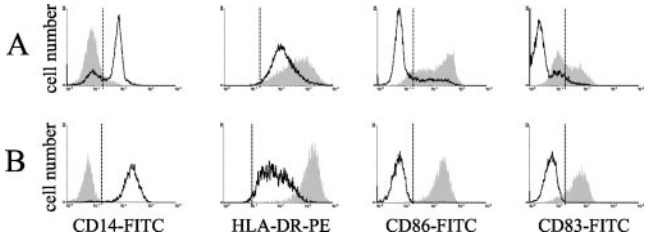

FIGURE 4. C5a-induced transformation of human monocytes into mature DC in vivo can be blocked by anti-TNF-α Ab, and indomethacin. Monocytes (1×10^7) were injected i.v. into SCID mice together with C5a (10 μg) i.p. After 24 h, C5a injection was repeated. Another 24 h later, peritoneal cells were harvested, counted, stained, and analyzed by flow cytometry. Gates were set on HLA-DR^+ human cells. Filled histograms, Cells from mice without further treatment; open histograms (solid lines), cells from mice that had been additionally treated with anti-TNF-α (500 μg) i.p. together with the first C5a injection (*A*) or with indomethacin (2×60 μg) i.p together with both C5a injections (*B*). Vertical bars mark negative control staining. Monocytes used in *A* and *B* were from separate donors. One representative experiment of at least three is shown.

Exogenous TNF-α and PGE_2 induced transformation of monocytes into DC in vivo

To establish that TNF-α and PGE_2 were involved in the differentiation of human monocytes into DC, these two inflammatory stimuli were injected i.p. together with MIP-1α. It was found that TNF-α and PGE_2 were sufficient to induce the phenotypic transformation of mobilized monocytes, as evidenced by the down-regulation of CD14 and the up-regulation of HLA-DR, CD86, and CCR7 (Fig. 5).

Monocytes phagocytosed latex beads before their transformation in vivo

To function as APCs, monocytes must take up Ag before transformation into mature DC abrogates their capacity for phago- and pinocytosis (30). Fig. 6 shows that human monocytes that migrated i.p. in response to MIP-1α or C5a efficiently phagocytosed latex beads, but differentiated into DC only if C5a was injected. These results demonstrate that phagocytosis was without impact on the differentiation state of monocytes. Confocal microscopy confirmed that latex beads were located intracellularly and did not stain cells by passive adherence (data not shown).

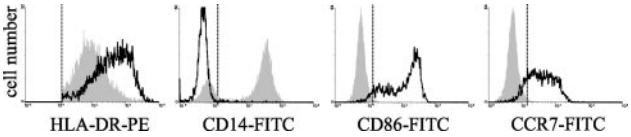

FIGURE 5. TNF-α and PGE_2 induce transformation of human monocytes into DC in vivo. Monocytes (1×10^7) were injected i.v. into SCID mice together with MIP-1α (10 μg) i.p. After 24 h, injection of MIP-1α (10 μg) was repeated. After another 24 h, peritoneal cells were harvested, counted, stained, and analyzed by flow cytometry. Gates were set on HLA-DR^+ human cells. Filled histograms, Cells from mice without further treatment; open histograms (solid lines), cells from mice that had been additionally treated with TNF-α (twice, 500 ng each time) plus PGE_2 (twice, 500 ng each time) together with both MIP-1α injections. Vertical bars mark negative control staining. One experiment of three is shown.

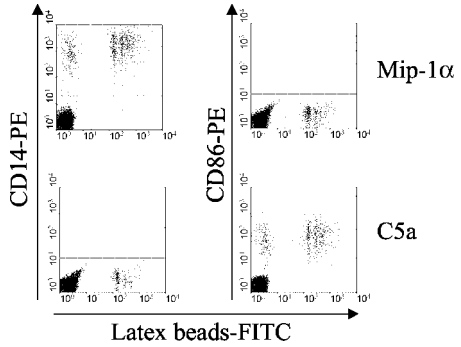

FIGURE 6. Monocytes phagocytose latex beads and differentiate into DC in vivo. Monocytes (1×10^7) were injected i.v. into SCID mice that at the same time obtained FITC-conjugated latex beads (1×10^7) together with C5a (20 μg) or MIP-1α (10 μg) i.p. After 24 h, peritoneal cells were collected, counted, stained, and analyzed by flow cytometry.

IL-12 and TNF-α release from C5a-induced DC ex vivo

We investigated the capacity of C5a-induced DC to secrete cytokines ex vivo after their isolation from the peritoneal cavity of SCID mice. Without further stimulation, constitutive secretion of TNF-α was very high, whereas only small amounts of IL-12 were released (Fig. 7*A*). In contrast, human monocytes that had been recruited i.p. by MIP-1α injection did not secrete detectable levels of IL-12 or TNF-α ex vivo. Stimulation with CD40L has been shown to augment maturation of DC and to induce the production of bioactive IL-12 that may promote the development of Th1 responses (13). In line with these findings, the release of IL-12 p70 from C5a-induced DC was strongly inducible by CD40L stimulation ex vivo (Fig. 7*B*). In addition to its impact on IL-12 secretion,

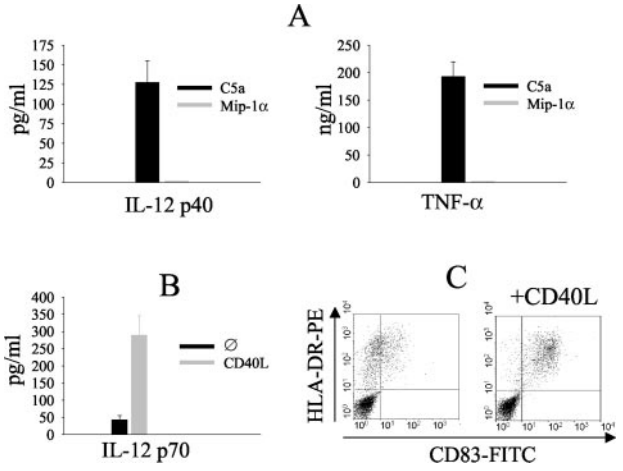

FIGURE 7. IL-12 and TNF-α release from DC ex vivo. Human monocytes (2×10^7) were injected i.v. into SCID mice together with C5a or MIP-1α (10 μg each) i.p. Twenty-four hours later, the injection of C5a or MIP-1α was repeated. Another 24 h later, peritoneal cells were harvested and counted in a hemocytometer, and the percentage of HLA-DR$^+$ human cells was determined by flow cytometry. Absolute numbers of migrated HLA-DR$^+$ cells were calculated from the percentage of red fluorescent cells and the total peritoneal cell count. HLA-DR$^+$ cells were cultured at 2×10^6/ml for 48 h. *A*, Supernatants of cultured cells were tested by ELISA for IL-12 p40 and TNF-α release ($n = 7$). *B*, IL-12 p70 was measured after culturing cells from C5a-treated mice for 48 h in the absence ($n = 7$) or the presence ($n = 4$) of CD40L/enhancer. The mean ± SEM are shown. *C*, CD83 expression on human cells that were obtained from C5a-treated mice and cultured in the absence or the presence of CD40L/enhancer was analyzed by flow cytometry.

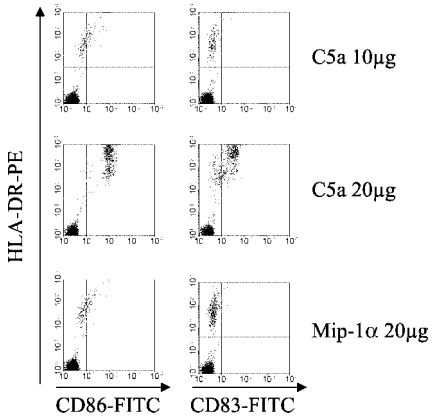

FIGURE 8. C5a but not MIP-1α induces maturation of human immature DC in vivo. Immature DC (1×10^7) generated in vitro from monocytes with GM-CSF and IL-4 were injected i.v. into SCID mice together with C5a or MIP-1α i.p. Twenty-four hours later, peritoneal cells were harvested, counted, stained, and analyzed by flow cytometry. DC were from one blood donor. One representative experiment of three is shown.

stimulation with CD40L was able to augment CD83 expression on DC, indicating their maturation (Fig. 7*C*).

Differentiation of human immature DC in the SCID mouse

Immature DC generated from monocytes in vitro in the presence of GM-CSF and IL-4 are widely used for experimental and clinical purposes. Therefore, we investigated the effects of MIP-1α and C5a on these cells in the SCID mouse model. Fig. 8 demonstrates that HLA-DR$^+$ human DC, which immigrated i.p. by 24 h in response to 10 μg of C5a or 20 μg of MIP-1α, remained phenotypically immature. In contrast, 20 μg of C5a not only recruited more DC, but also induced their maturation, as was shown by the upregulation of CD86 and CD83. It should be noted, however, that in a few experiments even 10 μg of C5a was sufficient to induce maturation of immature DC in vivo by 24 h (data not shown).

Blockage of TNF-α, but not PGE_2, prevented C5a-induced maturation of human immature DC

We speculated that the lower efficiency of 10 μg of C5a compared with 20 μg may be due to the lower TNF-α serum levels (see Fig.

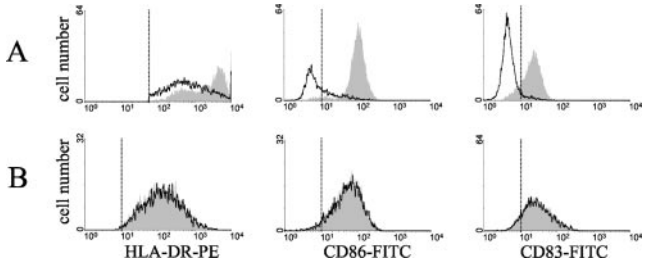

FIGURE 9. C5a-induced differentiation of immature into mature DC in vivo can be blocked by anti-TNF-α Ab, but not indomethacin. Immature DC (1×10^7) generated in vitro from monocytes with GM-CSF and IL-4 were injected i.v. into SCID mice that also received C5a (20 μg) with or without anti-TNF-α Ab (500 μg; *A*) or indomethacin (60 μg; *B*) i.p. After 24 h, peritoneal cells were harvested, counted, stained, and analyzed by flow cytometry. Gates were set on HLA-DR$^+$ human cells. Filled histograms, Cells from mice injected with C5a only; open histograms (solid lines), cells from mice that had been additionally treated with anti-TNF-α Ab (*A*) or indomethacin (*B*). Vertical bars mark negative control staining. Immature DC used in *A* and *B* were from separate donors. One representative experiment of at least three is shown.

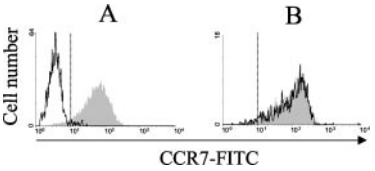

FIGURE 10. Indomethacin abrogates CCR7 neoexpression on C5a-transformed monocytes, but not immature DC in vivo. Monocytes (*A*) or immature DC (*B*; 1×10^7 each) were injected i.v. into SCID mice that also received C5a (10 μg) with or without indomethacin (60 μg) i.p. After 24 h, C5a injection was repeated. Another 24 h later, peritoneal cells were harvested, counted, stained, and analyzed by flow cytometry. Gates were set on HLA-DR$^+$ human cells. Filled histograms, Cells from mice injected with C5a only; open histograms (solid lines), cells from mice that had been additionally injected with indomethacin (60 μg) i.p. together with C5a. Vertical bars mark negative control staining. One representative experiment of at least two is shown.

3). To prove the involvement of TNF-α in C5a-induced DC maturation in vivo, anti-TNF-α Ab was injected together with C5a. Fig. 9*A* demonstrates that C5a-stimulated murine TNF-α was indeed responsible for the maturation of immature DC, as anti-murine TNF-α Ab blocked the up-regulation of HLA-DR, CD86, and CD83. We also examined whether prostanoids may play a role in DC maturation similar to their function in monocyte transformation. However, blockage of the cyclooxygenase pathway in vivo by indomethacin treatment was without effect on the expression of HLA-DR, CD86, and CD83 on DC (Fig. 9*B*).

The differential effect of indomethacin on C5a-induced transformation of monocytes vs immature DC was also investigated with regard to the neoexpression of CCR7 required for the migration of DC into lymphatic tissues to initiate immune responses (7). Indomethacin abrogated CCR7 neoexpression on C5a-recruited human cells if monocytes, but not if immature DC, were injected (Fig. 10).

Discussion

Our findings demonstrate that C5a is involved in the transformation of human monocytes into mature DC by 48 h after their injection into immunodeficient SCID mice. C5a functioned via the induction of murine TNF-α and PGE_2, as blockage of either one completely inhibited DC maturation in vivo. The importance of these two stimuli was further emphasized in experiments in which MIP-1α was injected instead of C5a to mobilize human monocytes into the peritoneal cavity of SCID mice. In this case, exogenous TNF-α and PGE_2 were required to induce monocyte transformation.

C5a-transformed monocytes represented activated, but unpolarized, DC, as they constitutively secreted high amounts of the proinflammatory TNF-α, whereas the Th1 cytokine IL-12 p70 was released only upon stimulation with CD40L. This finding may be surprising, as PGE_2 is generally believed to be an inflammatory mediator with a Th2-driving role (31, 32). Although PGE_2 has been shown to cooperate in the phenotypic maturation of immature DC in vitro (27, 33), secretion of inflammatory cytokines, including the Th1 cytokine IL-12 p70, was actually suppressed (34–37). When added to monocytes from the start of culture with GM-CSF and IL-4, PGE_2 even prevented down-regulation of CD14 on DC, and high levels of IL-10 could be stimulated (27, 34).

Our results suggest that beyond its reported Th2-driving activity, PGE_2 may also play an important physiological role in the induction of monocyte transformation into DC in vivo. If C5a is liberated in the course of complement activation during infection, local phagocytes may respond to C5a with TNF-α and PGE_2 release (22, 25). Thus, both TNF-α and PGE_2 may represent natural constituents of an inflammatory environment. In this micromilieu, C5a-recruited, blood-derived monocytes may take up Ag (e.g., pathogens), and some of these cells may further differentiate into DC. Approximately 25% of phagocytic (latex$^+$) monocytes were shown to differentiate into lymph node DC in an in vivo mouse model (5). A prerequisite for their migration into lymph nodes is CCR7 neoexpression on the surface of DC (11), which we also demonstrated to occur as a consequence of C5a-induced monocyte transformation. In lymphatic tissue DC may then encounter CD40L-expressing lymphocytes, which, similar to the soluble CD40L used in our experiments, may stimulate the secretion of Th1-driving IL-12 p70.

The anaphylatoxin C5a may thus represent an endogenous danger signal necessary for the initiation of primary and secondary immune responses (38) via the induction of PGE_2 and TNF-α. Notably, PGE_2 has also been demonstrated to play an essential role in the differentiation and maturation of the T lymphocyte lineage in the fetal thymus (39).

In the SCID mouse model, the pivotal impact of PGE_2 on DC differentiation was restricted to monocytes, whereas immature DC generated from monocytes in the presence of GM-CSF and IL-4 in vitro were exclusively dependent on C5a-stimulated TNF-α for their maturation in vivo. In agreement with our results, TNF-α has been shown to be sufficient for the induction of the phenotypic maturation of immature DC in vitro (1).

Furthermore, monocytes cultured in vitro for 4 days completely lost their potential for transformation into DC by TNF-α and PGE_2. In contrast to our results, however, macrophages could be reverted in vitro to cells with a DC phenotype following their transfer into culture medium with GM-CSF and IL-4 (40, 41). It is not clear, however, whether these in vitro culture conditions reflect a physiological environment, considering that even immediate precursors of end-stage neutrophilic granulocytes could be transformed into highly stimulatory DC in the presence of GM-CSF and IL-4 (42).

Recent data have shown that human monocytes exposed to collagen and endothelial cells in a model of transendothelial trafficking can acquire characteristics of DC within 2 days (4). These DC migrated across endothelium in the ablumenal-to-lumenal direction (reverse transmigration) reminiscent of the migration into lymphatic vessels. Full differentiation of monocytes into DC required an additional stimulus, such as the uptake of latex or zymosan particles. The exact molecular mechanism resulting in monocyte transformation by reverse transmigration in vitro, however, remained unresolved. It should be noted that phagocytosis of latex particles has been shown to induce secretion of TNF-α by monocytes (43). TNF-α itself has the potential to simulate PGE_2 secretion in monocytes (44). One may therefore speculate that TNF-α together with PGE_2 could also be involved in the transformation of monocytes by reverse transmigration.

TNF-α and PGE_2 were abundantly present in the blood of SCID mice following i.p. C5a injection. They were most likely released by hepatic Kupffer cells, which represent the majority of all mononuclear phagocytes present in the body, and may have come into contact with C5a leaking from the peritoneal cavity. The systemic effects of C5a via stimulation of Kupffer cells may be the main reason why not only monocytes mobilized to the peritoneal cavity, but also human monocytes trapped in animal lungs, differentiated into mature DC, as evidenced by up-regulation of CD83 (data not shown).

In summary, we identified for the first time a cascade of inflammatory signals that can induce the transformation of monocytes into DC in vivo. This novel function emphasizes the important

immunoregulatory role of C5a at the interface of innate and adaptive immunity.

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