The Formyl Peptide N-Formyl-methionyl-leucyl-phenylalanine Downregulates the Expression of Fc\(\gamma\)Rs in Interferon-\(\gamma\)-Activated Monocytes/Macrophages

In Vitro and In Vivo

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Abstract

N-Formyl peptides are cleavage products of bacterial and mitochondrial proteins that have pro-inflammatory activities and play an important role in antibacterial host defence. Fc\(\gamma\)RI is a receptor for the Fc portion of immunoglobulin G expressed in monocytes that mediates cytotoxicity and is upregulated by interferon-\(\gamma\) (IFN-\(\gamma\)) and interleukin-10 (IL-10). In this report, we demonstrate that N-formyl-methionyl-leucyl-phenylalanine (FMLP) downregulates the expression of Fc\(\gamma\)RI in IFN-\(\gamma\)-treated monocytes, but not in IL-10-treated monocytes. We determine that supernatants obtained from monocytes treated with IFN-\(\gamma\) and then exposed to FMLP induce the downregulation of Fc\(\gamma\)RI in naïve monocytes. This effect is abrogated by the protease inhibitors phenylmethylsulphonyl fluoride and phosphoramidon, which inhibit serine and metalloproteases, respectively. Supernatants from FMLP-treated neutrophils also induce the downregulation of Fc\(\gamma\)RI, when added to naïve monocytes. Similar observations were obtained in vivo in a mouse model of chronic inflammation. In vivo, FMLP also downregulates the expression of Fc\(\gamma\)Rs in IFN-\(\gamma\)-activated macrophages. Our results support the existence of a new mechanism through which FMLP could modulate the activity of monocytes/macrophages during bacterial infections.

Introduction

The N-formyl peptides are cleavage products of bacterial and mitochondrial proteins that attract phagocytes to sites of infection, and therefore play an important role in antibacterial host defence in vivo [1, 2]. In fact, it has been demonstrated that the interaction between formyl peptides and their receptors enhances the killing of Leishmania donovani [3], while the lack of these receptors renders mice more susceptible to Listeria monocytogenes infection [4] or impairs the recruitment of monocytes in a mouse model of pneumococcal pneumonia [5].

The receptors for the Fc portion of immunoglobulin G (IgG) (Fc\(\gamma\)Rs) belong to a series of receptors involved in different effector and inflammatory functions, such as phagocytosis, respiratory burst, antibody-dependent cell-mediated cytotoxicity (ADCC) and secretion of inflammatory cytokines [6, 7]. Although formyl peptides are considered potent pro-inflammatory agents, capable of triggering respiratory burst and degranulation of neutrophils and monocytes [8, 9], we have recently demonstrated that the prototypic chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP), identical in sequence to the major chemotactic peptide produced by Escherichia coli [10], exerted, paradoxically, a severe anti-inflammatory effect manifested by the downregulation of receptors for the Fc\(\gamma\)Rs on human leucocytes [11–13]. Thus, in neutrophils, we have observed that the exposure of the cells to FMLP downregulated both Fc\(\gamma\)RII and Fc\(\gamma\)RIIB [11, 12], while the pretreatment of monocytes with FMLP only impaired the upregulation of Fc\(\gamma\)RI induced by interferon-\(\gamma\) (IFN-\(\gamma\)) or interleukin-10 (IL-10) [13].

Inflammation involves several factors, and hence during the development of the inflammatory phenomenon, monocytes could be exposed first to cytokines and then
to formyl peptides. In this context, we evaluated the effect of the chemotactic peptide on the expression of FcγRI in monocytes previously activated with IFN-γ or IL-10. We also studied the effect of FMLP on the expression of FcγRII/III in IFN-γ-activated macrophages, using a mouse model of chronic inflammation.

Materials and methods

Reagents. FMLP, Ficoll 400, tissue culture medium (RPMI-1640), dextran, human recombinant IFN-γ, human recombinant IL-10, mouse recombinant IFN-γ, the protease inhibitors phenyl methyl sulphonlyl fluoride (PMSF), leupeptin, phosphoramidon and pepstatin were obtained from Sigma (St Louis, MO, USA). Percoll was obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Fluorescein isothiocyanate (FITC)-labelled anti-human FcγRI (clone 22), phycoerythrin-labelled anti-human CD14 (clone RM052), mouse IgG1 (clone 679.1Mc7) and mouse IgG2a (clone U7.27) isotype controls were from Immunotech (Marseille, France). FITC-labelled anti-HLA-DR (clone L243) was purchased from Becton Dickinson (San Jose, CA, USA). Rat antimouse FcγRII/III antibody (clone 2.4G2) and rat IgG2b isotype control were kindly provided by Dr M. Vermeulen. FITC-labelled goat antirat IgG (H+L) was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA).

Preparation of human monocytes. Fresh human blood was obtained by venipuncture from healthy adult volunteers and collected on citrate/dextrase/adenine. Blood was diluted 1:2 with saline, layered on a Ficoll–Hypaque cushion and centrifuged at 850 x g for 25 min, as previously described [14]. Peripheral blood mononuclear cells (PBMCs) were collected, centrifuged on a Percoll gradient and resuspended in RPMI 10% heat-inactivated (56°C, 30 min) fetal calf serum (FCS) and 50 μg/ml of gentamicin. Viability of monocytes was always more than 95% as measured by the Trypan blue exclusion test.

Preparation of human neutrophils. Fresh human blood was obtained by venipuncture from healthy adult volunteers and collected on citrate/dextrase/adenine. Polymorphonuclear leukocytes (PMNs) were isolated from the bulk of red cells by sedimentation with 3% dextran and Ficoll-Hypaque cushion, as previously described [11]. The pellet containing PMNs was collected, and the contaminating erythrocytes were removed by hypotonic lysis. The cell suspension contained 95–98% PMNs. Viability of PMN was always more than 95% as measured by the Trypan blue exclusion test.

FMLP treatment. FMLP was dissolved in dimethyl sulphoxide at a concentration of 0.1 M. The subsequent dilutions of FMLP were made in saline. Monocytes were incubated in polypropylene tubes with medium, IFN-γ (240 U/ml) or IL-10 (100 U/ml) at 37°C in a 5% CO₂ atmosphere for 24 h. Then, medium or FMLP 1 μM was added to the cells for two additional hours. After that period, the cells were used as effector cells in ADCC or for flow cytometry studies.

Supernatant collection. Monocytes (0.5 x 10⁶ cells/ml) were treated with medium, IFN-γ (240 U/ml) or IL-10 (100 U/ml) at 37°C in a 5% CO₂ atmosphere for 24 h. After that period, the cells were incubated with medium or FMLP 1 μM for two additional hours. Then, supernatants were collected, spun down to remove cellular debris (10 min at 700 g), stored at −70°C and dialysed exhaustively against RPMI to eliminate residual FMLP before their usage. PMNs (1 x 10⁶ cells/ml) were treated with medium or FMLP 1 μM at 37°C in a 5% CO₂ atmosphere for 3 h, in the presence or absence of different protease inhibitors. Then, supernatants were collected, spun down to remove cellular debris (10 min at 700 g), stored at −70°C and dialysed exhaustively against RPMI to eliminate residual FMLP before their usage.

ADCC assay. When PBMCs at 4 x 10⁶ cells/ml (100 μl) were used as effector cells, ADCC was performed in 96-well polystyrene plates. The adherent PBMC population (monocytes) was obtained from 4 x 10⁶ PBMCs/ml that were left to adhere in 96-well, round-bottom plates for 1 h at 37°C. After that, the nonadherent cells were removed, and ADCC was performed. The cells were incubated with 1 x 10⁵ ⁵¹Cr-labelled chicken red blood cells (⁵¹CrBC) and a suboptimal concentration of rabbit IgG anti-CRBC, as previously described [15]. After 18 h of incubation at 37°C and 5% CO₂, the culture plate was centrifuged, and the percentage of cytotoxicity was calculated as follows: % ADCC = ⁵¹Cr released to the supernatant/total radioactivity. This value was corrected by subtracting the percentage of ⁵¹Cr released in the absence of antibody (spontaneous release). Quadruplicates were set up for each sample.

In vivo studies. Glass cylinders of 2 cm length and 8 mm width were introduced subcutaneously into 8- to 10-week old BALB/c female mice. After 20 days, the cylinders caused a chronic inflammatory process with the open ends of the cylinders ‘closed’ by fibrotic tissue and predominantly infiltrated by macrophages. At that moment, 50 μl of pyrogen-free saline or 50 μl of IFN-γ (3000 U/ml) was injected into the cylinder. After 24 h, 40 μl of pyrogen-free saline or 40 μl of FMLP at a concentration of 50 μM was added for two additional hours. Considering that the volume of each cylinder is approximately 200 μl, the final concentration of FMLP should be approximately 10 μM. Then, the cylinders were extracted from the mice, and the cells that had been recruited were stained with an antibody against murine FcγRII/III (clone 2.4G2) and then with a goat FITC-labelled antirat IgG (H+L). Flow cytometry was performed, and the macrophage population was analysed using macrophage-specific forward light scatter (FSC) and side light scatter (SSC) gates.
Flow cytometry. After different treatments, 0.5 × 10^6 monocytes/ml or 0.35 × 10^6 cells obtained from the glass cylinders introduced into BALB/c mice, were washed and incubated with the monoclonal antibodies indicated above. The cells were washed and resuspended in ISOFLOW, and flow cytometry was performed on FACS Analyser (Becton-Dickinson Immunocytometry System, San Jose, CA, USA). The results corresponding to human monocytes were expressed as the percentage of median of fluorescence intensity (% MFI) of untreated cells (controls). Purity of monocytes, determined as CD14-positive cells, was >90% in all donors. Nonspecific binding was determined using the appropriate mouse IgG isotype-matched control antibody.

Statistical analysis. Statistical significance of the results was calculated using the nonparametric Mann–Whitney U-test or Wilcoxon tests (two-tailed). For the in vivo experiment, the unpaired t-test was used.

Results

Effect of FMLP on the expression of FcγRI in monocytes pretreated with IFN-γ or IL-10

We evaluated the effect of FMLP on the expression of FcγRI in monocytes that had been previously treated with either IFN-γ or IL-10. As shown in Fig. 1A, FMLP 1 μM did not alter the basal level of the expression of FcγRI. However, when monocytes were treated with IFN-γ for 24 h and then exposed to FMLP for two additional hours, a significant downregulation of the expression of FcγRI was observed (Fig.1B, Table 1). Lower concentrations of FMLP ranging from 1 to 100 nM were devoid of effect (data not shown). In contrast to the results obtained with IFN-γ-treated monocytes, FMLP was completely unable to downregulate the expression of FcγRI in monocytes previously exposed to IL-10 (Fig.1C). The levels of expression of other surface molecules, such as HLA-DR, were not altered by FMLP in monocytes previously treated with IFN-γ (% MFI of untreated cells: IFN-γ, 150%; IFN-γ + FMLP, 150%; FMLP, 99%; representative experiment of n = 3).

Effect of FMLP on the enhancement of ADCC by IFN-γ

As the upregulation of FcγRI induced by IFN-γ could correlate with FcγR-dependent functions, such as ADCC [16], this cytotoxic activity was evaluated. As shown in Fig. 2, the enhancement of ADCC induced by IFN-γ in human monocytes was completely inhibited when the cells were treated with FMLP.

Figure 1 Effect of N-formyl-methionyl-leucyl-phenylalanine (FMLP) on the expression of FcγRI in human monocytes treated with interferon-γ (IFN-γ) or interleukin-10 (IL-10). Monocytes (0.5 × 10^6 cells/ml) were incubated for 24 h with: (A) medium (B) IFN-γ (240 U/ml) or (C) IL-10 (100 U/ml). After that period, the cells were incubated with medium or FMLP 1 μM for two additional hours. Then, monocytes were stained with an anti-FcγRI antibody. The histograms correspond to a representative experiment of n = 26. Nonspecific binding (filled peak) was determined using control isotype antibodies. X-axis: fluorescence intensity (arbitrary units); y-axis: cell number.

Effect of supernatants obtained from monocytes treated with IFN-γ plus FMLP on the expression of FcγRI

In order to investigate whether the factors released by monocytes were responsible for the downregulation of FcγRI triggered by FMLP, experiments were carried out in which supernatants from IFN-γ-treated monocytes cultured with FMLP were collected, and their ability to modulate the expression of FcγRI in naïve monocytes was evaluated. Supernatants were dialysed exhaustively to eliminate FMLP before their usage. The results summarized in Table 2 indicate that these supernatants significantly reduce the expression of FcγRI in naïve
monocytes, confirming the role of released product(s) in this process. In contrast, supernatants obtained from FMLP- or IL-10 plus FMLP-treated monocytes were not capable of inducing the downregulation of FcRRI (Table 2).

**Effect of protease inhibitors on the downregulation of FcRRI**

With the purpose of investigating whether proteases released by monocytes were responsible for the downregulation of FcRRI, monocytes were incubated with IFN-γ for 24 h and then treated with FMLP for 2 h in the presence of different protease inhibitors. These inhibitors included PMSF 2 mM, which inhibits all serine proteases with a trypsin-like specificity [17]; pepstatin 1 mM, an inhibitor of aspartic protease [18]; phosphoramidon 10 μM, a metalloprotease inhibitor [19] and the cysteine protease inhibitor, leupeptin 50 μM [20]. The results shown in Fig. 3 indicate that the downregulation of FcRRI is completely abrogated by PMSF and phosphoramidon, supporting the role of a serine protease(s) and a metalloprotease(s) in this process. The lack of effect of the other inhibitors led us to discard the participation of a wide range of proteases. Similar results were obtained when supernatants from monocytes treated with IFN-γ plus FMLP in the presence of the protease inhibitors were added to naïve monocytes (data not shown).

**Effect of supernatants obtained from neutrophils stimulated with FMLP on the expression of FcRRI in human monocytes**

In previous reports [11, 12], we had demonstrated that supernatants obtained from FMLP 1 μM-stimulated human neutrophils (PMNs) induced the downregulation of FcRRII and FcRRIIIB present on the surface of naïve PMNs. As neutrophils are one of the most important cell populations in infectious/inflammatory processes, we studied whether supernatants from FMLP-treated PMNs could modify the expression of FcRRI in human monocytes. As shown in Fig. 4, these supernatants also induced the downregulation of FcRRI. Moreover, only the serine protease inhibitor PMSF completely inhibited this effect (Table 3). Supernatants obtained from PMNs treated with lower concentrations of FMLP (from 10 to 100 nM) did not show any downregulatory effect on FcRRI (data not shown). Other molecules, such as HLA-DR, were not affected by supernatants obtained from FMLP 1 μM-treated PMNs (% MFI of untreated cells: untreated, 100%; FMLP, 98%; representative experiment of n = 4). In all the experiments, supernatants were exhaustively dialysed to eliminate FMLP before their usage.

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**Table 1.** Effect of N-formyl-methionyl-leucyl-phenylalanine (FMLP) on the expression of FcRRI in interferon-γ (IFN-γ)-treated monocytes

| Treatments          | % MFI of untreated cells ± SEM |
|---------------------|--------------------------------|
| FMLP                | 100 ± 3                        |
| IFN-γ               | 232 ± 23*                      |
| IFN-γ + FMLP        | 185 ± 20†                      |

Monocytes (0.5 × 10⁶ cells/ml) 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 two additional hours. Then, monocytes were stained with an anti-FcRRI antibody. Data are expressed as percentage of median of fluorescence intensity (% MFI) of untreated cells ± standard error of the mean (SEM), n = 26. *P < 0.0006 significantly different from untreated cells. †P < 0.003 significantly different from untreated cells and P < 0.003 significantly different from IFN-γ-treated cells. Statistical significance was calculated using the Mann–Whitney U-test, two-tailed.

**Figure 2.** Effect of N-formyl-methionyl-leucyl-phenylalanine (FMLP) on the enhancement of antibody-dependent cell-mediated cytotoxicity (ADCC), induced by interferon-γ (IFN-γ) in human monocytes. Monocytes obtained from peripheral blood mononuclear cells (PBMCs) (4 × 10⁶ cells/ml) were incubated with medium or IFN-γ (240 U/ml) for 24 h and then incubated with medium or FMLP 1 μM for two additional hours. After that period, ADCC was performed. Data are expressed as percentage ADCC of untreated cells ± standard error of the mean (SEM), n = 9. *P < 0.008 significantly different from untreated cells. Statistical significance was calculated using the Mann–Whitney U-test, two-tailed.

**Table 2.** Effect of supernatants from interferon-γ (IFN-γ) plus N-formyl-methionyl-leucyl-phenylalanine (FMLP)-treated monocytes on the expression of FcRRI

| Supernatants from monocytes treated with          | % MFI of untreated cells ± SEM |
|-------------------------------------------------|--------------------------------|
| FMLP                                            | 99 ± 3                        |
| IFN-γ                                           | 108 ± 3                       |
| IFN-γ + FMLP                                     | 75 ± 2*                       |
| IL-10                                           | 105 ± 3                       |
| IL-10 + FMLP                                     | 105 ± 8                       |

Monocytes (0.5 × 10⁶ cells/ml) were incubated with medium, IFN-γ (240 U/ml) or interleukin-10 (IL-10) for 24 h. After that period, the cells were incubated with medium or FMLP 1 μM for two additional hours. Supernatants were collected, dialyzed to eliminate residual FMLP and added to naïve monocytes (0.5 × 10⁶ cells/ml). These cells were incubated with the supernatants for 3 h, and then were stained with an anti-FcRRI antibody. Data are expressed as percentage of median of fluorescence intensity (% MFI) of untreated cells ± standard error of the mean (SEM), n = 8. *P < 0.05 significantly different from untreated cells. Statistical significance was calculated using the Mann–Whitney U-test, two-tailed.
Effect of FMLP on the expression of FcγRs in a mouse model of chronic inflammation

In order to investigate the *in vivo* effect of FMLP on the expression of FcγRs, a glass cylinder was introduced subcutaneously into female BALB/c mice [21]. After 20 days, the cylinders caused a chronic inflammatory process. At that moment, the open ends of the cylinders had been ‘closed’ by fibrotic tissue, and were predominantly infiltrated by macrophages. Then, murine IFN-γ was injected into the cylinder, and 24 h later FMLP was added. Two hours after the injection of FMLP, the cylinders were extracted from mice, and the cells that had been recruited into the cylinder were stained with an antibody against murine FcγRII/III. Flow cytometry was performed, and

### Figure 3

Effect of different protease inhibitors on the downregulation of FcγRI exerted by N-formyl-methionyl-leucyl-phenylalanine (FMLP) on interferon-γ (IFN-γ)-treated human monocytes. Monocytes (0.5 × 10^6 cells/ml) 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 two additional hours in the presence or absence of different protease inhibitors. Then, monocytes were stained with an anti-FcγRI antibody. The figure shows a representative experiment of *n* = 8. Data are expressed as percentage of median of fluorescence intensity (% MFI) of untreated cells. The symbol (*) represents the degree of significance (*P* < 0.05) of eight experiments compared with IFN-γ-treated cells. Statistical significance was calculated using the Wilcoxon test, two-tailed.

### Table 3

Effect of different protease inhibitors on the downregulation of FcγRI induced by supernatants from *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)-treated polymorphonuclear leucocytes (PMNs)

| Inhibitor        | Supernatants from PMNs treated with | % MFI of untreated cells |
|------------------|------------------------------------|--------------------------|
| PMSF             | —                                  | 100                      |
| FMLP             | —                                  | 99                       |
| Phosphoramidon   | —                                  | 100                      |
| FMLP             | —                                  | 77                       |
| Leupeptin        | —                                  | 100                      |
| FMLP             | —                                  | 78                       |
| Pepstatin        | —                                  | 100                      |
| FMLP             | —                                  | 75                       |

PMNs (1 × 10^6 cells/ml) were incubated with medium or FMLP 1 μM for 3 h in the presence or absence of different protease inhibitors. After that period, supernatants were collected, dialysed to eliminate residual FMLP and added to naive monocytes (0.5 × 10^6 cells/ml). These cells were incubated with the supernatants for 3 h, and then were stained with an anti-FcγRI antibody. The table summarizes a representative experiment of *n* = 4. Data are expressed as percentage of median of fluorescence intensity (% MFI) of untreated cells. PMSF, phenyl methyl sulphonyl fluoride.
the macrophage population was studied. Figure 5 shows that FMLP did not modify the basal level of expression of FcγRII/III, but induced a drastic downregulation of these receptors in macrophages that had been previously treated with IFN-γ. This result is in accordance with our \textit{in vitro} experiments with human monocytes although, in this case, we have not observed any upregulatory effect of IFN-γ in the murine FcγRs studied.

**Discussion**

The lysis of bacteria at the site of infection provokes the release of formyl peptides [8, 22] and generates a concentration gradient that induces the unidirectional migration of neutrophils and monocytes to the inflammatory focus [23]. The aim of this report was to study the effect of FMLP, a prototype of N-formyl peptides, on the expression of FcγRs in human monocytes as well as in murine macrophages.

In this report, we demonstrate that when monocytes are previously activated by IFN-γ, FMLP 1 μM induces a significant downregulation of FcγRI that have been already expressed. However, this effect of FMLP is not observed in naive monocytes, indicating that the activity of FMLP depends on the pretreatment of the cells with IFN-γ. This result could be owing to the well-known effect of priming induced by IFN-γ, which enhances the activity of different agonist on monocytes [24]. This is different from our previous observations, showing that preincubation of monocytes with lower concentrations of FMLP, ranging from 10 to 100 nM, inhibited the ability of IFN-γ to upregulate FcγRI, without affecting the already-expressed FcγRI. Taken together, these results suggest that FMLP could downregulate FcγRI in monocytes through their way to the inflammatory site in two different ways: at a relatively low concentration, it could inhibit the overexpression of FcγRI induced by IFN-γ or IL-10, while at higher concentrations, it could induce the secretion of enzymes which downregulate the FcγRI induced only by IFN-γ. The relevance of these mechanisms in the context of an ongoing bacterial induced inflammation is that, independently of the sequence of events, the expression of FcγRI will be downregulated, either before or after its expression has been induced by cytokines.

The downregulation of FcγRII correlates with the complete inhibition of ADCC (Fig. 2), a well-known FcγR-dependent cytotoxic mechanism. The mechanism of downregulation of FcγRI by FMLP has not been completely elucidated. However, it seems to be mediated by the action of serine and metalloproteases released by monocytes (Fig. 3). The role of secretory products is supported by the fact that the downregulation of FcγRI is also observed when using supernatants from IFN-γ plus FMLP-treated monocytes (Table 2).

In contrast to these observations, FMLP does not induce any effect on the expression of FcγRI in IL-10-treated monocytes, despite the fact that this cytokine also induces the overexpression of FcγRI (Fig. 1C). In addition, supernatants from monocytes that have been stimulated with IL-10 are devoid of effects (Table 2). These results could be explained by the fact that IL-10 is a powerful inhibitor of monocyte secretion of pro-inflammatory products, including proteases [25], and suggests that the state of activation of the cells is a crucial event to allow FMLP to mediate the downregulation of FcγRI.

In previous reports, we had demonstrated that FMLP was capable of inducing the downregulation of both FcγRII and FcγRIIIB in human neutrophils [11, 12], and that this effect was owing to the secretion of serine protease(s) by these cells. In this report, we observe that serine proteases secreted not only by PMNs but also by monocytes are involved in the downregulation of FcγRI in human monocytes, showing the existence of autocrine and paracrine mechanisms, leading to the downregulation of FcγRs. This mechanism is not unique to FcγRs, as it has been demonstrated that CD14 expressed in human monocytes is downregulated by serine proteases released by FMLP-stimulated PMNs [26]. Moreover, proteases released by activated human monocytes downregulate the expression of major histocompatibility complex class I (MHC-I) [27] as well as that of MHC-II molecules [28].

When we assayed FMLP in a mouse model of chronic inflammation [21], the formyl peptide was capable of inducing the downregulation of FcγRII/III in IFN-γ-activated macrophages present at the inflammatory focus,
suggesting that our observations performed in vitro could be relevant to the development of the inflammatory phenomenon in vivo. This is supported by the fact that previous reports have shown that formyl peptides, through their receptors, are important in antibacterial host defence in vivo, because mice lacking these receptors are more susceptible to challenge with *L. monocytogenes* [4] or *L. donovani* [3]. Also, it has been demonstrated that FMLP mediates the recruitment of monocytes and macrophages in a mouse model of pneumococcal pneumonia [5].

We can speculate that when cells are gradually entering into the infectious focus, they come in contact with different products such as IFN-γ, which is produced early by activated natural killer and T cells in response to pathogens like *L. monocytogenes* and *Leishmania* sp. [29, 30]. At the site of infection, the cells are exposed to the highest concentration of formyl peptides (i.e. FMLP 1 μM), which induces both the release of products such as proteases and oxygen species, enabling inflammatory cells to kill micro-organisms, and the downregulation of receptors (FcγRs). The regulation of the expression of FcγRI is a key event in the control of bacterial infections, as it has been demonstrated that FcγRI knockout mice show impaired antibacterial host defence [31]. According to our results, formyl peptides are able to downregulate the expression of FcγRI even in IFN-γ-activated monocytes, and this event could help control an exacerbated host response to infection as well as to operate as a bacterial mechanism of evasion of the immune response.

Further studies about the nonconventional (anti-inflammatory) effects of formyl peptides will be necessary in order to have a comprehensive view of inflammation.

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