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Monocytes and neutrophils from tuberculosis patients are insensitive to anti-inflammatory effects triggered by the prototypic formyl peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP)

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SUMMARY

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis* where formyl peptides, which are cleavage products of bacterial and mitochondrial proteins, are present. In this study, we demonstrated that interferon gamma (IFN)-γ and interleukin (IL)-10 induced the overexpression of the receptor for the Fc portion of IgG I (FcγRI) in monocytes from tuberculosis (TB) patients, showing that these cells respond to IFN-γ and IL-10 signals. We also demonstrated that lower doses of IL-10 render monocytes from TB patients less responsive to higher doses of the cytokine. Although the prototypic formyl peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) is a well-known proinflammatory agonist, we have demonstrated previously that preincubation of monocytes with FMLP inhibited the up-regulation of FcγRI induced by IFN-γ or IL-10. This effect was not observed in monocytes from TB patientes. FMLP also induced the down-regulation of the expression of FcγRI in monocytes that had been activated already with IFN-γ. However, this effect of FMLP was not observed in monocytes from TB patients and supernatants from monocytes obtained from these patients were incapable of inducing the down-regulation of FcγRI. In contrast to normal donors, supernatants from FMLP-treated neutrophils from TB patients did not modify the basal level of expression of FcγRI in monocytes from normal donors. In conclusion, in this study we demonstrated the existence of two novel mechanisms that may contribute to the pathological effects generated by *M. tuberculosis*: the enhancement of FcγRI in response to IFN-γ and IL-10, and the unresponsiveness to the anti-inflammatory effects induced by formyl peptides.

Keywords formyl peptides leucocytes tuberculosis

INTRODUCTION

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis*, which is responsible for approximately 2 million deaths each year [1]. Cytokines play an important role in the regulation of host immune response against the mycobacteria by controlling effector functions of antigen-presenting cells. Because it has been demonstrated that individuals defective in genes for interferon-gamma (IFN-γ) or the IFN-γ receptor are more susceptible to serious mycobacterial infections [2], this cytokine has been individualized as a key in control of *M. tuberculosis* infection. Moreover, it has been demonstrated recently that *M. tuberculosis* can prevent human macrophages from responding adequately to IFN-γ by inhibiting IFN-γ signalling [3]. In contrast, cytokines such as interleukin (IL)-10 have been associated with immunosuppression in active tuberculosis [4].

It has been demonstrated that formyl peptides, which are cleavage products of bacterial and mitochondrial proteins, are antigens present in *M. tuberculosis* infection, as they are able to elicit a protective cytotoxic T cell response when displayed by H2–M3 molecules on the surface of infected macrophages [5]. However, less is known about the effect of formyl peptides acting through the binding to their receptors on monocytes from patients with mycobacterial infections. Although formyl peptides are well-known proinflammatory agonists [6,7], we have demonstrated previously that the prototypic formyl peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) was capable of exerting an anti-inflammatory activity in human monocytes and neutrophils by inhibiting the up-regulation of the receptors for the Fc portion of IgG (FcγR) induced by IFN-γ or IL-10 [8–10]. In the

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sera of tuberculosis patients opsonizing IgG1 and IgG3, which bind preferably to FcγRI, were found in high ammounts [11,12]. IgG1 and IgG3 were capable of enhancing the release of proinflammatory cytokines such as tumour necrosis factor (TNF)-α and IL-6, as well as blocking the release of IL-10 by human monocytes [13].

Taking all these data into account, in this study we investigated whether peripheral blood monocytes from tuberculosis (TB) patients were able to respond to IFN-γ or IL-10 by up-regulating the expression of FcγRI, even though the basal expression of this receptor was already up-regulated in those patients [14]. In addition, we investigated whether FMLP was able to exert its anti-inflammatory effects by down-regulating the expression of FcγRI in monocytes from TB patients.

MATERIALS AND METHODS

Reagents

FMLP, Ficoll 400, dextran, tissue culture medium (RPMI-1640), human recombinant IFN-γ, human recombinant IL-10, monoclonal antihuman IL-10 antibody (clone 23738·11) and nitroblue tetrazolium (NBT) were obtained from Sigma, St Louis, MO, USA. FITC-labelled antihuman FcγRI (clone 22), PE-labelled antihuman CD14 (clone RMO52), mouse IgG1 (clone 679·1Mc7) and mouse IgG2a (clone U7·27) isotype controls were from Immunotech, Marseille, France.

Patients

A total of 14 hospitalized active TB patients was studied. Informed consent for experimentation was obtained according to the Ethics Commission of Hospital Francisco J. Muñiz. All the patients were male and had extensive pulmonary involvement confirmed by chest radiographs. All the patients had a sputum smear positive for acid-fast bacilli and the presence of *M. tuberculosis* was confirmed by sputum-positive culture. All of them were seronegative for HIV, and those with other infectious or underlying disease were not included in the study. All were treated with a standard 4 regimen (1–30 days) and patients who had not received any antituberculosis treatment were also studied. Fourteen normal healthy volunteer blood donors were studied, all had received BCG vaccination in childhood and their tuberculin-test status was unknown.

Preparation of human mononuclear cells

Fresh human blood was obtained by venipuncture and collected with heparine. Blood was diluted 1 : 2 with saline, layered on a Ficoll-Hypaque cushion and centrifuged at 400 ***g*** for 25 min as described previously [15]. Peripheral blood mononuclear cells (PBMC) were collected, washed and resuspended in RPMI 10% heat-inactivated (56°C, 30 min) fetal calf serum and 50 μg/ml gentamycin. Viability of mononuclear cells was always more than 98% as measured by the trypan blue exclusion test.

Preparation of human neutrophils

Fresh human blood was obtained by venipuncture and collected on heparin. Neutrophils were isolated from the bulk of red cells by sedimentation with 3% dextran and Ficoll-Hypaque cushion, as described previously [10]. The pellet containing neutrophils was collected and the contaminating erythrocytes were removed by hypotonic lysis. The cell suspension contained 95–98% of neutrophils. Viability of neutrophils was always more than 95% as measured by the trypan blue exclusion test.

Cytokine treatment

PBMC from TB patients or normal donors were incubated with medium, IFN-γ (240 U/ml), IL-10 (100 U/ml) or IFN-γ (240 U/ml) plus IL-10 (100 U/ml) for 24 h at 37°C, 5% CO_2. In other experiments, PBMC from TB patients or normal donors were incubated with IL-10 (10 U/ml) for 2 h and then IL-10 (100 U/ml) was added for 24 h at 37°C, 5% CO_2. PBMC were also incubated with medium or an anti-IL-10 antibody (10 μg/ml) and 2 h later IFN-γ (240 U/ml) was added for 24 h at 37°C, 5% CO_2. In all these experiments, PBMC were incubated in polypropylene tubes and used for flow cytometry studies.

FMLP treatment

FMLP was diluted in dimethylsulphoxide (DMSO) at a concentration of 0·1 M. The subsequent dilutions of FMLP were made in saline. PBMC from TB patients or normal donors were incubated with medium or FMLP at a concentration of 1 μM in polypropylene tubes. After 1 h of incubation at 37°C, 5% CO_2, the cells were washed and incubated with medium or IFN-γ (240 U/ml) for 24 h. After that period, the cells were used for flow cytometry studies. In other experiments, PBMC from TB patients or normal donors were incubated in polypropylene tubes with medium or IFN-γ (240 U/ml) at 37°C, 5% CO_2 for 24 h. Then, medium or FMLP (1 μM) were added to the cells for an additional 2 h. After that period, the cells were used for flow cytometry studies.

Supernatants collection

PBMC (1×10^6 cells/ml) from TB patients or normal donors were treated with medium, IFN-γ (240 U/ml) or IL-10 (100 U/ml) at 37°C in a 5% CO_2 atmosphere for 24 h. After that period, the cells were incubated with medium or FMLP (1 μM) for an additional 2 h. Then, supernatants were collected, spun down to remove cellular debris (10 min at 6000 r.p.m) and stored at −70°C until their use. Neutrophils (1×10^6 cells/ml) from TB patients or normal donors were treated with medium or FMLP (1 μM) at 37°C in a 5% CO_2 atmosphere for 3 h. Then, supernatants were collected, spun down to remove cellular debris (10 min at 6000 r.p.m), stored at −70°C and dialysed exhaustively against RPMI to eliminate residual FMLP before usage.

Flow cytometry

After different treatments, PBMC (1×10^6 cells/ml) from TB patients or normal donors were washed and incubated with the monoclonal antibodies indicated above. The cells were washed and resuspended in ISOFLOW and flow cytometry was performed on a FACS Analyser (Becton-Dickinson Immunocytometry System, San Jose, CA, USA). The results were expressed as median of fluorescence intensity (MFI) of the CD14-positive ($CD14^+$) population of PBMC. Non-specific binding was determined using the appropriate mouse IgG isotype matched control antibody.

NBT assay

Nitroblue tetrazolium (NBT) is a colourless compound which is reduced to dark blue formazan by the superoxide anion formed by leucocytes [16]. Briefly, 0·1 ml of PBMC resuspended in medium at a concentration of 30×10^6 cells/ml were mixed with 0·1 ml of FMLP (final concentration of 1 μM). Then, 0·2 ml of

0·1% NBT in Hanks's solution was added and the suspensions were incubated for 30 min in a 37°C waterbath with shaking. The reaction was stopped by adding 1 ml of 0·5% HCl. The tubes were centrifuged and the supernatant was removed. The formazan formed was extracted by adding 1 ml of dimethylsulphoxide (DMSO) and the optical density (OD) of the solution was determined by spectrophotometry at 550 nm.

Statistical analysis

Statistical significance of results was calculated using the non-parametric Mann–Whitney test (two-tailed).

RESULTS

IFN-γ and IL-10 up-regulate the expression of FcγRI in monocytes from TB patients

It has been described previously that FcγRI, which is expressed constitutively on human monocytes, was significantly up-regulated by IFN-γ after 24 h of incubation [17]. As the expression of FcγRI is already up-regulated in monocytes from TB patients [14], we investigated whether the incubation of these cells with IFN-γ could increase the expression of the receptor. Figure 1 shows that monocytes from TB patients increase the expression of FcγRI after 24 h of incubation with IFN-γ. The magnitude of this effect varies in monocytes from different TB patients and could be attributed to individual susceptibility to IFN-γ and/or to the heterogeneity of monocyte subpopulations. However, these variations could not be attributed to the severity of the disease, the PPD status or a previous overcome tuberculosis. Even though 50% of the patients do not respond significantly to IFN-γ this does not mean that monocytes from these patients are unresponsive, because they do augment FcγRI in response to IFN-γ compared to untreated cells (data not shown). The fact that monocytes from TB patients increase the level of expression of FcγRI after treatment with IFN-γ indicates that the cells are still able to respond to this cytokine.

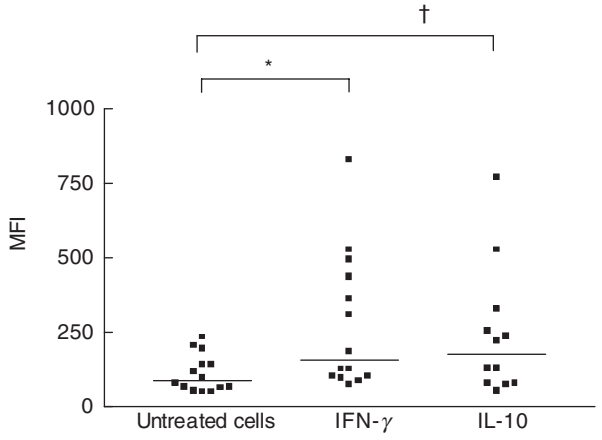

Fig. 1. Effect of IFN-γ and IL-10 on the expression of FcγRI in monocytes from TB patients. PBMC (1×10^6 cells/ml) from TB patients were incubated with medium, IFN-γ (240 U/ml) or IL-10 (100 U/ml) for 24 h. After that period, PBMC were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) and correspond to the $CD14^+$ population of PBMC, $n = 14$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. *$P < 0{\cdot}02$ significantly different from untreated cells. †$P < 0{\cdot}05$ significantly different from untreated cells.

Although IL-10 is a well-known anti-inflammatory cytokine, it is also capable of increasing the expression of FcγRI in human monocytes [18]. As shown in Fig. 1, IL-10 induces the overexpression of FcγRI in monocytes from TB patients after 24 h of incubation. The differences in the magnitude of the increase, again, could not be attributed to the severity of the disease, the PPD status or a previous overcome tuberculosis. The same results were obtained with four patients who had not received any antituberculosis treatment (data not shown). Monocytes from all the patients evaluated augmented the expression of FcγRI in response to IL-10 compared to untreated cells although, in some cases, the level of increment was not statistically significant (data not shown).

The incubation of monocytes from normal donors with IFN-γ + IL-10 enhance the expression of FcγRI in an additive manner (MFI: untreated cells, 93; IFN-γ, 246; IL-10, 235; IFN-γ + IL-10, 411; $n = 6$). A similar effect was observed in monocytes from TB patients (MFI: untreated cells, 201; IFN-γ, 437; IL-10, 331; IFN-γ + IL-10, 906; $n = 6$).

Pretreatment of monocytes from TB patients with lower doses of IL-10 renders the cells less responsive to the challenge with higher doses of the cytokine

As shown in Fig. 1, IL-10 (100 U/ml) is capable of inducing the overexpression of FcγRI in monocytes from TB patients. Nevertheless, if TB cells are incubated previously in the presence of a lower dose of IL-10 (10 U/ml) for 2 h before the addition of a higher dose of the cytokine (100 U/ml), FcγRI is enhanced to a lesser extent (Fig. 2). The lower dose used in the experiments (10 U/ml) was incapable of inducing the overexpression of FcγRI (data not shown).

In order to investigate whether the endogenous production of IL-10 by cultured cells could alter the basal level of expression of FcγRI as well as the action of IFN-γ, PBMC from TB patients were incubated in the presence of an anti-IL-10 antibody for 24 h. Figure 3 shows that the incubation of the cells with this specific antibody does not modify the basal level of expression of FcγRI or the up-regulation of the receptor induced by IFN-γ. However, we cannot rule out the possibility that small amounts of IL-10 produced by cells from TB patients could alter the expression of FcγRI *in vivo*.

FMLP does not alter the up-regulation of FcγRI induced by IFN-γ in monocytes from TB patients

Although FMLP is a well-known proinflammatory agonist [6,7], we have demonstrated previously that preincubation of monocytes with FMLP significantly reduced the up-regulation of FcγRI induced by IFN-γ or IL-10 [8]. Thus, we investigated whether the anti-inflammatory effect exerted by FMLP could be reproduced in monocytes from patients with a chronic inflammatory disease such as tuberculosis. Figure 4a shows that, in contrast to normal donors, the up-regulation of FcγRI induced by IFN-γ is not altered by FMLP in monocytes from TB patients. The same results were obtained with four patients who had not received any antituberculosis treatment (data not shown).

FMLP does not alter the expression of FcγRI in IFN-γ-activated monocytes from TB patients

We have demonstrated previously that FMLP also induced the down-regulation of the expression of FcγRI in monocytes that had been already activated with IFN-γ [19]. However, as shown in Fig. 4b, this effect is not observed in monocytes from TB patients.

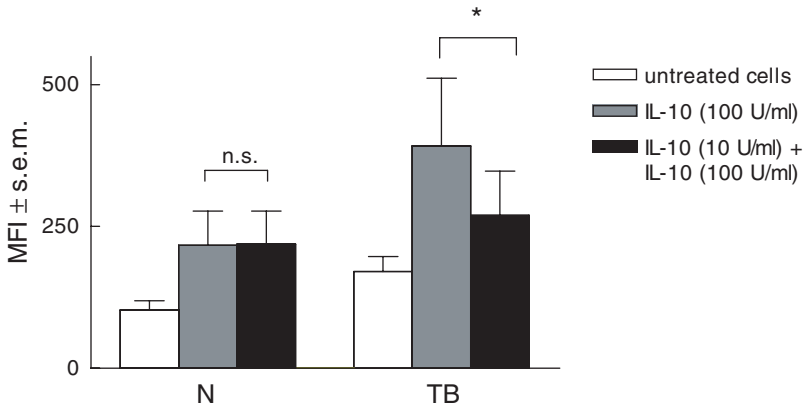

Fig. 2. Effect of low doses of IL-10 on the IL-10-induced expression of FcγRI in monocytes from TB patients. PBMC (1×10^6 cells/ml) from normal donors (N) or TB patients were incubated with medium or IL-10 (10 U/ml) for 2 h. Then, medium or IL-10 (100 U/ml) were added. After 24 h of incubation, PBMC were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) ± s.e.m. and correspond to the $CD14^+$ population of PBMC, $n = 5$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. *$P < 0{\cdot}05$ significantly different from cells treated with IL-10 (100 U/ml) alone.

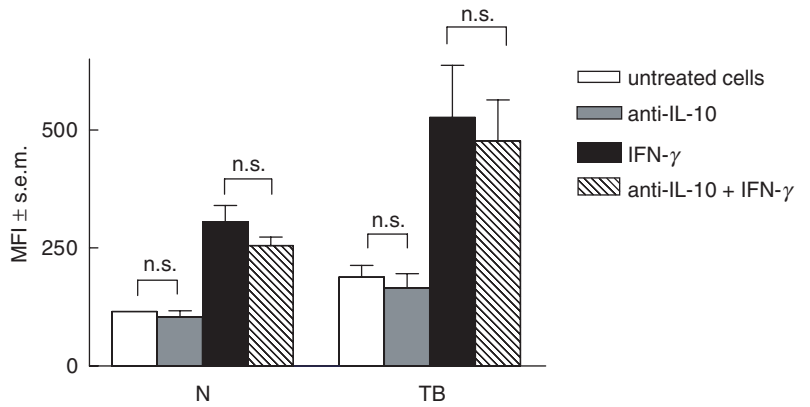

Fig. 3. Effect of an anti-IL-10 antibody on the basal and IFN-γ-induced expression of FcγRI in monocytes from TB patients. PBMC (1×10^6 cells/ml) from normal donors (N) or TB patients were incubated with medium or an anti-IL-10 antibody (10 μg/ml) for 2 h. Then, medium or IFN-γ (240 U/ml) were added. After 24 h of incubation, PBMC were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) ± s.e.m. and correspond to the $CD14^+$ population of PBMC, $n = 5$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. ns: $P > 0{\cdot}05$ not significantly different from untreated or IFN-γ-treated cells.

The down-regulation of FcγRI in monocytes is dependent on the secretion of serine and metalloproteases to the extracellular milieu, and supernatants from monocytes treated with IFN-γ + FMLP induce the down-regulation of FcγRI when added to naive monocytes. To investigate further whether TB monocytes were themselves capable of secreting the enzymes that mediate the effect of FMLP, supernatants from TB PBMC were added to naive monocytes from normal donors. The results shown in Fig. 5a indicate that these supernatants do not induce the down-regulation of FcγRI. Nevertheless, we incubated monocytes from TB patients with supernatants from PBMC from normal donors to investigate whether the products released by these cells had any effect on TB monocytes. As shown in Fig. 5b, FcγRI expressed in the surface of monocytes from TB patients is not down-regulated by the supernatants obtained from monocytes from normal donors, suggesting that TB monocytes do not respond to FMLP themselves and are also insensitive to FMLP-released products.

Supernatants from TB neutrophils treated with FMLP do not modify the basal level of expression of FcγRI in monocytes from normal donors

We have demonstrated previously that supernatants from FMLP-stimulated neutrophils induced the down-regulation of FcγRI when added to monocytes from normal donors [19]. As neutrophils are one of the most important cell populations in infectious/inflammatory processes, including mycobacterial infections [20–22], we studied whether supernatants from FMLP-treated neutrophils obtained from normal donors could modify the expression of FcγRI in monocytes from TB patients. Similar to our previous results, these supernatants induce the down-regulation of FcγRI on TB monocytes (Fig. 6a). However, when supernatants came from FMLP-stimulated neutrophils from TB patients, there was no effect on the expression of FcγRI (Fig. 6b). These results suggest that TB neutrophils do not release the anti-inflammatory products responsible for the down-regulation of FcγRI but are

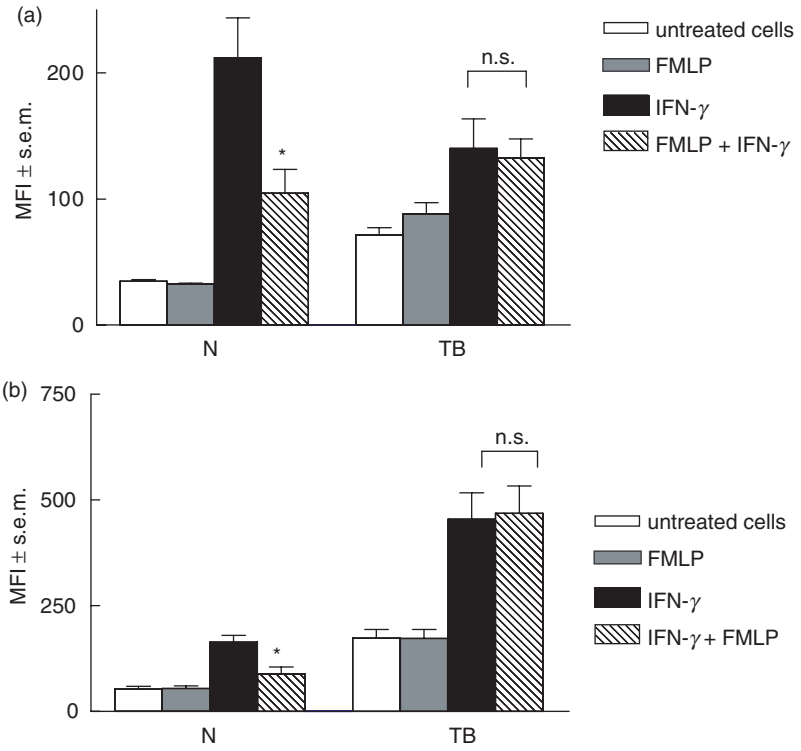

Fig. 4. Effect of FMLP on the IFN-γ-induced expression of FcγRI in monocytes from TB patients. (a) PBMC (1×10^6 cells/ml) from normal donors (N) or TB patients were incubated with medium or FMLP 1 μM for 1 h. Then, the cells were washed and medium or IFN-γ (240 U/ml) were added. After 24 h of incubation, PBMC were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) ± s.e.m. and correspond to the $CD14^+$ population of PBMC, $n = 6$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. $^*P < 0{\cdot}0001$ significantly different from untreated cells and $P < 0{\cdot}0001$ significantly different from IFN-γ-treated cells. (b) PBMC (1×10^6 cells/ml) from normal donors (N) or TB patients were incubated with medium or IFN-γ (240 U/ml) for 24 h. After that period, the cells were incubated with medium or FMLP 1 μM for an additional 2 h. Then, PBMC were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) ± s.e.m. and correspond to the $CD14^+$ population of PBMC, $n = 6$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. $^*P < 0{\cdot}05$ significantly different from untreated cells and $P < 0{\cdot}05$ significantly different from IFN-γ-treated cells.

themselves capable of responding to these mediators secreted by normal neutrophils in response to FMLP.

Monocytes from TB patients show an enhanced release of reactive oxygen intermediates in response to FMLP

We also evaluated whether monocytes from TB patients were capable of responding to proinflammatory signals triggered by FMLP such as the release of reactive oxygen intermediates. As shown in Fig. 7, monocytes from normal donors do not show a significant release of reactive oxygen intermediates when using a suboptimal cell concentration. In contrast, monocytes from TB patients show an enhanced release of these intermediates. The same has been demonstrated previously for neutrophils from TB patients, which also show an enhanced release of reactive oxygen intermediates [23,24].

The difference between monocytes from normal donors and TB patients in their responses to FMLP could not be attributed to differences in the amount of FMLP receptors, as shown by the binding of FMLP–FITC to these cells (MFI ± s.e.m.: normal, 87 ± 4; TB, 80 ± 6, $n = 4$). The FMLP–FITC bound to the surface of the cells was released by the addition of FMLP, showing that the binding was specific (data not shown).

DISCUSSION

Infection with *M. tuberculosis* is accompanied by an initial and persistent intense local inflammatory response in which cytokines play important roles in the regulation of host immune response against the infection. IFN-γ was identified as a key cytokine in the control of tuberculosis as it has been demonstrated that patients with defects in IFN-γ or IFN-γ receptor genes had severe manifestations of the disease [2]. *M. tuberculosis* itself is capable of inhibiting the IFN-γ signalling *in vitro* [3], and this could be interpreted as a mechanism of evasion of the immune response. However, in this study we have demonstrated that peripheral monocytes from TB patients are able to respond to IFN-γ by enhancing the expression of FcγRI (Fig. 1), even though this receptor is up-regulated already in those patients [11]. The response to IFN-γ varies among individuals, but this variation does not correlate with the severity of the disease, the PPD status or a previous overcome tuberculosis. Although some studies suggest that IFN-γ production is depressed in patients with active tuberculosis [25,26], this cytokine may be unreliable as an immune correlate of protection [27]. Nevertheless, according to our results, monocytes from TB patients are capable of responding to this proinflammatory cytokine. Monocytes from

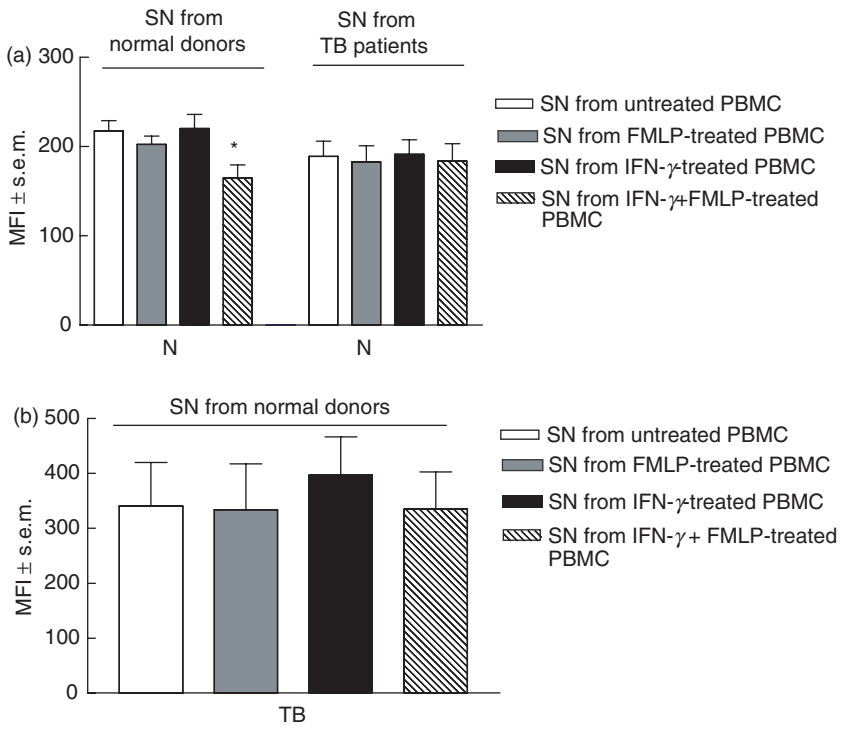

Fig. 5. Effect of supernatants from FMLP + IFN-γ-treated PBMC on the expression of FcγRI in monocytes from TB patients. (a) PBMC (1×10^6 cells/ml) from normal donors (N) or TB patients were incubated with medium or IFN-γ (240 U/ml) for 24 h. After that period, the cells were incubated with medium or FMLP 1 μM for an additional 2 h. Supernatants were collected, dialysed exhaustively and added to naive PBMC from normal donors (1×10^6 cells/ml) for 3 h. Then, these cells were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) ± s.e.m. and correspond to the $CD14^+$ population of PBMC, $n = 5$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. $^*P < 0{\cdot}05$ significantly different from untreated cells. (b) PBMC (1×10^6 cells/ml) from normal donors (N) were incubated with medium or IFN-γ (240 U/ml) for 24 h. After that period, the cells were incubated with medium or FMLP 1 μM for an additional 2 h. Supernatants were collected, dialysed exhaustively and added to naive PBMC from TB patients (1×10^6 cells/ml) for 3 h. Then, these cells were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) ± s.e.m. and correspond to the $CD14^+$ population of PBMC, $n = 5$. Statistical significance was calculated using the Mann–Whitney test, two-tailed.

all the patients included in this study augmented the level of expression of FcγRI in response to IFN-γ although, in some cases, this increment was not statistically significant. A possible explanation of this fact is that FcγRI is up-regulated already in monocytes fromTB patients [11], so the degree of increment after IFN-γ treatment may not be statistically significant.

IL-10, which is considered generally to be primarily anti-inflammatory, is produced by macrophages and T cells during *M. tuberculosis* infection. This cytokine is capable of deactivating macrophages and suppressing T cell proliferation *in vitro* [28] as well as blocking immune responses in anergic TB patients [29]. However, it can also exert proinflammatory activities in TB patients such as the enhancement of the expression of FcγRI, as shown in Fig. 1. Moreover, IL-10 collaborates with IFN-γ to increase the level of expression of FcγRI, although these cytokines seem to have opposite effects in tuberculosis. Also, we have demonstrated that while low doses of IL-10 render monocytes from TB patients less responsive to higher doses of the cytokine (Fig. 2), anti-IL-10 antibodies do not affect the IFN-γ-induced expression of FcγRI (Fig. 3). The dual effect of IL-10 in tuberculosis is supported by the demonstration that this cytokine converts dendritic cells into macrophage-like cells with increased antibacterial activity as well as reducing their ability to present mycobacterial lipids to T cells. Thus, it has been suggested that IL-10 may collaborate to maintain a balance between protective immune response and excessive cellular activation [30].

Selective up-regulation of IgG1 and IgG3, which bind preferably to FcγRI, has been reported in advanced tuberculosis [11,12]. IgG1 and IgG3 were capable of enhancing the release of proinflammatory cytokines such as TNF-α and IL-6, as well as blocking the release of IL-10 [13]. Thus, the fact that TB patients up-regulate FcγRI in response to IFN-γ and IL-10 seems to be a mechanism for augmenting cytokines that may contribute to the pathogenesis of the disease.

Formyl peptides could be prevalent antigens during *M. tuberculosis* infection, as they might be released by infected macrophages due to necrosis in the caseatious bodies. Mitochondrial formyl peptides from necrotic tissues might also be present in the granuloma or displayed by H2–M3 molecules in the surface of infected macrophages [5]. However, less is known about the effect of these peptides acting through their receptors expressed on monocytes and neutrophils from TB patients. Even though FMLP is a well-known proinflammatory agonist, we have demonstrated recently that it could exert, paradoxically, an anti-inflammatory effect by down-regulating the induced-expression of FcγRI in

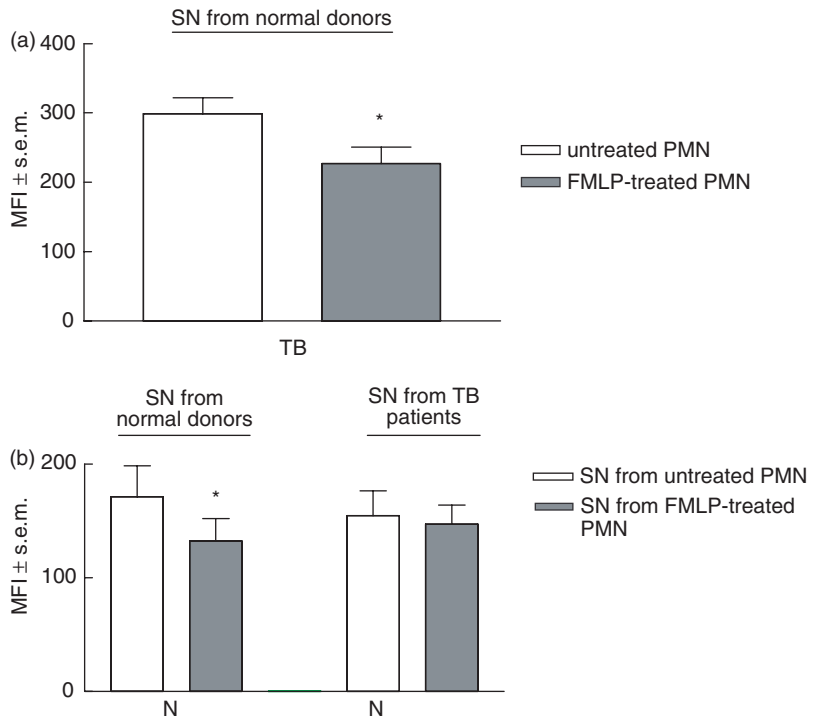

Fig. 6. Effect of supernatants from FMLP-treated neutrophils on the expression of FcγRI in monocytes from TB patients. (a) Neutrophils (1×10^6 cells/ml) from normal donors (N) were incubated with medium or FMLP 1 μM for 3 h. After that period, supernatants were collected, dialysed exhaustively and added to naive PBMC from TB patients (1×10^6 cells/ml) for 3 h. Then, these cells were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) ± s.e.m. and correspond to the $CD14^+$ population of PBMC, $n = 5$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. $^*P < 0{\cdot}05$ significantly different from untreated cells. (b) Neutrophils (1×10^6 cells/ml) from normal donors (N) or TB patients were incubated with medium or FMLP 1 μM for 3 h. After that period, supernatants were collected, dialysed exhaustively and added to naive PBMC from normal donors (1×10^6 cells/ml) for 3 h. Then, these cells were washed and stained with anti-FcγRI and anti-CD14 antibodies. Data are expressed as median of fluorescence intensity (MFI) ± s.e.m. and correspond to the $CD14^+$ population of PBMC, $n = 9$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. $^*P < 0{\cdot}05$ significantly different from untreated cells.

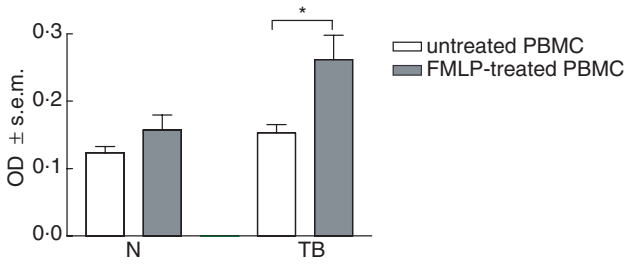

Fig. 7. Effect of FMLP in the generation of reactive oxygen intermediates in monocytes from TB patients. PBMC (3×10^6 cells) from normal donors (N) or TB patients were incubated with medium or FMLP 1 μM for 30 min in the presence of NBT. After that period, PBMC were washed and the optical density (OD) at 550 nm of the solution was evaluated (see Materials and methods). Data are expressed as OD ± s.e.m., $n = 4$. Statistical significance was calculated using the Mann–Whitney test, two-tailed. $^*P < 0{\cdot}03$ significantly different from untreated cells.

human monocytes [8]. Figures 4a,b show that monocytes from TB patients are insensitive to the anti-inflammatory activity of FMLP. Moreover, monocytes from TB patients do not respond to FMLP-released products from normal monocytes (Fig. 5b). However, monocytes from TB patients are able to respond to FMLP-released products from normal neutrophils (Fig. 6a). These differences could be attributed to the release of antagonists of the anti-inflammatory mediators by monocytes but not by neutrophils. Further studies will be necessary in order to elucidate the cause of this difference.

From a more general viewpoint, our previous studies [8–10,19,31] indicate that FMLP, which is usually viewed as a proinflammatory agent, can assume an anti-inflammatory role in certain specific circumstances. Thus, the anti-inflammatory effect of FMLP could be interpreted as a mechanism to avoid an excessive host response against infection. However, as shown in Figs 4, 5 and 6, the anti-inflammatory effect exerted by FMLP is not observed in cells from TB patients and this may contribute to enhancemeny of the host response against the mycobacteria leading to pathological consequences. The augmented generation of oxygen radicals observed in monocytes from TB patients (Fig. 7) reinforces this interpretation.

In conclusion, in this study we have demonstrated the existence of two mechanisms that may contribute to the pathological effects generated by *M. tuberculosis*: the enhancement of FcγRI in response to IFN-γ as well as to IL-10, and the unresponsiveness to the anti-inflammatory effects induced by formyl peptides.

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