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ARTICLE *in* EUROPEAN JOURNAL OF IMMUNOLOGY · JUNE 2007

Impact Factor: 4.03 · DOI: 10.1002/eji.200636625 · Source: PubMed

European Journal of Immunology Leukocyte signaling

IL-13 induces expression of CD36 in human monocytes through PPARγ activation

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The class B scavenger receptor CD36 is a component of the pattern recognition receptors on monocytes that recognizes a variety of molecules. CD36 expression in monocytes depends on exposure to soluble mediators. We demonstrate here that CD36 expression is induced in human monocytes following exposure to IL-13, a Th2 cytokine, *via* the peroxisome proliferator-activated receptor (PPAR)γ pathway. Induction of CD36 protein was paralleled by an increase in CD36 mRNA. The PPARγ pathway was demonstrated using transfection of a PPARγ expression plasmid into the murine macrophage cell line RAW264.7, expressing very low levels of PPARγ, and in peritoneal macrophages from PPARγ-conditional null mice. We also show that CD36 induction by IL-13 *via* PPARγ is dependent on phospholipase A2 activation and that IL-13 induces the production of endogenous 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, an endogenous PPARγ ligand, and its nuclear localization in human monocytes. Finally, we demonstrate that CD36 and PPARγ are involved in IL-13-mediated phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes. These results reveal a novel role for PPARγ in the alternative activation of monocytes by IL-13, suggesting that endogenous PPARγ ligands, produced by phospholipase A2 activation, could contribute to the biochemical and cellular functions of CD36.

Received 25/8/06
Revised 22/1/07
Accepted 15/3/07

[DOI 10.1002/eji.200636625]

Key words: CD36 · Human monocytes · IL-13 · Nuclear receptors · Phagocytosis

Abbreviations: 15d-PGJ$_2$: 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 · **HODE:** hydroxyoctadecadienoic acid · **LDL:** low-density lipoprotein · **LysM:** lysozyme M · **MAFP:** methyl arachidonyl fluorophosphonate · **PLA2:** phospolipase A2 · **PPAR:** peroxisome proliferator-activated receptor · **PPRE:** PPAR-responsive element · **SFM:** serum-free medium

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Introduction

The innate immune system protects the host in the early phase of infection. Circulating monocytes and tissue macrophages (Mφ) mediate much of this innate immune response [1]. The strategy of recognition in the innate response is mediated by the coordinated action of pathogen-associated molecular patterns and pattern recognition receptors. Scavenger receptors and mannose receptor are important pattern recognition receptors in monocytes/Mφ [2, 3]. The modulation of the expression of these receptors may be critical in the role of these cells in antigen processing, scavenging, and host defence against pathogens.

Several members of the scavenger receptor family, including Mφ class A scavenger receptors and CD36, have been identified as receptors for modified lipoproteins on Mφ, and their relevance to lipid uptake has been demonstrated *in vitro* and *in vivo* [4]. The scavenger receptor CD36, an 88-kDa integral membrane protein, is a class B scavenger receptor expressed on a wide variety of cells, in particular on monocytes and monocyte-derived Mφ [5, 6]. CD36 is known as a receptor for the uptake of oxidatively modified low-density lipoprotein (LDL) [7] and is also able to bind anionic phospholipid phosphatidylserine [8]. This scavenger receptor is implicated in the clearance of apoptotic cells [9]. Recently, McGilvray and colleagues [10] described a CD36-dependent nonopsonic phagocytosis of erythrocytes containing *P. falciparum*, by monocytes and culture-derived Mφ, and a decrease in the parasite-induced TNF secretion by monocytes/Mφ. These processes were accentuated by CD36 up-regulation by peroxisome proliferator-activated receptor (PPAR) activators [11]. PPARγ is a member of a family of ligand-activated nuclear transcription factors that form heterodimers with the retinoic X receptors, and binds to PPAR-responsive elements (PPRE) in the promoter regions of target genes.

PPARγ is activated by a range of natural and synthetic substances. These include modified fatty acids, members of the prostanoid family, in particular 15-deoxy-$\Delta^{12,14}$–prostaglandin J_2 (15d-PGJ_2) and linoleic acid derivatives [12, 13], and insulin-sensitizing thiazolidinedione compounds such as BRL-49653 (rosiglitazone), pioglitazone, and troglitazone [14–16].

IL-4, a cytokine produced by Th2-polarized $CD4^+$ cells, induces expression of CD36 mRNA in monocytes [17]. The mechanism by which IL-4 up-regulates CD36 involves the generation of PPARγ ligands *via* 12/15-lipoxygenases and the activation of this transcription factor [18]. IL-13, an immunoregulatory cytokine secreted predominantly by activated Th2 cells, shares many functional properties with IL-4, as they have a common receptor subunit, the α subunit of the IL-4Rα [19, 20]. These cytokines, which produce an alternative activation of Mφ, [21] up-regulate expression of the mannose receptor and MHC class II molecules, and stimulate endocytosis and antigen presentation.

Several arguments suggest the possibility that IL-13 could be implicated in the over-expression of CD36 in monocytes/Mφ. Firstly, IL-4 up-regulates monocyte/Mφ CD36 by the generation of an arachidonic acid metabolite that is a PPARγ agonist [18], and we have shown that mouse Mφ stimulated by IL-13 are also able to produce these metabolites [22]. Additionally, we have recently demonstrated with resident peritoneal Mφ in mice that IL-13 activates PPARγ *via* the endogenous production of 15d-PGJ_2, an arachidonic acid metabolite, and induces the expression of mannose receptors [23]. In this study we demonstrate that IL-13 induces CD36 expression on human blood monocytes *via* a PPARγ-dependent signaling pathway involving activation of cytosolic phospholipase A2 (PLA2).

Results

IL-13 up-regulates human monocyte CD36

Since IL-4 increases CD36 expression in monocytes/Mφ *via* PPARγ, we investigated the expression of CD36 on human monocytes treated with IL-13 or rosiglitazone, a PPARγ agonist used as positive control [15]. Flow cytometry showed a significant enhancement of CD36 receptors on the surface of human monocytes treated with IL-13 or rosiglitazone (Fig. 1). The IL-13 effects were maximal at 50 ng/mL (Fig. 1).

To determine whether PPARγ-specific ligands and IL-13 increase the mRNA level of CD36, we used a quantitative real-time PCR. As shown in Fig. 2, treatment with IL-13 or rosiglitazone for 6 h resulted in a significant induction of CD36 mRNA. The increase of the CD36 mRNA level was similar for both IL-13 and rosiglitazone treatment, with a 4.22- and 3.86-fold elevation, respectively (Fig. 2).

The observation that CD36 was increased following treatment with PPARγ-specific ligands or IL-13 raised the possibility that IL-13 could induce CD36 *via* a PPARγ signaling pathway. To evaluate the role of PPARγ in the up-regulation of CD36 we used GW9662, an irreversible antagonist of PPARγ [24]. Fig. 1 shows that the increase in CD36 induced by IL-13 and rosiglitazone was inhibited by GW9662. The effect of GW9662 was only observed on the CD36 induction by IL-13 and not on the constitutive CD36 level. The inhibition was total for 5 ng/mL of IL-13 and at least 50% for the other concentrations used. GW9662 treatment also inhibited the increase in the CD36 mRNA level induced by IL-13 (Fig. 2).

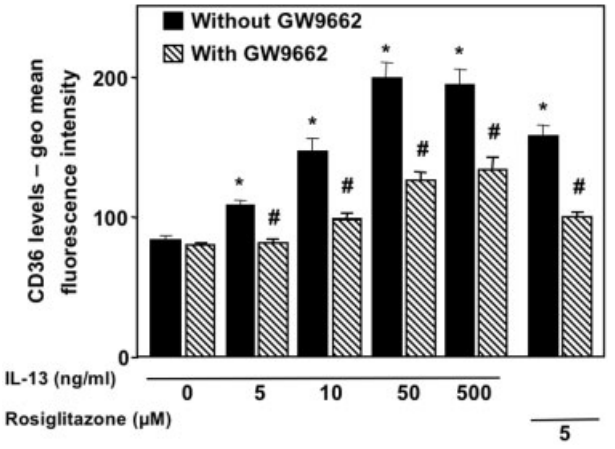

Figure 1. IL-13 and rosiglitazone (PPARγ-specific ligand) induce expression of human monocyte CD36. Monocytes were cultured in Mφ SFM for 12 h in the presence of the following activators as indicated: 5, 10, 50 or 500 ng/mL of IL-13 or 5 μM of rosiglitazone, in the presence or absence of GW9662 (1 μM). The cells were stained with an anti-CD36-PE antibody, and CD36 expression analyzed by a flow cytometer. Values are means ± SE of three separate experiments; *$p<0.01$ compared with untreated monocytes; #$p<0.01$ compared with the respective control (monocytes without GW9662).

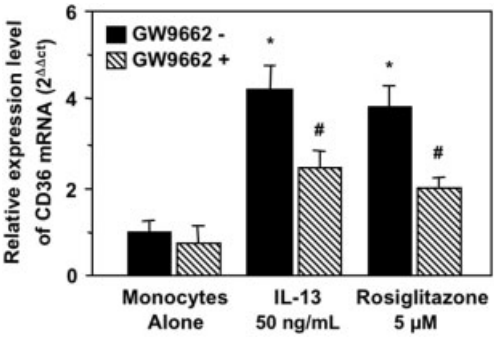

Figure 2. IL-13 and rosiglitazone stimulate CD36 transcription. Human monocytes were treated for 6 h with 50 ng/mL IL-13 and with 5 μM of rosiglitazone in the presence or not of GW9662 (1 μM). CD36 mRNA expression was analyzed by quantitative RT-PCR. To examine the linearity of the assay, a serial dilution of the positive control sample was used. The linearity of the mRNA concentrations *versus* Ct values of the PCR reaction in the serial diluted samples was calculated by linear regression analysis ($y = 3.28x - 26.5$). The *n*-fold differential expression of CD36 mRNA samples compared with the control was expressed as 2^{Ct}. Values are means ± SE of three separate experiments; *$p<0.01$ compared with untreated monocytes; #$p<0.01$ compared with the respective control (monocytes without GW9662).

IL-13 induces PPARγ activation in human monocytes

Given that the previous experiment strongly suggested that IL-13 activated PPARγ, we studied the role of IL-13 on PPARγ activation in human monocytes. Fig. 3A shows

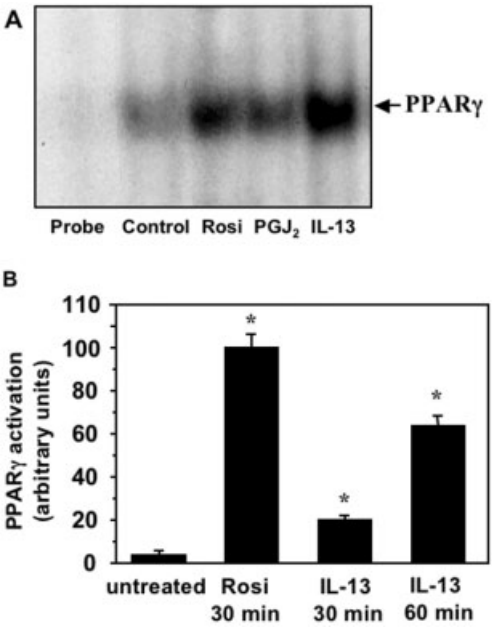

Figure 3. IL-13 induces PPARγ activation in human monocytes. Activation of PPARγ was determined by EMSA (A) and by ELISA with TransAM® technology (B). (A) Cells were untreated (control) or treated with rosiglitazone (Rosi; 5 μM) or IL-13 (50 ng/mL) or 15d-PGJ_2 (1 μM) for 1 h. Nuclear extracts were collected and EMSA carried out as described in the *Materials and methods*. The experiment was repeated twice with similar results. (B) Human monocytes were untreated or exposed to rosiglitazone (Rosi; 5 μM for 1 h) or IL-13 (50 ng/mL for 30 min or 1 h). Ten micrograms of nuclear proteins were used to perform an immunodetection of activated PPARγ using a TransAM® kit. The data are expressed as relative arbitrary units with rosiglitazone treatment set at 100. The results are representative of three different experiments. Values are means ± SE of three separate experiments; *$p<0.01$ compared with the control (untreated monocytes).

the enhancement of PPARγ DNA binding activity in nuclear protein extracts of monocytes after treatment with 15d-PGJ2, rosiglitazone or IL-13. A TransAM® method using a specific PPARγ antibody confirmed the EMSA results and demonstrated that PPARγ was specifically activated in human monocytes after 30 or 60 min of treatment with IL-13 (Fig. 3B).

IL-13 regulates CD36 expression through PPARγ

To establish that IL-13 regulates CD36 expression through PPARγ, we performed transfection experiments on the murine Mφ cell line RAW264.7, which expresses very low levels of PPARγ mRNA [25]. IL-13 and rosiglitazone did not induce an increase in CD36 expression in this cell line transiently transfected with a control plasmid (RSV-βGal) (Fig. 4A). Direct proof of the role of PPARγ in CD36 over-expression by IL-13 (as by rosiglitazone) was provided by the fact that CD36 over-expression was restored when the PPARγ expres-

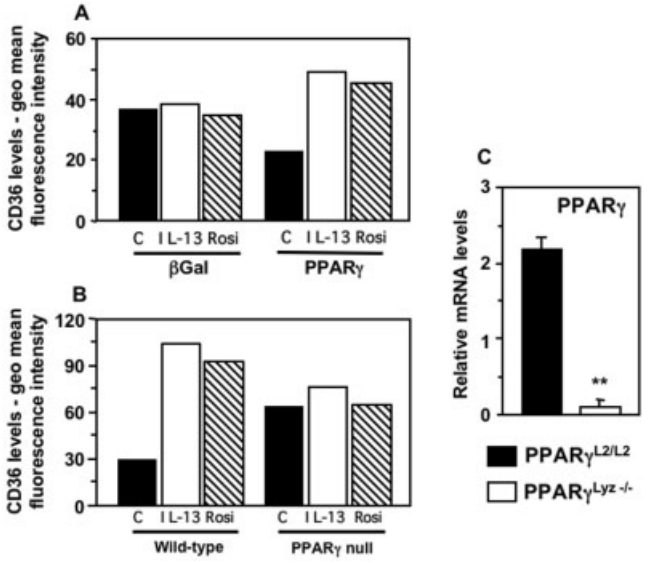

Figure 4. Up-regulation of CD36 by IL-13 is dependent on PPARγ. (A) RAW264.7 Mφ were transfected with 2 μg of CMV-PPARγ or RSV-βGal plasmid. After stimulation with rosiglitazone at 5 μM or IL-13 at 50 ng/mL over 24 h, the cells were stained with an anti-CD36-PE antibody, and CD36 expression analyzed by a flow cytometer. The data represented here are the percent increase in the CD36 staining obtained with the sample compared with the corresponding control (unstimulated PPARγ- or Gal-transfected cells). The experiment was repeated three times with similar results. (B) PPARγ-deficient peritoneal Mφ from C57BL/6 mice (PPARγ null) or peritoneal Mφ from wild-type mice were incubated *in vitro* with rosiglitazone at 5 μM or IL-13 at 50 ng/mL over 24 h. The cells were stained with an anti-CD36-PE antibody and CD36 expression analyzed by a flow cytometer. The experiment was repeated twice with similar results. (C) PPARγ mRNA levels in the peritoneal Mφ from wild-type mice ($PPAR\gamma^{L2/L2}$; black bars, n=3) or from PPARγ-null mice ($PPAR\gamma$ $Lyz^{-/-}$; open bars, n=3) was determined by RT-PCR analysis. $^{**}p<0.01$ compared with the control.

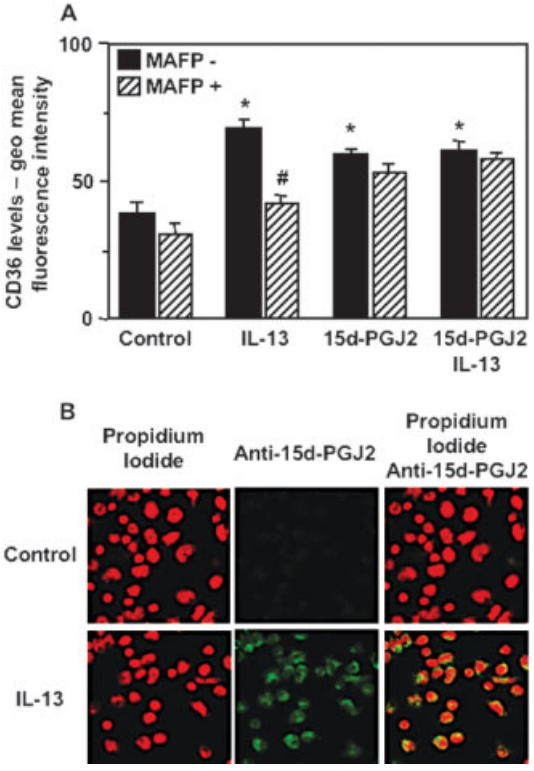

Figure 5. Participation of cytosolic PLA2 in the induction of CD36 and production of 15d-PGJ_2 by IL-13 in human monocytes. (A) Human monocytes were treated or not for 10 min with MAFP (MAFP+ or MAFP–), a PLA2 inhibitor (10 μM). The cells were then incubated for 24 h in the presence or absence of IL-13 (50 ng/mL) with or without 15d-PGJ_2 (1 μM). The cells were stained with an anti-CD36-PE antibody and CD36 expression analyzed by flow cytometry. Values are means ± SE of three separate experiments; $^{*}p<0.01$ compared with the respective controls (monocytes without or with MAFP); $\#p<0.01$ compared with the monocytes treated with IL-13 without MAFP. (B) Human monocytes were treated for 30 min with IL-13 (50 ng/mL). The fixed cells were treated with anti-15d-PGJ_2 mouse polyclonal antibody, followed by fluorescein isothiocyanate-conjugated anti-mouse Ig. Nuclear staining was performed using propidium iodide. The red color represents the nucleus (left-hand panels); the green color represents 15d-PGJ_2 (middle panels). Merged images of the green and red colors are shown in the right-hand panels.

sion vector (pCMV-PPARγ) was transfected into this cell line (Fig. 4A).

To confirm the role of the PPARγ signaling pathway, we studied CD36 expression by flow cytometry in murine peritoneal Mφ in which PPARγ had been selectively disrupted [lysozyme M (LysM)-$Cre^{(tg/0)}$/$PPAR\gamma^{L2/L2}$ mice]. The deficiency of PPARγ in these cells has been validated by RT-PCR (Fig. 4C). Consistent with previous data, the expression of CD36 was induced by rosiglitazone and IL-13 in control Mφ harvested from $PPAR\gamma^{L2/L2}$ mice, which contain LoxP-flanked PPARγ alleles. In contrast the over-expression of CD36 induced by rosiglitazone or IL-13 was not observed in PPARγ-deficient Mφ (Fig. 4B).

IL-13 induces PLA2 activation and 15d-PGJ_2 production by human monocytes

As cytosolic PLA2 induces the production of endogenous PPARγ ligands such as PGJ_2, we investigated the role of cytosolic PLA2 on CD36 induction by IL-13. Fig. 5A reveals that CD36 expression on 15d-PGJ_2-treated human monocytes was not influenced by methyl arachidonyl fluorophosphonate (MAFP), a specific cytosolic PLA2 inhibitor. Conversely, MAFP inhibited up-regulation of CD36 expression by IL-13. Moreover, the induction of CD36 was restored by the addition of 15d-PGJ_2 in the presence of MAFP and IL-13.

To confirm that IL-13, which activates cytosolic PLA2, triggers arachidonic acid metabolism, we studied the synthesis and cellular localization of 15d-PGJ_2 by immunofluorescence using an anti-PGJ_2 polyclonal antibody. Fig. 5B shows that IL-13 generates production of 15d-PGJ_2 and induces partial nuclear localization of 15d-PGJ_2 in human monocytes. This finding suggests that IL-13 can regulate gene expression in monocytes partly by controlling the production of endogenous ligands of PPARγ such as 15d-PGJ2. This study demonstrates a physiological role for cytosolic PLA2 in the generation of endogenous ligands for PPARγ in human monocytes.

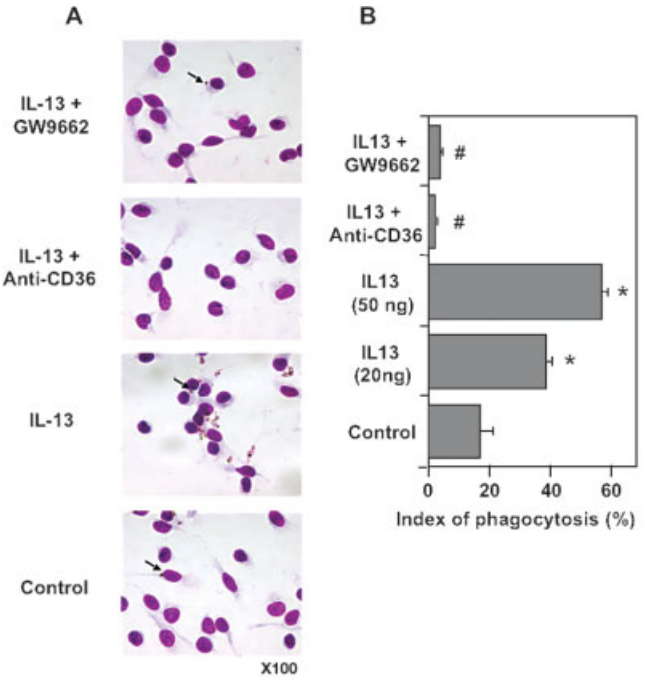

Figure 6. Participation of CD36 and PPARγ in the IL-13-induced phagocytosis of *P. falciparum*-parasitized erythrocytes by human monocytes. Human monocytes were treated for 24 h with 20 or 50 ng/mL of IL-13 in the presence or absence of GW9662 (1 μM). *P. falciparum*-parasitized erythrocytes phagocytosis assays with or without CD36-blocking antibodies were performed. (A) After Giemsa staining, cells were visualized by light microscopy and photographed. The arrow shows the hemozoin pigment produced by the phagocytosed *P. falciparum*-parasitized erythrocytes. (B) Between 500 and 1000 monocytes were counted for each coverslip and scored for the presence or absence of phagocytosed *P. falciparum*-parasitized erythrocytes. The phagocytic index was calculated as the percentage of monocytes/Mφ with clear evidence of phagocytosis. The results are representative of three different experiments. Values are means ± SE of three separate experiments; $^{*}p<0.01$ compared with the control (untreated monocytes); $\#p<0.01$ compared with the group monocytes treated with IL-13.

IL-13 enhances the phagocytosis of parasitized erythrocytes *via* PPARγ and CD36

It has previously been shown that nonopsonic phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes is CD36-dependent [10] and that PPARγ ligands increase this phagocytosis [11]. That is why, to demonstrate that IL-13-induced CD36 over-expression has functional and immunological consequences on human monocytes, we tested whether this cytokine enhances nonopsonic phagocytosis of *P. falciparum*-parasitized erythrocytes (Fig. 6). The phagocytosis was assessed by light microscopy after Giemsa staining to reveal hemozoin, a specific product of degradation of the erythrocyte hemoglobin produced by the parasite. This method has previously been described by our team [26] and validated by other researchers [10, 11]. The results show that IL-13 significantly enhanced the phagocytosis of *P. falciparum*-parasitized erythrocytes by human monocytes in a CD36-dependent manner as demonstrated by the use of CD36-specific antibodies. Furthermore, we show that this effect is PPARγ-depen--dependent as the use of GW9662, an irreversible antagonist of PPARγ, inhibited the IL-13 effects.

Discussion

The class B scavenger receptor type 2, CD36, is involved in the Mφ response to oxidized LDL [7, 27] and mediates microglial and Mφ responses to β-amyloid [28]. This scavenger receptor also recognizes anionic phospholipids [8], apoptotic cells [9], thrombospondin [29] and *P. falciparum*-infected erythrocytes [10]. Thus, CD36 is a necessary signaling component of a pattern recognition receptor complex on monocytes/Mφ that recognizes modified host proteins.

CD36 can be up-regulated by different molecules, in particular by high glucose concentrations [30], oxidatively modified LDL [31] and two oxidized linoleic acid metabolites, 9-hydroxyoctadecadienoic acid (9-HODE) and 13-HODE [13]. IL-4, a Th2 cytokine, has been shown to increase monocyte/Mφ expression of CD36 [17], whereas expression of CD36 is down-regulated in response to a Th1 cytokine like IFN-γ [32] and to LPS or dexamethasone [17].

Here, we study the effects of IL-13, another Th2 cytokine, on CD36 surface expression in human monocytes. Indeed, even though IL-4 and IL-13 share many structural characteristics, they also have important differences. *In vivo* studies using IL-$4^{-/-}$, IL-$13^{-/-}$, STAT$6^{-/-}$ and IL-4R$\alpha^{-/-}$ mice revealed different effects of IL-4 and IL-13 in models of allergic inflammation [33] and helminth infections [34].

The mechanism by which oxidized LDL or linoleic acid metabolites up-regulate CD36 involves activation of the transcription factor PPARγ [13, 35]. Other PPARγ ligands such as 15d-PGJ2 and the thiazolidinedione class of anti-diabetic drugs also increase CD36 expression notably in Mφ [13] but also in mature and immature human monocyte-derived dendritic cells [36]. The CD36 promoter contains a PPARγ-9-*cis*-retinoic acid receptor binding site, and the PPARγ-9-*cis*-retinoic acid receptor complex can modulate CD36 gene expression through direct promoter interaction [35].

Several authors report that scavenger receptors can cooperate with Toll-like receptors (TLR) in microbial sensing, enhancing inflammatory signals [37]. In particular, it has been shown recently that several components of Gram-positive bacteria such as lipoteichoic acid can form TLR2/6 heterodimers with CD36 [38]. In addition, some TLR2 ligands, but not all, are dependent on CD36 [39]. This cooperation significantly enhances NF-kB activation and TNF-α production after TLR2 ligands bind to cells. Thus, all these results suggest that CD36 could be considered as functioning in an analogous way to CD14, which concentrates the LPS signal for transduction through TLR4 [40]. On the contrary, CD36 also has an established role in the phagocytosis of endogenous ligands (such as apoptotic cells) [41] or with *P. falciparum*, without enhancing pro-inflammatory signaling [10, 11]. These data suggest that, depending on the ligands and the environment, CD36 could enhance different mechanisms to favor immune response activation or to eliminate endogenous ligands without inflammation.

The results presented here suggest that PPARγ is a key molecule involved in the anti-inflammatory properties of Th2 cytokines through the enhancement of CD36-dependent phagocytosis independently of TLR2 recruitment. Support for this hypothesis came from the fact that previous studies have demonstrated that the phagocytosis of *P. falciparum*-parasitized erythrocytes is enhanced by PPARγ ligands without TNF-α production [11]. In addition, it has previously been shown that Th2 cytokines like IL-4 activate PPARγ in Mφ [18] while this cytokine down-regulates TLR2 expression [42], suggesting that TLR2 and PPARγ are opposite signaling pathways.

Here, we have provided evidence that IL-13 up-regulates CD36 surface expression in human monocytes and increases CD36 mRNA. Our results demonstrate for the first time that IL-13 up-regulates CD36 *via* PPARγ. Indeed, GW9662 (1 μM), an irreversible antagonist of PPARγ, inhibits the up-regulation of CD36 surface expression on human monocytes triggered by rosiglitazone (5 μM) or IL-13. GW9662 is a potent irreversible PPARγ ligand that functions as a selective PPARγ antagonist at concentrations of 1–10 μM in cell-based assays [24]. Furthermore, in the murine Mφ cell line RAW264.7, which expresses little PPARγ, IL-13 or rosiglitazone do not induce the increase of CD36 expression. Moreover, the effect of PPARγ agonists or IL-13 on this expression is restored by transfection with a PPARγ expression vector in these cells. To confirm the implication of PPARγ in this phenomenon, we have used resident peritoneal Mφ from mice in which PPARγ is selectively disrupted. We showed that IL-13, as was rosiglitazone, was ineffective on CD36 induction on these Mφ compared with wild-type cells.

Quite surprisingly, in the absence or deficit of PPARγ, in RAW cells as well as in PPARγ$^{-/-}$ Mφ, the basal level of CD36 (in the absence of IL-13) is considerably higher than in cells with restored PPARγ expression or in wild-type Mφ. This observation could suggest a negative role of PPARγ in CD36 expression and that other transcription factors, different from PPARγ, would be implicated in CD36 expression. Recently it has been shown that Nrf2 is a novel signaling pathway, distinct from PPARγ, that also up-regulates CD36 expression in murine Mφ treated with oxidized LDL. Nrf2 is a key transcription factor controlling antioxidant gene expression and that regulates antioxidant defence in Mφ, implicating up-regulation of CD36 in oxidative stress [43]. The recent study reporting that PPARγ inhibits Nrf2-induced expression of the gene encoding thromboxane synthase in Mφ, suggests that the transcriptional regulators PPARγ and Nrf2 may interact [44]. Future studies *in vitro* using PPARγ-deficient Mφ should enable us to determine whether Nrf2 modulates CD36 gene expression in these PPARγ-deficient cells.

Lastly, the use of AG 490, a specific inhibitor of the JAK2-STAT6 signaling pathway that can be activated by IL-13 [45], did not modify the CD36 expression in the presence of IL-13 in human monocytes (data not shown). Furthermore, in order to independently confirm PPARγ activation, EMSA analysis and immunodetection of activated PPARγ were performed, demonstrating PPARγ migration into the nucleus and PPARγ binding to its responsive element on DNA under IL-13 treatment of human monocytes. These data strengthened the evidence of a single PPARγ signaling pathway of CD36 up-regulation by IL-13.

Finally, to determine the functional consequences of CD36 over-expression and PPARγ activation mediated by IL-13, we analyzed the phagocytosis of *P. falciparum*-parasitized erythrocytes by human monocytes after IL-13 treatment. Indeed, previous studies have shown that PPARγ ligands enhance the phagocytosis of *P. falciparum*-parasitized erythrocytes *via* CD36 over-expression [11]. Furthermore, these ligands inhibit TNF-α production by Mφ and could explain the absence of inflammatory cytokines during this phagocytosis of *P. falciparum*-parasitized erythrocytes [11].

Here, we show for the first time that a type-2 cytokine, IL-13, significantly enhances the CD36-mediated phagocytosis of *P. falciparum*-parasitized erythrocytes and that this effect is dependent on PPARγ activation. This result reveals that IL-13 treatment could have important immunological or physiological consequences *via* the modulation of monocyte functions through the activation of the nuclear receptor PPARγ.

PPARγ is a member of the nuclear hormone receptor superfamily that functions as a transcriptional regulator of genes and has been implicated in Mφ development and functions. PPARγ is activated by a number of natural or synthetic ligands, including polyunsaturated fatty acids, 15d-PGJ2, components of oxidized LDL, such as 13-HODE and 15-hydroxyeicosatetraenoic acid, and anti-diabetic thiazolidienediones. Interestingly, Huang *et al*. [18] reported that IL-4 may generate natural PPARγ ligands by enzymatic oxidation of polyunsaturated fatty acids. Indeed, the pharmacological and genetic manipulation of specific metabolic pathways has added support for an important role for 12/15-lipoxygenase products (13-HODE and 15-hydroxyeicosatetraenoic acid) as endogenous regulators of PPARγ produced in the Mφ by IL-4, and required for a maximal induction of CD36 gene by this cytokine [18]. Recently we have shown, in murine peritoneal Mφ, that IL-13 upregulates mannose receptor expression *via* PPARγ activation. We have also shown that this induction of the mannose receptor by IL-13 was dependent on cytosolic PLA2 activation [23].

These two results demonstrating PPARγ involvement in IL-13-induced mannose receptor expression or in IL-4 induced CD36 expression were obtained using murine Mφ. The extrapolation of these data between different species and between different stages of Mφ differentiation remains difficult. Indeed, there are numerous species differences between mice and humans in lipid metabolism concerning different enzyme pathways and notably PPAR. For example, PPARα, the target of hypolipidemic fibric acids, has an essential role in regulating cholesterol efflux from human but not from mouse Mφ [46]. Furthermore, different anti-inflammatory responses of human monocytes and sinoval fluid Mφ have been shown for IL-13 and IL-4 depending on different patterns of the receptors for these cytokines [47].

We show here, in human monocytes, that inhibition of cytosolic PLA2 blocks the CD36 induction by IL-13, and that this CD36 over-expression is restored by the addition of 15d-PGJ_2. In addition, the participation of 15d-PGJ_2 in CD36 over-expression by IL-13 is also suggested by confocal microscopy analysis that shows that IL-13 generates 15d-PGJ_2 production and its nuclear localization in human monocytes. Thus, we demonstrated that CD36 induction by IL-13 was dependent on cytosolic PLA2 activation, and that IL-13 could positively regulate CD36 expression by controlling the production of endogenous PPARγ ligands, particularly 15d-PGJ2.

In summary, we have demonstrated in this study that IL-13 induces CD36 over-expression on human monocytes *via* PPARγ activation. We have also shown that this mechanism has a functional consequence on human monocytes, as PPARγ is involved in the IL-13-mediated phagocytosis of *P. falciparum*-parasitized erythrocytes by human monocytes.

It is tempting to assume that this mechanism could also contribute to other anti-inflammatory properties of IL-13. Indeed, Yang *et al*. [48] showed recently that IL-13 stops brain inflammation *via* cyclooxygenase-2 products, like 15d-PGJ_2, through PPARγ activation, enhancing the death of activated microglia. Altogether, these data suggest that this Th2 cytokine could favor the elimination of abnormal or inflammatory cells, while reducing inflammation, probably through PPARγ activation and CD36 over-expression.

Materials and methods

Media and reagents

Ficoll-Hypaque was from AbCys (Paris, France). RPMI 1640, DMEM, and PBS without calcium or magnesium, Mφ serum-free medium (SFM), optimized for monocytes/Mφ culture, and Trizol reagent were from Invitrogen Corporation (Cergy Pontoise, France). Paraformaldehyde, isopropanol, and ethanol were from Sigma-Aldrich (Saint Quentin Fallavier, France). Triton X-100 was from Merck Sharp (Riom, France). Rosiglitazone, GW9662, 15d-PGJ_2 and MAFP were from Cayman Chemical (Ann Arbor, MI); AG 490 was from Biomol International LP (Plymouth, PA). IL-13 was from Sanofi Synthelabo (Labège, France).

R-PC5-conjugated mouse monoclonal antibody against human CD14 and R-PC5-conjugated mouse monoclonal isotype antibody IgG2a (negative control) were from Beckman Coulter (Villepinte, France). R-PE-conjugated mouse monoclonal antibody against human CD36, and R-PE-conjugated mouse monoclonal isotype antibody IgM (negative control) were from BD Biosciences (Pont de Claix, France). The murine PE-conjugated CD36 antibody was from TEBU (Le Peray en Yvelines, France). For the phagocytosis assays, the CD36-blocking antibody (FA6-152) was purchased from Immunotech (France).

FuGENE 6 Transfection Reagent was purchased from Roche Diagnostics (Mannheim, Germany). The pCMX-mPPARγ, a gift from Ron Evans (The Salk Institute, San Diego, CA), encoded for the mouse nuclear receptor PPARγ.

Monocyte isolation

PBMC were obtained from healthy blood donor buffy coats and isolated by a standard Ficoll-Hypaque gradient method.

Monocytes were isolated from mononuclear cells by adherence to plastic for 2 h in Mφ SFM at 37°C in a humidified atmosphere containing 5% CO_2. Non-adherent cells were removed by three washings with PBS. The remaining adherent cells (>85% monocytes) were incubated in Mφ SFM.

Production of PPARγ-KO mice and harvesting of macrophages

All animal experimentation was conducted in accordance with accepted standards of humane animal care. Mouse experiments were approved by the Animal Studies Committee of the Midi-Pyrénées Region and were performed in accordance with their guidelines.

Mice harboring LoxP-flanked PPARγL2 alleles [49] were crossed with LysM-Cre-transgenic mice in which the Cre recombinase is expressed under the control of the Mφ-selective lysozyme promoter [50]. The resulting LysM-$Cre^{(tg/0)}$/$PPAR\gamma^{L2/L2}$ mice, in which PPARγ is selectively disrupted in Mφ, and age- and sex-matched $PPAR\gamma^{L2/L2}$ control littermates were used to harvest peritoneal Mφ.

The analysis of PPARγ mRNA deficiency on Mφ was realized using a quantitative real-time PCR. RNA was extracted as described next for human monocytes. cDNA was synthesized using the SuperScript System (Invitrogen, Carisbad, CA), random hexamer primers and dNTP. Quantitative RT-PCR was performed on a LightCycler as described previously [23]. The 18S rRNA transcript was used as the invariant control.

To determine CD36 expression, resident peritoneal Mφ were obtained by injection into the peritoneal cavity of sterile 199 medium with Hank's salts. The cells collected were centrifuged and the cell pellet was suspended in Mφ SFM. Cells were allowed to adhere for 2 h at 37°C in a 5% CO_2 atmosphere in 24-well culture plates. Non-adherent cells were removed by washing with PBS, and the remaining adherent cells were stimulated with 15d-PGJ_2 or rosiglitazone, or IL-13 diluted in Mφ SFM as described in Fig. 4B. After 2 h of adhesion, 98% of adherent cells were positive for non-specific esterase and had the morphological appearance of Mφ as judged by May–Grünwald–Giemsa staining.

Parasite culture

The laboratory strain FcB1-K+ of *P. falciparum* was continuously cultured according to Trager and Jensen [51]. The parasites were maintained *in vitro* in human red blood cells O+ (French blood bank). The culture medium was RPMI 1640 (Gibco, Invitrogen) supplemented with 5% human serum (French blood bank) and containing 25 mM HEPES and L-glutamine. The culture was performed at 37°C with a 2–4% hematocrit and in an atmosphere of 5% CO_2.

Analysis of CD36 expression using flow cytometry

About 5.0×10^5 adherent monocytes were cultured in 24-well plastic culture plates. The cells were incubated with one or more of the following products: IL-13, rosiglitazone (synthetic PPARγ agonist), 15d-PGJ_2, GW9662 (specific PPARγ inhibitor), MAFP (specific cytosolic PLA2 inhibitor) and AG 490, a potent and specific inhibitor of the JAK-2 tyrosine kinase and STAT6 signaling pathway.

After culture, monocytes were collected with a scraper and assayed for CD36 expression by flow cytometry. Each experiment was matched with an appropriate non-specific isotype antibody (negative control). The cells were assayed by FACScan flow cytometry, with at least 500 monocytes per sample being analyzed. Data were analyzed using Cell Quest software.

Analysis of CD36 mRNA using a quantitative real-time PCR

About 1.0×10^6 adherent monocytes were cultured in 12-well plastic culture plates without or with IL-13 (20 ng/mL). Rosiglitazone was used as positive control at 5 μM. After 6 h of incubation the mRNA was extracted using Trizol reagent and purified by a chloroform/isopropanol/ethanol procedure. The reverse transcription was performed with the First-strand cDNA Synthesis Kit (Promega, Charbonnières les bains, France).

The PCR for CD36 and α-actin cDNA was performed with the LC FastStart DNA master SYBER Green I (Roche Diagnostics, Meylan, France). Amplification and detection were performed in a LightCycler system (Roche Diagnostics) as follows. Twenty microliters of reaction mixture was incubated initially for 8 min at 95°C to activate the Fast Start Taq DNA; amplifications were performed for 40 cycles (15 s at 95°C and 30 s at 68°C) for CD36 and α-actin. The primers were designed with the software Primer Express (Applied Biosystems, Foster City, CA). The primers were: 5′-TGTAACCCAGGACG-CAGGACGCTGAGG-3′ (sense) and 5′-GAAGGTTCGAAG-TTCGAAGATGGCACC-3′ (antisense) for CD36; 5′-CCTC-CCTCACCCTGAAGTACCCCA-3′ (sense) and 5′-TGCCAGAT-GCCAGATTTTCTCCATGTCG-3′ (antisense) for α-actin. Results of the real-time PCR data were represented as Ct values as described [26].

Nuclear protein extraction

About 6×10^6 adherent monocytes were cultured in 6-well plastic culture plates. After treatment with human IL-13 (20 ng/mL), 15d-PGJ_2 (1 μM) and rosiglitazone (5 μM) for 1 h, nuclear protein was isolated for the EMSA and analysis of the DNA-binding activity. For the EMSA, protein extraction was performed using a standard method, and for analysis of the DNA-binding activity, protein extraction was performed using a lysis buffer included in the TransAM® kit (Active Motif, Rixensart, Belgium). We used the Bradford method for measuring protein concentration.

Electrophoretic mobility shift assay

Double-stranded PPAR gel shift consensus oligonucleotides were purchased from Santa Cruz Biotechnology (Santa Cruz, CA): 5′-CAAAACTAGGTCAAAGGTCA-3′. Underlined letters correspond to the consensus PPRE half-site sequences. Oligonucleotides were radioactively end-labeled with [^{32}P]ATP (Perkin-Elmer Life Science, Paris, France) using T4 polynucleotide kinase (Promega) and purified from unin-

corporated nucleotides by the QIAquick Nucleotides Removal Kit (Qiagen, Courtaboeuf, France). Cells were cultured as above and were stimulated with IL-13 (20 ng/mL), 15d-PGJ_2 (1 μM) and rosiglitazone (5 μM) for 1 h. Nuclear protein extraction and assay were done as above. About 15 000 cpm of oligonucleotide probe and 5 μg of the various nuclear extracts were subjected to 5% PAGE. The gel was then dried and autoradiographed.

DNA-binding activity

A TransAM® kit (Active Motif) was used to evaluate the DNA-binding activity of PPARγ. Nuclear protein was extracted according to the manufacturer's protocol. Ten micrograms of total extract was incubated in 96-well plastic culture plates coated with a PPRE. The detection of linked PPARγ was carried out with a specific antibody and a secondary antibody coupled to horseradish peroxidase supplied in the kit. After incubation with the substrate at the indicated time, the fluorescence was read at 450 nm using a Wallac 1420 Victor fluorimeter (Wallac, Turku, Finland). All the data shown represent the amount of activated PPARγ in arbitrary units.

Transfection assays

The Mφ murine cell line RAW264.7 was maintained in an exponential growth phase by subsequent splitting in DMEM complemented with 10% of FBS. The cells were transfected with a mouse PPARγ expression vector (pCMX-mPPARγ) or a beetle β-galactosidase expression vector as control as previously described [23]. Cells were collected 24 h later and analyzed as described in *Analysis of CD36 expression using flow cytometry*.

Confocal microscopy

Human monocytes were cultured on round glass coverslips in 24-well plastic culture plates and were stimulated by IL-13 (50 ng/mL) for 30 min. The cells were fixed, permeabilized and stained with anti-15d-PGJ_2 mouse polyclonal antibody (1:50) as previously described [52]. Nuclei were stained with propidium iodide at 10 μg/mL. A confocal laser-scanning microscope (LSM 510; Zeiss, Le Pecq, France) was used to visualize the production and localization of 15d-PGJ_2.

Phagocytosis assay

About 10^6 adherent human monocytes were plated on round glass coverslips in 24-well plastic culture plates. To study the impact of IL-13 on phagocytosis mediated by the CD36 receptor, monocytes were incubated with IL-13 with or without GW9662, an irreversible antagonist of the nuclear receptor PPARγ, for 24 h at 37°C. Fc receptors were first blocked by incubating the cells with human IgG Fc fragments (Sigma) at 20 μg/mL for 25 min at room temperature. To analyze CD36 involvement in the phagocytosis of *P. falciparum*-parasitized erythrocytes, monocytes were then incubated with 10 μg/mL of monoclonal anti-CD36. The *P. falciparum* culture was grown and synchronized by sorbitol lysis followed by 24 h of culture as described previously. Synchronized trophozoite stage-infected erythrocytes were carefully washed in RPMI 1640 prior to the phagocytosis assay.

P. falciparum-parasitized erythrocytes were suspended in 500 μL RPMI 1640 and added to the monocytes at an erythrocyte to monocyte ratio of 20:1. Control monocytes were exposed to equivalent numbers of uninfected erythrocytes. The plates were incubated for 4 h at 37°C in a 5% CO_2 atmosphere. Then, non-adherent erythrocytes were washed away with three changes of RPMI and adherent but non-phagocytosed erythrocytes were lysed in ice-cold distilled water for 30 s. Cell preparations were fixed and stained with Giemsa. Phagocytosis was assessed by light microscopy. Between 500 and 1000 monocytes were counted for each coverslip and scored for the presence or absence of phagocytosed *P. falciparum*-parasitized erythrocytes. The criteria for phagocytosis required the parasitized erythrocytes to be completely within the outline of the monocyte/Mφ cells. The phagocytic index was calculated as the percentage of monocytes/Mφ with clear evidence of phagocytosis.

Statistics

All experiments were performed in duplicate or triplicate and repeated at least three times. Data were expressed as means ± SE, unless otherwise noted. Statistical significance was determined using bilateral Student's *t*-tests. $p < 0.05$ was considered as the level of statistical significance.

Acknowledgements: This study was supported by a grant of the Ministère de l'Education Nationale de la Recherche et de la Technologie awarded to P. Balard. The authors are grateful to A. Minty and Sanofi-Synthelabo (Toulouse Labège, France) for supplying IL-13, and Dr. Förster for providing LysM-Cre-transgenic mice. The authors also thank K. Uchida for providing the anti-15d-PGJ_2 mouse polyclonal antibody and Dr. John Woodley for manuscript revision.

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