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# Interferon-gamma (IFN-γ) and prostaglandin $E_2$ ($PGE_2$) regulate differently IL-12 production in human intestinal lamina propria mononuclear cells (LPMC)

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## SUMMARY

IL-12 modulates Th1 immune response during chronic colitis. Mechanisms regulating IL-12 synthesis in human intestine are poorly understood. The aim of this study was to investigate the effect of IFN-γ and $PGE_2$ on lipopolysaccharide (LPS)-stimulated LPMC IL-12 production. Normal LPMC cultures were run in the presence or absence of IFN-γ and/or $PGE_2$ before LPS stimulation. To examine the role of endogenous $PGE_2$ on LPS-stimulated IL-12 release, LPMC cultures were added of indomethacin before LPS stimulation. IL-12, IL-10 and IL-8 were measured by ELISA. No IL-12 was detected in either unstimulated or LPS-stimulated LPMC cultures. In contrast, LPMC released IL-8 ($650 \pm 125$ pg/ml) and IL-10 ($75 \pm 25$ pg/ml) in response to LPS. Treatment of LPMC with IFN-γ facilitated LPS-stimulated IL-12, whereas it completely abrogated IL-10 production. IL-12 release by LPMC stimulated with IFN-γ and LPS was significantly inhibited by exogenous IL-10. The addition of $PGE_2$ to IFN-γ-treated LPMC cultures inhibited in a dose-dependent manner LPS-induced IL-12 secretion. Furthermore, IL-12 was detectable ($85 \pm 25$ pg/ml) in the supernatants of LPMC cultures treated with indomethacin and LPS. In contrast to the effect on IL-12, $PGE_2$ significantly augmented LPS-stimulated LPMC IL-10 production. However, the inhibition of IL-12 by $PGE_2$ was only partially reversed by anti-IL-10. In a simplified model of LPS tolerance, we finally showed that monocyte-derived macrophages exhibited reduced IL-12 production after repeat LPS stimulation. In these cell cultures, indomethacin abrogated the induction of LPS desensitization. IFN-γ and $PGE_2$ modulate differently the LPMC responsiveness to LPS in terms of IL-12 synthesis.

**Keywords** IL-12 interferon-gamma lipopolysaccharide prostaglandin-$E_2$ intestinal inflammation

## INTRODUCTION

IL-12, a heterodimeric cytokine composed of two subunits (p35 and p40), is produced by activated antigen-presenting cells (APC) mainly in response to bacteria or bacterial products [1–3]. IL-12 plays a pivotal role in generating Th1-mediated inflammatory responses by priming T and natural killer (NK) cells for high IFN-γ production [1–3]. IFN-γ enhances in turn the ability of APC to produce IL-12, triggering a positive feedback mechanism capable of amplifying APC activation and maintaining a Th1-type immune response [1,2,4]. In murine models, IFN-γ proved to be necessary for inducing macrophage IL-12 production, supporting the hypothesis that during some Th1-mediated diseases IL-12 may not be the first cytokine generated [5]. It is, however, widely shown that the synthesis of IL-12 by APC in response to bacterial stimulation is also regulated by cytokines/factors with deactivating effects on the producer cells [1–3].

IL-12 is expressed and actively released by intestinal LPMC in Crohn's disease (CD) [6,7], a chronic inflammatory process involving the alimentary tract and characterized by Th1-mediated phenomena [8–11]. Evidence has also accumulated to indicate that IL-12 modulates the differentiation of Th1-type lymphocytes during intestinal inflammation [7,12,13].

Mechanisms regulating IL-12 production in the human intestinal mucosa are not understood. Studies from experimental models suggest that the development of Th1-type cytokine-mediated chronic colitis requires the presence of non-pathogenic

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bacterial flora and that the inflammation may be driven by IL-12 [14,15]. Lipopolysaccharide (LPS), a bacterial product largely present in the gut lumen, is a powerful inducer of IL-12 *in vitro* [1–4]. It has been shown, however, that the capability of releasing IL-12 in response to LPS is impaired in macrophages isolated from the normal gut mucosa [6]. In addition, IL-12 has been hardly detected in normal intestinal mucosa [6,7]. Taken together, these observations suggest that in normal intestine IL-12 production is a tightly regulated function and that local factors may operate in down-regulating IL-12.

$PGE_2$ is an important mediator of the acute inflammatory response. At the site of tissue injury $PGE_2$ induces erythema, oedema, and hyperalgesia [16,17]. $PGE_2$ is also known to inhibit B and T lymphocyte proliferation, T lymphocyte IL-2 and IFN-$\gamma$ synthesis, and IL-2 receptor expression [18]. Furthermore, in the presence of bacterial products, such as LPS, $PGE_2$ acts as an inhibitor of APC functions [18–21]. Consistent with these observations, $PGE_2$ is normally synthesized in human intestine where it seems to be determinant in preventing APC-derived cytokine secretion [22,23]. We therefore, hypothesized that in the human intestinal mucosa $PGE_2$ contributes to down-regulate IL-12 production, counterbalancing the effects of IFN-$\gamma$.

In the present study, we report data indicating that IFN-$\gamma$ and $PGE_2$ modulate differently the responsiveness of normal LPMC to LPS in terms of IL-12 production.

## MATERIALS AND METHODS

*Mucosal samples and mononuclear cell isolation*

Mucosal samples were obtained from the macroscopically and microscopically unaffected areas of 16 surgical colon cancer specimens. Autologous peripheral blood mononuclear cells (PBMC) were obtained from nine patients. PBMC from six healthy subjects were also available. The study was approved by the local Department Ethical Committee. LPMC were isolated by the DTT–EDTA–collagenase sequence as previously described [6,9]. The isolated cells were counted and checked for viability using 0·1% trypan blue (viability ranged from 91% to 95%). PBMC were isolated by density gradient centrifugation (Lymphoprep; Nycomed Pharma, Oslo, Norway). When used, purified macrophages and monocytes were obtained by seeding LPMC or PBMC, respectively ($3\times10^6$/ml), in microtitre plates and on chamber slides (Lab-Tek; Nunc, Roskilde, Denmark) coated with 0·1% (w/v) gelatin (Sigma Chemical Co., St Louis, MO). After 3 h at 37°C, non-adherent cells were removed by washings with pre-warmed PBS 1× (Sigma), while adherent cells were collected by gentle washings with cold PBS 1×. Adherent cell preparations contained consistently $<5\%$ contaminating $CD3^+$ cells, as assessed by FACS analysis (data not shown). In order to control monocyte/macrophage morphology in adherence-separated LPMC or PBMC cultures, adherent cells on chamber slides were fixed in ethanol and stained by haematoxylin.

*Cell cultures*

Both total and adherence-separated cells were cultured in complete medium consisting of RPMI 1640 supplemented with 10% fetal calf serum (FCS), 1% L-glutamine, 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin (all Sigma). Both LPMC and PBMC (concentration $3\times10^6$ cells/ml) cultures were run with or without the initial addition of LPS (*Escherichia coli*) (1 $\mu$g/ml) or Staphylococcal enterotoxin B (SEB; 1 $\mu$g/ml) (both Sigma) for 24 h. Parallel experiments were performed using adherent cells (at a concentration of $5\times10^5$ cells/ml). After 24 h of culture, cell-free supernatants were collected and stored at −80°C until tested. LPMC and PBMC were also treated with graded doses of recombinant human IFN-$\gamma$ (Becton Dickinson Labware, Bedford, MA) (final concentration ranging from 1 ng/ml to 250 ng/ml) or recombinant human IL-4 (Sigma) (final concentration ranging from 1 ng/ml to 250 ng/ml) for 32 h before LPS stimulation (1 $\mu$g/ml) for a further 24 h. Parallel cultures were added of an IFN-$\gamma$ antiserum (1:100 final dilution; kindly supplied by Dr M. R. Capobianchi, Istituto di Virologia, Università La Sapienza, Roma, Italy) or a neutralizing IL-4 antibody (1:100 final dilution; R&D Systems, Minneapolis, MN). Experiments were also performed by stimulating LPMC with IFN-$\gamma$ (100 ng/ml final concentration) and recombinant human IL-10 (10 ng/ml final concentration; Sigma). At the end of the culture period, cell-free supernatants were collected and stored at −80°C until tested.

To examine the effect of $PGE_2$ on IL-12 production, LPMC were treated with IFN-$\gamma$ (100 ng/ml) in the presence or absence of $PGE_2$ (final concentration ranging from $10^{-5}$ M to $10^{-8}$ M; provided by Dr G. De Sarro, University of Catanzaro) for 32 h before LPS (1 $\mu$g/ml) stimulation for a further 24 h. Cultures were also treated with $PGE_2$ alone before LPS stimulation. To prove further that endogenous $PGE_2$ modulates LPS-stimulated IL-12 production, LPMC cultures were provided with 100 $\mu$g/ml indomethacin for 32 h before LPS (1 $\mu$g/ml) stimulation. In order to explore the possibility that $PGE_2$ modulates IL-12 release through the induction of IL-10, LPMC cultures treated with IFN-$\gamma$ (100 ng/ml) and $PGE_2$ ($10^{-5}$ M) were added of a neutralizing human IL-10 MoAb (100 ng/ml final concentration) (clone 23738.11; Sigma). At the end of the culture period, cell-free supernatants were collected and stored at −80°C until tested.

*IL-12 production by monocyte-derived macrophages pretreated with low doses of LPS*

To investigate whether monocyte-derived macrophages pretreated with low concentrations of LPS produce IL-12 upon restimulation with LPS, adherent mononuclear cells from peripheral blood were resuspended in complete medium at a concentration of $1\times10^6$ cells/ml and cultured for 7 days. Thereafter, cells were washed and cultured again in complete medium in the presence or absence of LPS (200 pg/ml final concentration) or LPS plus indomethacin (100 $\mu$g/ml final concentration) for 2 days (primary culture). At the end of the culture period, cells were extensively washed and stimulated again with medium alone or 100 ng/ml LPS for a further 24 h (secondary culture). Thereafter, cell-free supernatants were collected and stored at −80°C until tested.

*Cytokine assay*

IL-12, IL-10 and IL-8 were measured in the cell culture supernatants using sensitive ELISA kits (Amersham Int. plc, Slough, UK). The minimum detectable concentration was 5 pg/ml for both IL-12 and IL-10, and 10 pg/ml for IL-8. IL-12 was measured using a commercially available kit that detects both IL-12 p40 and the bioactive IL-12 p70 heterodimer.

*Statistical analysis*

Student's *t*-test was used for statistical analysis of the data.

**Table 1.** IL-12 release by LPMC or peripheral blood mononuclear cells (PBMC)

| | Preincubation | | | | | |
|---|---|---|---|---|---|---|
| | Medium alone | | IL-4 | | IFN-γ | |
| Stimulus | LPMC | PBMC | LPMC | PBMC | LPMC | PBMC |
| Medium alone | Und. | Und. | Und. | Und. | Und. | Und. |
| SEB | 60 ± 20 | 1400 ± 180 | 210 ± 35 | 2000 ± 140 | 1050 ± 160 | 3500 ± 125 |
| LPS | Und. | 180 ± 65 | Und. | 1700 ± 120 | 320 ± 70 | 2800 ± 100 |

Cells were cultured and preincubated in the presence of medium or IL-4 (100 ng/ml final concentration) or IFN-γ (100 ng/ml final concentration) for 32 h before stimulating with complete medium or Staphylococcal enterotoxin B (SEB; 1 μg/ml final concentration) or lipopolysaccharide (LPS; 1 μg/ml final concentration) for a further 24 h. Results are mean ± s.d. of six experiments. IL-12 values are expressed as pg/ml. Und., Undetectable.

## RESULTS

*Normal LPMC fail to release IL-12 after LPS stimulation*

No IL-12 was detected in the supernatants of total or adherence-separated LPMC cultures incubated with medium alone. Similarly, LPMC failed to release IL-12 after LPS stimulation. In contrast, IL-12 was measured in the supernatants of both PBMC (160 ± 10 pg/ml) and monocyte-derived macrophage (290 ± 40 pg/ml) cultures provided with LPS. Both total and adherence-separated LPMC were, however, able to release IL-12 in response to SEB (38 ± 8·5 pg/ml and 25 ± 4·0 pg/ml, respectively). Importantly, the inability of LPS-stimulated LPMC to produce IL-12 was not the result of a global reduction in protein synthesis, because these cells released IL-8 (650 ± 125 pg/ml *versus* 120 ± 30 pg/ml in unstimulated LPMC; $P < 0{\cdot}001$). Furthermore, LPMC viability in the presence of LPS was >90% as assessed by trypan blue exclusion.

*IFN-γ facilitates IL-12 release in LPS-stimulated LPMC cultures*

IFN-γ is a major activator of monocytes/macrophages [24]. There is now evidence indicating that IFN-γ specifically influences monocyte IL-12 production [4,25]. In addition, studies from human and animal models indicate that IFN-γ promotes cytokine secretion by endotoxin-resistant cells [26,27]. To examine whether IFN-γ could restore IL-12 production by normal intestinal mononuclear cells, LPMC were preincubated with graded doses of IFN-γ before LPS stimulation. IFN-γ alone did not induce IL-12 production. IFN-γ, however, promoted, in a dose-dependent fashion, IL-12 release by LPS-stimulated LPMC (Table 1 and Fig. 1). In both total and adherence-separated LPMC cultures, the amount of IL-12 produced in response to LPS after treatment with 100 ng/ml IFN-γ (320 ± 70 pg/ml and 250 ± 20 pg/ml, respectively) was two and four times higher than that measured in cell cultures provided with 25 ng/ml or 10 ng/ml IFN-γ, respectively (180 ± 35 pg/ml and 140 ± 30 pg/ml in 25 ng/ml IFN-γ-stimulated cells; 80 ± 15 pg/ml and 60 ± 10 pg/ml in 10 ng/ml IFN-γ-stimulated cells) (Fig. 1). The addition of an IFN-γ antiserum decreased by nearly 10 times the amount of IL-12 released by total LPMC treated with 100 ng/ml IFN-γ (320 ± 70 pg/ml *versus* 45 ± 18 pg/ml; $P < 0{\cdot}0001$), and completely abolished that measured in cell cultures treated with 25 ng/ml or 10 ng/ml IFN-γ. No inhibitory effect was seen with a non-relevant control antibody (anti-IL-4).

The ability of phagocytes to produce IL-12 is regulated by several cytokines with activating or deactivating effects on the producer cells. IL-10 has been described as a strong inhibitor of monocyte IL-12 synthesis [28]. Since IFN-γ is capable of affecting IL-10 production in monocytic cell lines [29], we hypothesized that IFN-γ could modulate LPS-stimulated LPMC IL-12 synthesis through the inhibition of endogenous IL-10. To address this issue, we first measured IL-10 in the culture supernatants of LPMC treated with 100 ng/ml IFN-γ before LPS stimulation. IFN-γ completely inhibited LPS-stimulated LPMC IL-10 secretion. To prove further that the inhibition of endogenous IL-10 may be a mechanism by which IFN-γ promotes IL-12 synthesis, we treated LPMC with IFN-γ (100 ng/ml) in the presence or absence of recombinant human IL-10 (10 ng/ml final concentration) for 32 h before LPS stimulation. Exogenous IL-10 significantly decreased the release of IL-12 by IFN-γ-treated LPMC after LPS stimulation. The inhibitory effect of IL-10 was completely abolished by the addition of a neutralizing IL-10 antibody (100 ng/ml final concentration) (Table 2).

IL-4, originally described as a factor secreted by type 2 cells, can prime peripheral monocytes for optimal IL-12 production in

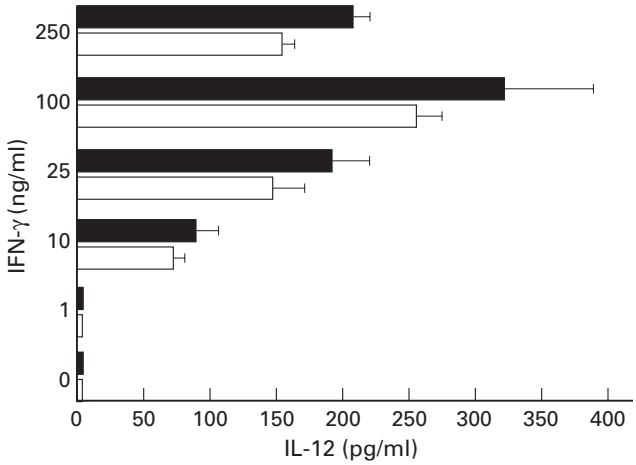


**Fig. 1.** IFN-γ promotes IL-12 release by both total (■) and adherence-separated LPMC (□) after exposure to lipopolysaccharide (LPS). Cells were incubated with graded doses of IFN-γ or medium for 32 h before stimulation with 1 μg/ml LPS for a further 24 h. IL-12 was measured by ELISA. Data are the mean of all experiments; bars indicate 1 s.d.

**Table 2.** Effect of recombinant human IL-10 on IL-12 release by LPMC

| Preincubation | Medium | IL-10 | IL-10+ anti-IL-10 |
|---|---|---|---|
| Medium | Und. | Und. | Und. |
| IFN-γ | 320 ± 70 | 125 ± 30 | 352 ± 90 |

LPMC were cultured and preincubated with medium alone or IFN-γ (100 ng/ml final concentration) in the presence or absence of rhIL-10 (10 ng/ml final concentration) or rhIL-10 and anti-IL-10 (100 ng/ml) for 32 h before lipopolysaccharide (LPS) stimulation. IL-12 was measured by ELISA. Results are indicated as mean ± s.d. IL-12 values are expressed as pg/ml. Und., Undetectable. Pre-incubation with IL-10 significantly decreased IL-12 secretion by IFN-γ-treated LPMC after LPS exposure ($P < 0{\cdot}01$). The effect of IL-10 was completely inhibited by anti-IL-10.

response to LPS stimulation [28]. To investigate whether IL-4 facilitated IL-12 synthesis by LPS-stimulated LPMC, cell cultures were added of IL-4 for 32 h before LPS stimulation. No IL-12 was measured in the culture supernatants of either total or adherence-separated LPMC preincubated with IL-4 and then exposed to LPS. In contrast, IL-4 significantly enhanced SEB-stimulated LPMC IL-12 production (Table 1) ($P < 0{\cdot}001$). In addition, IL-4 induced a significant increase in the amount of IL-12 released by LPS-stimulated monocytes (Table 1) ($P < 0{\cdot}0001$).

*$PGE_2$ mediates LPMC hyporesponsiveness to LPS*

$PGE_2$, in the presence of bacterial products, acts as a powerful inhibitor of APC cytokine production [18–23]. In order to investigate whether $PGE_2$ modulates IL-12 production in human intestine, LPMC were preincubated with graded doses of $PGE_2$ and 100 ng/ml IFN-γ for 32 h before LPS stimulation.

As shown in Fig. 2, $PGE_2$ inhibited, in a dose-dependent manner, the production of IL-12, yielding complete inhibition at $10^{-5}$ M $PGE_2$. As LPS stimulates the induction of $PGE_2$ [18], in selected experiments indomethacin was added to LPMC cultures to examine LPS-stimulated IL-12 production in the absence of *de novo* $PGE_2$ synthesis. No IL-12 was detected in the supernatants of LPMC cultures provided with indomethacin alone. In contrast, pre-exposure of LPMC to indomethacin facilitated IL-12 release in response to LPS (85 ± 25 pg/ml). The addition of $PGE_2$ ($10^{-5}$ M final concentration) to the LPMC cultures provided with indomethacin resulted in complete inhibition of LPS-stimulated LPMC IL-12 release. Taken together, these data indicate that $PGE_2$ is a powerful inhibitor of IL-12 production in human LPMC. Our next goal was to analyse potential mechanisms by which $PGE_2$ down-regulates LPS-stimulated LPMC IL-12 production. Since it is known that $PGE_2$ enhances IL-10 production in human PBMC [19], we investigated the possibility that the inhibitory effect of $PGE_2$ was mediated through the induction of IL-10. Treatment of LPMC with $PGE_2$ significantly augmented LPS-stimulated IL-10 release (265 ± 15, 180 ± 20 and 135 ± 22 pg/ml after $10^{-5}$ M, $10^{-6}$ M, and $10^{-7}$ M $PGE_2$, respectively, *versus* 74 ± 25 pg/ml) ($P < 0{\cdot}03$) (Fig. 3). In agreement with these results, treatment of LPMC with indomethacin resulted in a reduced secretion of IL-10 after LPS stimulation (18 ± 5 pg/ml) ($P = 0{\cdot}03$). Further experiments were therefore performed to establish the role of endogenous IL-10 in $PGE_2$-induced inhibition of IL-12 production. LPMC treated with 100 ng/ml IFN-γ were cultured in the presence or absence of $PGE_2$ ($10^{-5}$ M) and anti-IL-10 (100 ng/ml) before LPS stimulation. As shown in Fig. 4, $PGE_2$-induced inhibition of IL-12 release in IFN-γ-treated LPMC cultures was only partially reversed by anti-IL-10. This result was not dependent on possible efficacy problems of the anti-IL-10, because this antibody was capable of completely blocking the effect of rhIL-10 on IL-12 secretion in LPMC cultures preincubated with IFN-γ and stimulated with LPS (Table 2).

*Human monocyte-derived macrophages exhibit reduced IL-12 production after repeat LPS stimulation*

Although monocytes/macrophages respond to primary encounter with LPS by releasing cytokines, repeat LPS exposure renders these cells refractory to a further challenge with LPS [26,30]. This

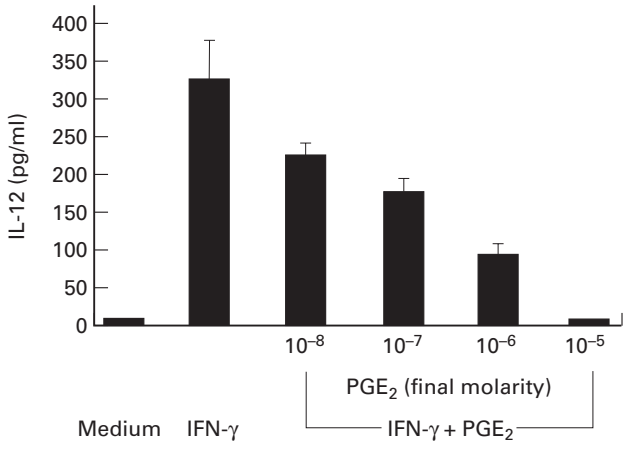


**Fig. 2.** Effect of $PGE_2$ on IL-12 release by IFN-γ-stimulated LPMC exposed to lipopolysaccharide (LPS). Cells were stimulated with medium or 100 ng/ml IFN-γ in the presence or absence of graded doses of $PGE_2$ for 32 h before treating 1 μg/ml LPS for a further 24 h. IL-12 was measured by ELISA. Data are the mean of all experiments; vertical bars indicate 1 s.d.

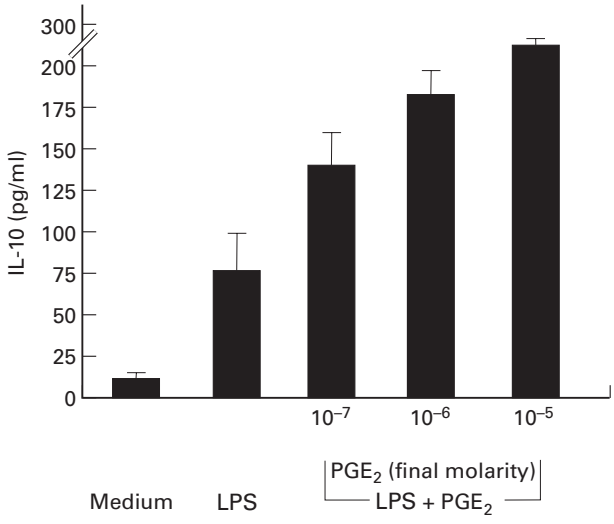


**Fig. 3.** Effect of $PGE_2$ on IL-10 release by LPMC exposed to lipopolysaccharide (LPS). Cells were stimulated with nothing or LPS (1 μg/ml) for 24 h. In parallel experiments LPMC were treated with LPS and graded doses of $PGE_2$ for 24 h. IL-10 was measured by ELISA. Data are the mean of all experiments; vertical bars indicate 1 s.d.

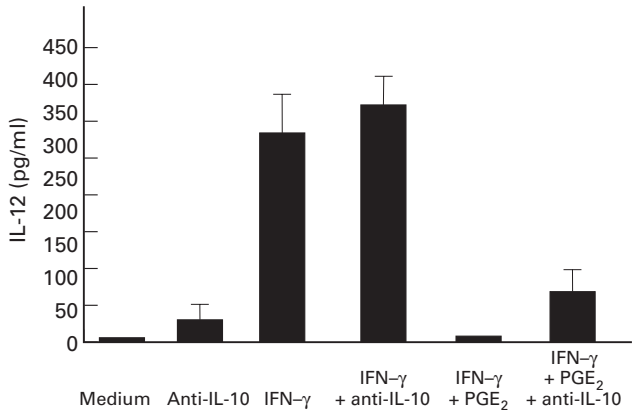


**Fig. 4.** Effect of anti-IL-10 on IL-12 release by LPMC stimulated with lipopolysaccharide (LPS). Cells were treated with medium or 100 ng/ml IFN-$\gamma$ in the presence or absence of $PGE_2$ ($10^{-5}$ M) for 32 h (first stimulation) before treating with 1 $\mu$g/ml LPS for a further 24 h (second stimulation). Parallel cultures were added of an anti-IL-10 (100 ng/ml final concentration) at the time of the first stimulation. IL-12 was measured by ELISA. Data are the mean of all experiments; vertical bars indicate 1 s.d.

phenomenon, termed LPS tolerance or desensitization, may be reproduced *in vitro* by using different types of APC [26,30]. During the isolation procedure LPMC may be exposed to substantial concentrations of LPS. LPMC might therefore be desensitized to further LPS stimulation. To examine whether IL-12 production by LPS-stimulated APC can be affected by pre-exposure to LPS, monocyte-derived macrophages were treated with low concentrations of LPS for 2 days, washed and stimulated again with 100 ng/ml LPS for a further 24 h. No IL-12 was measured in the supernatants of unstimulated monocyte-derived macrophages. Macrophages did respond to the primary LPS exposure by releasing IL-12 ($100 \pm 15$ pg/ml and $270 \pm 42$ pg/ml after stimulation with 200 pg/ml and 100 ng/ml LPS, respectively). However, macrophages cultured in the presence of low-dose LPS exhibited a decreased IL-12 release after a secondary challenge with LPS (Table 3). No inhibition of IL-8 release was observed in LPS-desensitized macrophage cultures (Table 3).

$PGE_2$ is a major product of LPS stimulation [18]. We therefore explored the possibility that $PGE_2$ could inhibit IL-12 production in LPS-tolerant macrophage cultures. To examine this issue, macrophages were treated with low-dose LPS in the presence or absence of indomethacin for 32 h, then washed and stimulated again with 100 ng/ml LPS and/or medium for 24 h. Macrophages cultured in the presence of indomethacin maintained the capability of releasing IL-12 in response to a secondary stimulation with LPS ($180 \pm 25$ pg/ml) (Table 3).

## DISCUSSION

Our study confirms and expands the concept that molecules released during inflammation may influence IL-12 production by LPS-stimulated APC. We here provide evidence that IFN-$\gamma$ and $PGE_2$ have opposing effects on IL-12 production by LPS-stimulated LPMC.

No IL-12 was measured in LPMC cultures provided with IFN-$\gamma$ alone, confirming the observation that IFN-$\gamma$ is not sufficient to completely activate IL-12 genes [4,25]. However, in both total and adherence-separated LPMC exposed to LPS, IFN-$\gamma$ evoked IL-12 release in a dose-dependent manner. The effect of IFN-$\gamma$ on IL-12 release appeared to be specific because it was inhibited by an IFN-$\gamma$ antiserum. These findings are supported by the observations that IFN-$\gamma$ is capable of promoting the synthesis of other cytokines in LPS-desensitized cell cultures and that endotoxin-resistant macrophages bearing the $lps^d$ mutation respond to IFN-$\gamma$ in terms of cytokine secretion [26,27]. Our data are moreover consistent with studies showing that, in other cell systems, IFN-$\gamma$ may specifically enhance LPS-induced IL-12 secretion [1–4,25]. Since IFN-$\gamma$ is capable of affecting the intestinal permeability and facilitating the influx of bacterial products from the lumen [31,32], these results suggest that IFN-$\gamma$ can play an important role in inducing IL-12 within the gut mucosa. In contrast to the effect on IL-12, IFN-$\gamma$ completely inhibited IL-10 secretion by LPS-stimulated LPMC. When recombinant IL-10 was added to IFN-$\gamma$-treated

**Table 3.** IL-12 and IL-8 production by control and lipopolysaccharide (LPS)-primed monocyte-derived macrophages

| Primary culture | | Secondary culture | | |
|---|---|---|---|---|
| LPS | Indomethacin | (LPS 100 ng/ml) | IL-12 | IL-8 |
| − | − | − | und. | $40 \pm 22$ |
| − | − | + | $270 \pm 42$ | $1660 \pm 96$ |
| + | − | − | Und. | $50 \pm 10$ |
| + | − | + | $20 \pm 10$ | $1700 \pm 180$ |
| + | + | − | Und. | $65 \pm 20$ |
| − | + | + | $380 \pm 12$ | $1450 \pm 150$ |
| + | + | + | $235 \pm 25$ | $1560 \pm 220$ |

Human monocyte-derived macrophages were cultured in complete medium at a concentration of $1 \times 10^6$/ml in the presence or absence of 200 pg/ml LPS and/or indomethacin for 2 days (primary culture). At the end of the culture period, cells were extensively washed and treated with medium or LPS (100 ng/ml) for a further 24 h (secondary culture). IL-12 and IL-8 release was measured by ELISA and their values are expressed as pg/ml. Und, Undetectable. Results are mean ± s.d. of six experiments.

cultures, IL-12 release by LPS-stimulated LPMC was remarkably decreased, suggesting that inhibition of endogenous IL-10 may be a mechanism by which IFN-$\gamma$ modulates LPS-stimulated LPMC IL-12 production.

At the intestinal mucosal level, where luminal (dietary, bacterial and viral) antigens are continuously interacting with immune cells, multiple and complex mechanisms operate in promoting local tolerance. Particularly, counter-balancing molecules seem to be determinant in promoting or blocking the occurrence of chronic mucosal inflammation [33,34]. $PGE_2$, normally synthesized by human intestinal cells, has been shown to modulate selectively both humoral and cellular response at multiple levels [18]. Particularly, $PGE_2$ and similar cAMP-elevating agents inhibit Th1-type cytokines [35,36] and IL-12 production induced by LPS [19]. Furthermore, $PGE_2$ seems to play a role in inducing tolerance *in vivo* [21]. In agreement with these observations, we found that $PGE_2$ in a dose-dependent manner inhibited IL-12 release by LPMC stimulated with IFN-$\gamma$ and LPS. As indomethacin, a powerful inhibitor of $PGE_2$ synthesis, facilitated IL-12 release by LPS-stimulated LPMC, it is plausible that endogenous $PGE_2$ acts in preventing IL-12 synthesis in human intestine. The ability of locally released $PGE_2$ to suppress intestinal macrophage cytokine production has been previously reported. Rugtveit *et al*. showed that LPMC tumour necrosis factor-alpha (TNF-$\alpha$) release was enhanced by indomethacin [22], whereas Nathens *et al*. suggested that, after exposure to bacterial products, endogenous $PGE_2$ down-regulates TNF-$\alpha$ secretion in co-cultures of intestinal epithelial and mononuclear cells [23]. Taken together, these observations suggest that $PGE_2$ may alter the activation state of intestinal macrophages, making them refractory to microbial stimulation. This hypothesis is also supported by *in vivo* studies showing that indomethacin and other cyclooxygenase inhibitors may contribute to the abnormal activation of the immune system and promote specific inflammatory enteropathies in the presence of luminal bacteria [37,38].

Consistent with other studies [19], we also showed that $PGE_2$ augmented in a dose-dependent fashion IL-10 secretion by LPS-stimulated LPMC. Together with the demonstration that IL-10 is a strong inhibitor of IL-12 production, these findings prompted us to test the hypothesis that $PGE_2$ could modulate LPS-stimulated LPMC IL-12 synthesis through the induction of IL-10. However, inhibition of IL-12 production by $PGE_2$ was only partially reversed by anti-IL-10, suggesting that the induction of IL-10 is not the main mechanism by which $PGE_2$ modulates LPMC IL-12 production.

Repeat LPS stimulation renders cells refractory to further LPS challenges. This phenomenon may be successfully reproduced *in vitro* [26,30]. We here report data indicating that human monocyte-derived macrophages treated with low concentrations of LPS release scarce amounts of IL-12 upon restimulation with LPS. This phenomenon does not seem to be due to a state of complete macrophage deactivation, because the same cells were capable of producing IL-8 in response to LPS. The capacity of indomethacin to restore IL-12 production by macrophages cultured in the presence of low concentrations of LPS strongly supports the role of $PGE_2$ in promoting the LPS tolerance phenomenon. Interestingly, while this manuscript was in preparation, it was reported that LPS-desensitized dendritic cells are poor sources of IL-12 and that $PGE_2$ may replace LPS for the induction of LPS tolerance [39]. As intestinal macrophages may be exposed to trace amounts of bacterial products either during the isolation procedure or *in vivo*, it is therefore conceivable that the defective LPMC IL-12 production in response to LPS may be due to a LPS tolerance phenomenon.

In conclusion, our study shows that IFN-$\gamma$ or $PGE_2$ regulate differently LPMC IL-12 production in response to LPS.

## REFERENCES


1 Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 1995; **13**:251–76.

2 Trinchieri G. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. Blood 1994; **84**:4006–27.

3 Hendrzak JA, Brunda MJ. Biology of disease. Interleukin-12. Biology activity, therapeutic utility, and role in disease. Lab Invest 1995; **72**:619–37.

4 Ma X, Chow JM, Cri G *et al*. The interleukin 12 p40 gene promoter is primed by interferon-$\gamma$ in monocytic cells. J Exp Med 1996; **183**:147–57.

5 Flesch IEA, Hess JH, Huang S *et al*. Early interleukin 12 production by macrophages in response to mycobacterial infection depends on interferon $\gamma$ and tumour necrosis factor $\alpha$. J Exp Med 1995; **181**:1615–21.

6 Monteleone G, Biancone L, Marasco R *et al*. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. Gastroenterology 1997; **112**:1169–78.

7 Parronchi P, Romagnani P, Annunziato F *et al*. Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. Am J Pathol 1997; **150**:823–32.

8 Pallone F, Fais S, Boirivant M. The interferon system in inflammatory bowel disease. In: Fiocchi C, eds. Cytokines in inflammatory bowel disease. Austin, Texas: R G Landes Co., 1995:57–67.

9 Fais S, Capobianchi MR, Pallone F *et al*. Spontaneous release of interferon gamma by intestinal lamina propria lymphocytes in Crohn's disease. Kinetics of in vitro response to interferon gamma inducers. Gut 1991; **32**:403–7.

10 Fais S, Capobianchi MR, Silvestri M *et al*. Interferon expression in Crohn's disease patients: increased interferon-gamma and -alpha mRNA in the intestinal lamina propria mononuclear cells. J Interferon Res 1994; **14**:235–8.

11 Fuss IJ, Neurath M, Boirivant M *et al*. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-$\gamma$, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. J Immunol 1996; **157**:1261–70.

12 Neurath MF, Fuss I, Kelsall BL *et al*. Antibodies to interleukin 12 abrogate established experimental colitis in mice. J Exp Med 1995; **182**:1281–90.

13 Monteleone G, Parrello T, Luzza F *et al*. Response of human intestinal lamina propria T lymphocytes to interleukin 12: additive effects of interleukin 15 and 7. Gut 1998; **43**:620–8.

14 Kuhn R, Lohler J, Rennick D *et al*. Interleukin-10 deficient mice develop chronic enterocolitis. Cell 1993; **75**:263–74.

15 Sellon RK, Tonkonogy S, Schultz M *et al*. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun 1998; **66**:5224–31.

16 Portanova JP, Zhang Y, Anderson GD *et al*. Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. J Exp Med 1996; **184**:883–91.

17 Williams TJ, Morley J. Prostaglandins as potentiators of increased vascular permeability in inflammation. Nature 1973; **246**:215–7.

18 Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. Immunol Today 1991; **12**:349–52.

19 van der Pouw Kraan TCTM, Boeije LCM, Smeenk RJT *et al*. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. J Exp Med 1995; **181**:775–9.

20 Strassmann G, Koota VP, Finkelman F *et al*. Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin E2. J Exp Med 1994; **180**:2365–70.
21 Scheuer WV, Hobbs MV, Weigle WO. Interference with tolerance induction in vivo by inhibitors of prostaglandin synthesis. Cell Immunol 1987; **104**:409–16.
22 Rugtveit J, Nilsen EM, Bakka A *et al*. Cytokine profiles differ in newly recruited and resident subsets of mucosal macrophages from inflammatory bowel disease. Gastroenterology 1997; **112**:1493–505.
23 Nathens AB, Rotstein OD, Dackiw APB *et al*. Intestinal epithelial cells down-regulate macrophage tumor necrosis factor-alpha secretion: a mechanism for immune homeostasis in the gut-associated lymphoid tissue. Surgery 1995; **118**:343–51.
24 Young HA, Hardy KJ. Role of interferon-gamma in immune cell regulation. J Leuk Biol 1995; **58**:373–81.
25 Hayes MP, Wang J, Norcross MA. Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-$\gamma$ of lipopolysaccharide-inducible p35 and p40 genes. Blood 1995; **86**:646–50.
26 Docke W-D, Randow F, Syrbe U *et al*. Monocyte deactivation in septic patients: restoration by IFN-$\gamma$ treatment. Nature Med 1997; **3**:678–81.
27 Beutler B, Tkacenko V, Milsark I *et al*. Effect of $\gamma$ interferon on cachetin expression by mononuclear phagocytes. Reversal of the $lps^d$ (endotoxin resistance) phenotype. J Exp Med 1986; **164**:1791–6.
28 D'Andrea A, Ma X, Aste-Amezaga M *et al*. Stimulatory and inhibitory effects of interleukin (IL)-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor $\alpha$ production. J Exp Med 1995; **181**:537–46.
29 Li C, Goodrich JM, Yang X. Interferon-gamma (IFN-$\gamma$) regulates production of IL-10 and IL-12 in human herpesvirus-6 (HHV-6)-infected monocyte/macrophage lineage. Clin Exp Immunol 1997; **109**:421–5.
30 Takasuka N, Tokunaga T, Akagawa KS. Preexposure of macrophages to low doses of lipopolysaccharide inhibits the expression of tumor necrosis factor-$\alpha$ mRNA but not of IL-1$\beta$ mRNA. J Immunol 1991; **146**:3824–30.
31 Madara JL, Stafford J. Interferon-$\gamma$ directly affects barrier function of cultured intestinal epithelial monolayers. J Clin Invest 1989; **83**:724–7.
32 Adams RB, Planchon SM, Roche JK. IFN-$\gamma$ modulation of epithelial barrier function. Time course, reversibility, and site of cytokine binding. J Immunol 1993; **150**:2356–63.
33 Strober W, Kelsall B, Fuss I *et al*. Reciprocal IFN-$\gamma$ and TGF-$\beta$ responses regulate the occurrence of mucosal inflammation. Immunol Today 1997; **18**:61–64.
34 Duchmann R, Schmitt E, Knolle P *et al*. Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. Eur J Immunol 1996; **26**:934–8.
35 Betz M, Fox BS. Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. J Immunol 1991; **146**:108–13.
36 Snijdewint FGM, Kalinski P, Wierenga EA *et al*. Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. J Immunol 1993; **150**:5321–9.
37 Stewart TH, Hetenyi C, Rowsell H *et al*. Ulcerative enterocolitis in dogs induced by drugs. J Pathol 1980; **131**:363–78.
38 Bjarnason I, Smethurst P, Levi AJ *et al*. Indomethacin induced chronic small intestinal ulceration in the rat. Gastroenterology 1988; **94**:1937.
39 Rieser C, Papesh C, Herold M *et al*. Differential deactivation of human dendritic cells by endotoxin desensitisation: role of tumor necrosis factor-$\alpha$ and prostaglandin E2. Blood 1998; **91**:3112–7.