LYPRINOL® INHIBITS LTB_4 PRODUCTION BY HUMAN MONOCYTES

LYPRINOL INHIBE LA PRODUCTION DE LTB_4 PAR LES MONOCYTES HUMAINS

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Summary

The effect of Lyprinol was evaluated on LTB4-induced human monocytes (normal and allergic donors) activation. Peripheral blood normal monocyte-derived monocytes when stimulated by Interleukin-4 (IL-4) produced high amounts of leukotriene B4 (LTB4) through the activation of the 5-lipoxygenase pathway. Maximal effect was observed in the presence of 10 ng/ml IL-4, and maximal LTB4 production was reached 40 min after the onset of stimulation. When stimulated for 48 h with IL-4, resting human monocytes expressed and released the low affinity receptor for IgE (CD23), and were inhibited in the presence of Lyprinol, or of the non redox 5-lipoxygenase inhibitor (BW B70C), suggesting that the production of LTB4 partially contributed to the IL-4-induced CD23 expression and release. In addition to these phenotypical changes, IL-4 primed the phorbol-12-myristate-13-acetate (PMA)-induced luminol-dependent chemiluminescence response (LDCL) by normal human monocytes ; this priming effect was abrogated in the presence of Lyprinol, or of BW B70C. Monocyte-derived monocytes from allergic patients spontaneously produced high amounts of LTB4, expressed CD23 expression, and had an increased oxidative metabolism. In the presence of Lyprinol, or of BW B70C, the hyper-activation of monocytes from allergic patients was significantly suppressed. Taken together, these data indicated that the pharmacological control of the 5-lipoxygenase pathway in human monocytes can be achieved with Lyprinol, and that the activation of this pathway could up-regulate the expression and release of CD23 and the respiratory burst of human monocytes.

Keys-words : Monocytes - CD23 - LTB4 - Respiratory burst - Allergy.

Résumé

L'effet du Lyprinol a été évalué sur l'activation du LTB4 induit par les monocytes humains (sujets normaux et allergiques). Les monocytes du sang périphérique, lorsqu'ils sont stimulés par l'IL4, produisent de grandes quantités de LTB4, par activation de la voie de la 5-lipoxygénase. L'effet maximal a été observé en présence de 10 ng/ml d'IL4 et la production maximale de LTB4 était atteinte 40 mn après le début de la stimulation. Lorsqu'ils sont stimulés pendant 48 heures, les monocytes expriment et libèrent le récepteur de faible affinité pour l'IgE (CD23) et sont inhibés en présence de Lyprinol ou en présence de l'inhibiteur redox 5-lipo-xygénase, suggérant que la production de LTB4 contribue partiellement à la synthèse d'IL4 induisant la libération et l'expression du CD23. De plus, à ces modifications phénotypiques, IL4 prime le phorbol-12-myristate-13-acétate (PMA) induisant une réponse de luminol chemiluminescence-dépendante par les monocytes humains normaux. Cet effet primaire est supprimé en présence de Lyprinol ou de BW B70C. Les monocytes dérivés des monocytes de patients allergiques produisent spontanément de grandes quantités de LTB4, expriment le CD23, et ont un métabolisme oxydatif élevé. En présence de Lyprinol ou BW B70C, l'hyperactivation de monocytes de patients allergiques est significativement supprimée. Au total, ces résultats indiquent que le contrôle pharmacologique de la 5-lypoxygénase des monocytes humains peut être amélioré par le Lyprinol, et que l'activation de cette voie peut réguler l'expression et la libération du CD23 et de la poussée oxydative des monocytes humains.

Mots-clés : Monocytes - CD23 - LTB4 - Poussée oxydative - Allergie.

Abbreviations :
IL-4 : Interleukin-4; CD23 : the low affinity receptor for IgE ; sCD23 : the soluble form of CD23 ; LTB4: leukotriene B4.

INTRODUCTION

Interleukin-4 (IL-4), a cytokine that displays a broad spectrum of biological activities (1), is produced by T cells, mast cells, and/or basophils (2, 4). IL-4 displays potent immunoregulatory properties that are either enhancing or inhibitory depending on the activation state of the cells that are being studied (5). IL-4 acts on monocytes/macrophages by enhancing the expression of class II MHC antigens, and adhesion molecules of the LFA-1 family [6], and by inducing expression of the low affinity receptor for IgE

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(FcεRIIb/CD23b) (7). In contrast, IL-4 was found to down-regulate the expression of FcγRI (CD64) and FcγRII (CD32) (8), and to inhibit expression of FcγRIII (CD16) induced by either interferon-g (IFN-g), or TGF-ß (9). Expression of CD14 on monocytes was also decreased (10), and associated with a decreased production of pro-inflammatory mediators in response to stimulation induced by lipopolysaccharide (LPS) (11, 12). On the other hand, IL-4 stimulates the production of G- and M-CSF (13). Depending on :

- the differentiation stage of monocytes/macrophages,
- and the activation signal provided (LPS or IFN-γ), IL-4 differentially regulates the generation of oxygen derivatives, H_2O_2, O_2°- (14, 16), and nitric oxide (17-19).

Recent observations from our laboratory indicated that LTB4, a 5-lipoxygenase metabolite produced by macrophages during inflammatory reactions, enhanced some of the biological effects of IL-4, namely CD23 expression and its release by human monocytes and B lymphocytes (22, 23), and IgE production by normal human peripheral blood mononuclear cells (PBMC) (25), possibly by interfering with the signaling pathway of IL-4 in target cells. However, the biochemical mechanism of action in human monocytes/macrophages of IL-4 is still poorly understood ; recent studies suggested that IL-4 regulates the lipoxygenase activity in human macrophages, and more precisely the expression of the 15-lipoxygenase (20), suggesting the existence of an important link between lipoxygenase function and the IL-4 induced immune/inflammatory responses.

We evaluated the effect of IL-4 on the activation of the 5-lipoxygenase pathway in normal human monocytes, as well as phenotypical and functional alterations induced by such a stimulation.

MATERIALS AND METHODS

REAGENTS

Recombinant human IL-4 (1 x 10^7 U/mg), and rabbit polyclonal anti-IL-4 were purchased from Immugenex (Los Angeles, CA, USA). Indomethacin, phorbol-12-myristate-13-acetate (PMA), and luminol were purchased from Sigma Co (St Louis, MO, USA) ; 5-lipoxygenase and BW B70C were provided by Wellcome Ltd (Beckenham, England) ; Lyprinol was kindly provided by Pharmalink International Ltd. (Hong Kong).

ISOLATION OF HUMAN MONOCYTES

Human peripheral blood mononuclear cells (PBMC) from healthy volunteers (n = 12) and allergic patients (n = 10, 4 asthmatics and 6 atopic dermatitis) were isolated as already described (26). Briefly, after centrifugation at 900 rpm for 10 min to eliminate the most platelets, blood samples were diluted in RPMI 1640 medium (Bioproduct, Les Ulis, France), and PBMC were harvested after 20 min centrifugation at 2,000 rpm on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient. Adherent cell populations were obtained by incubation of PBMC (1 x 10^7 cells/ml) in either Petri dishes (Nunc, Rocksille, Denmark), or 6-well culture plates (Nunc) for 30 min at 37° C in RPMI 1640 medium supplemented with 10% v/v fetal calf serum (FCS) (Flow, Les Ulis, France). Monocytes were then harvested by scraping the plates after addition of cold phosphate-buffered saline solution (PBS) containing 1 mM ethylene-diamine-tetra-acetic acid (EDTA). Cell preparations contained more than 95% viable monocytes, as assessed by trypan blue exclusion method, and non-specific esterase staining. Other cells present were lymphocytes and granulocytes, that never exceeded 2% of the total cell number.

MONOCYTE CULTURES

Mcarophages were obtained from monocytes cultured at a final cell concentration of 2 x 10^6 cells/ml in 12 or 24-well plastic culture plates (Nunc), and in Iscove medium supplemented with 100 U/ml penicillin + 100 µg/ml streptomycin + 5% FCS, after 5 days of culture in the absence of cytokines. This culture medium was shown to be endotoxin-free as assessed by the limulus amoebocyte lysate assay. The cells were incubated at 37° C under a 5% CO_2, 95% air and 100% humidity atmosphere, for different periods of times, in presence or absence of IL-4. Cell viability was assessed by trypan exclusion staining after each culture period, and cell mortality never exceeded 85%

LTB_4 PRODUCTION

As described above, monocytes were stimulated for various times (0 to 45 min), in medium alone or with IL-4 (0.1 to 30 ng/ml), and with or without 10 µM Indomethacin, BW B70C, or Lyprinol. Supernatants were then harvested and frozen at -80° C until the LTB_4 assay was performed.

LTB_4 was assayed by ELISA (Stallergènes, Fresnes, France) according to the manufacturer's recommendations. The sensitivity of the test was 20 pg/ml, and cross-reactivity with LTC4, LTD4, and LTE4 was < 0.1%.

CD23 EXPRESSION AND RELEASE

Monocytes were stained [immunofluorescence] according to published techniques (26). Briefly, cells (0.5 x

106/ml) were incubated at 4° C for 30 min in the presence of anti-CD23 directly coupled to phycoerythrin (Leu20 mAb, Becton Dickinson, Grenoble, France). Fluorescence was measured with a cytofluorograph (FACScan, Becton Dickinson, France). Data analysis was performed by reading 5,000 cells/sample, and results were expressed as % of CD23+ compared to control fluorescence (isotype matched mAb IgG_1, Becton Dickinson), using the lysis program (Becton Dickinson). Cell-free supernatants of monocytes, treated or not with the different stimuli for 3 to 5 days, were harvested and assayed for their sCD23 content using a commercial sCD23 ELISA kit (Immunotech, Luminy, France). All assays were performed according to the manufacturer's specific recommendations.

LUMINOL-DEPENDENT CHEMILUMINESCENCE (LDCL)

Chemiluminescence was evaluated as previously described (27), using a Berthold 9150 luminometer (Berthold, France). The resulting light output was recorded, and data were expressed in cpm. Temperature within the reaction vial was thermostatically controlled (37° C). Reaction mixes were conducted in polyethylene tubes : 700 µl of luminol (20 µg/ml), 200 µl of cell suspension (106 cells/ml), and 100 µl of PMA (200 mM).

RESULTS

Effects of Lyprinol and BW B70C on the IL-4-inducced LTB4 production by normal human monocytes : normal human monocytes spontaneously produced low quantities of LTB4. In the presence of IL-4 this production was dose-dependently increased with a maximal effect observed at a final concentration of 10 ng/ml IL-4 (figure 1) ; this effect was maximal after 40 min (data not shown). This IL-4-induced LTB4 production was decreased by BW B70C (10 µM), a 5-lipoxygenase inhibitor, but not by Indomethacin, a cycloxygenase inhibitor (table 1) ; it was also dose-dependently suppressed by Lyprinol (figure 2). Taken together, these data indicate that IL-4 stimulated the production of LTB4 from normal human monocytes by activation of the 5-lipoxygenase pathway, and that Lyprinol affects IL-4 signaling likely by effecting this pathway.

Stimulation	LTB4 (pg/ml)
Medium	20 + 5
IL-4	195 + 25
BW B70C	3 + 1
IL-4 + BW B70C	55 + 6
Indomethacin	22 ± 2
IL-4 + Indomethacin	205 ± 7

Table 1 : Effects of a 5-lipoxygenase inhibitor (BW B70C), and of Lyprinol on the IL-4-induced LTB4 production by human monocytes, from healthy human volunteers and allergic patients.
Human monocytes were stimulated or not either with an optimal dose (10 ng/ml) of IL-4, in the presence or in the absence of 10 µM of BW B70C or indomethacin. After 40 min of stimulation, cell-free supernatants were collected for LTB4 determinations as described in materials and methods. Data are the mean + SEM of 4 different experiments.

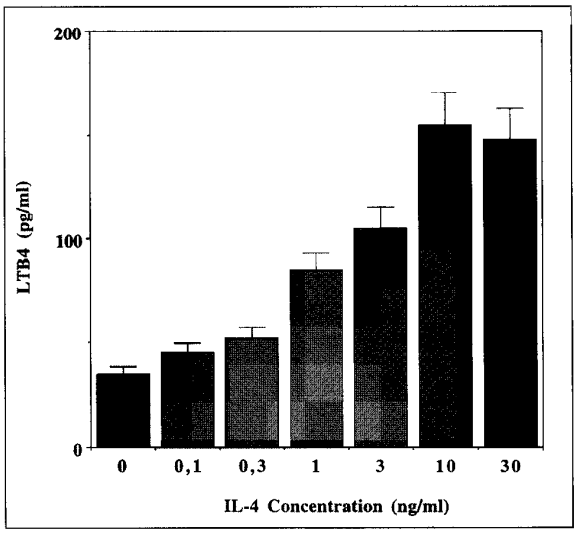

Figure 1 : Dose effect of IL-4 on the production of LTB4 by normal human monocytes.
Normal human monocytes (2 x 106) were cultured in the presence of various doses of IL-4 ; the cell-free supernatants were harvested after 40 min, and frozen at - 20° C prior to LTB4 assays, as described in materials and methods. Data are mean + SEM of 4 different experiments.

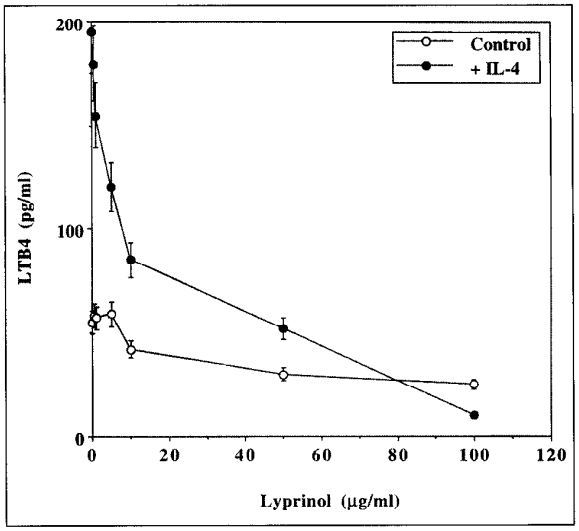

Figure 2 : Dose-effect of Lyprinol on the IL-4-induced LTB4 production by human monocytes from healthy volunteers.
Monocytes (2 x 106) from healthy donors were cultured in the presence of 10 ng/ml of IL-4 and/or in the presence of various doses of Lyprinol (0.1 to 30 µg/ml) ; the cell-free supernatants were harvested after 30 min and frozen at - 20° C prior to LTB4 assays, as described in materials and methods. Data are mean + SEM of 4 different experiments.

Effects of Lyprinol and BW B70C on the IL-4-induced CD23 expression and release by normal human mono-

trated that Lyprinol, by affecting the 5-lipoxygenase pathway, reduced the pro-inflammatory features of allergy.

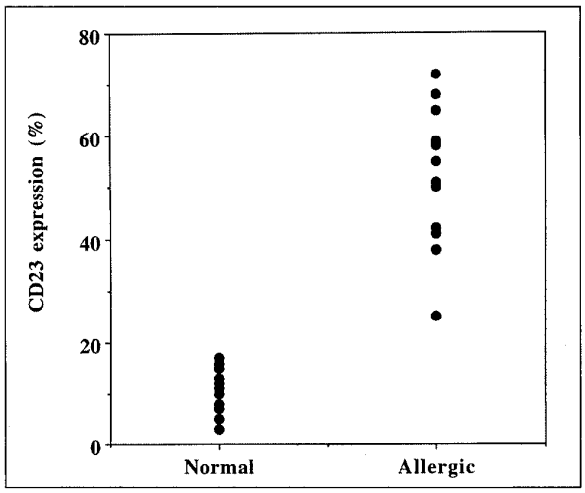

Figure 5 : Spontaneous expression of CD23 (A), release of LTB4 (B) and LDCL (C) by human monocytes, from healthy volunteers and allergic patients.
Monocytes (2 x 106) from healthy volunteers and allergic patients were cultured for 48 h; then cell-free supernatants were harvested after 30 min and frozen at - 20° C prior to LTB4 (B) assays, as described in materials and methods. Cells were also stained with anti-CD23 mAb, and analyzed by flow cytometry (A), and spontaneous LDCL response (C) was studied, as described in materials and methods. Data are mean + SEM of a quadruplicate experiment from one representative experiment out of 5.

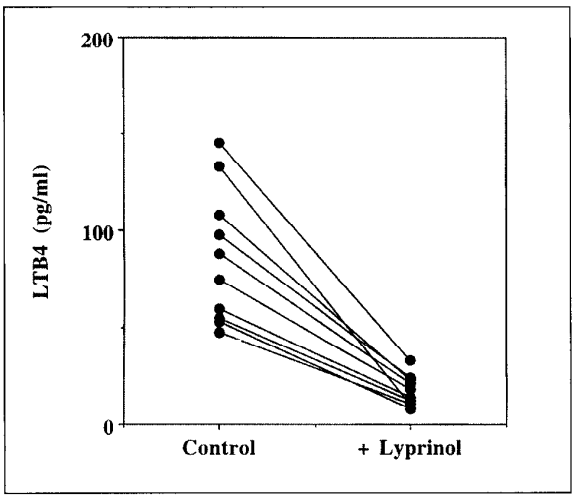

Figure 6 : Effect of Lyprinol on the spontaneous LTB4 production (A), CD23 release (B), and LDCL response (C) by human monocytes from allergic patients.
Monocytes (2 x 106) from allergic patients were cultured in the presence of 10 µg/ml of Lyprinol for 48 h ; then cell-free supernatants were harvested after 30 min and frozen at - 20° C prior to LTB4 assays, as described in materials and methods. Cells were also stained with anti-CD23 mAb, and analyzed by flow cytometry (B), and spontaneous LDCL response (C) was studied, as described in materials and methods. Data are mean + SEM of a quadruplicate experiment from one representative experiment out of 5.

DISCUSSION

Our study indicates that marine lipids, e.g. Lyprinol, affect the release of LTB4 by human monocytes from healthy and allergic patients, and help control their pro-inflammatory status. In the presence of Lyprinol, both the expression and release of CD23 by monocytes from allergic patients, or by IL-4-stimulated normal human macrophages, were partially inhibited. This suggests that the activation of the 5-lipoxygenase pathway, and specifically the LTB4 production, could play an important regulatory role in the IgE-dependent process. Previous reports have suggested that LTB4 was able to potentiate the IL-4-induced IgE production by normal human PBMC [24, 25], through enhanced expression of IL-4 receptors, and production of soluble CD23. Importantly, IL-4, as well as GM-CSF (31), is able to promote simultaneously an immune (CD23 expression and release) and an inflammatory (LTB4 production) response. Specific immune responses are triggered by an inflammatory reaction following antigen penetration of the organism. IL-4 could play an important role during the initial phase of IgE-dependent inflammation by inducing the production of LTB4, and, conversely, LTB4 could act as an amplifier of the IL-4-dependent immune response. Preliminary data indicate that Lyprinol inhibits concurrently the IL-4-induced production of IgE (B. Dugas et al, manuscript in preparation).

These results, suggesting that IL-4 stimulated the arachidonate cascade, and more specifically the lipoxygenase pathway, in normal human monocytes, are in accordance with previous studies confirming that IL-4 also regulates the expression of 15-lipoxygenase (20, 21). Based on referenced studies and the data we present, it is likely that Lyprinol elicits its anti-allergic and, more broadly, its anti-inflammatory properties by controlling the 5-lipoxygenase pathway.

IL-4 regulates the IgE-dependent immune and inflammatory processes [33] and has been shown to affect the oxygen metabolism of mononuclear phagocytes. IL-4 was found to promote LDCL response in peritoneal murine monocytes (32), and in normal human monocytes. This effect appeared to be due to the activation of the lipoxygenase pathway, since IL-4 was unable to promote this LDCL response in the presence of Lyprinol.

Taken together these data indicate that Lyprinol inhibits the lipoxygenase pathway (e.g. LTB4 production) in human monocytes from allergic patients, or healthy human donors, after stimulation by IL-4. Lyprinol may regulate both the inflammatory and immune responses, and specifically the CD23-dependent one, induced by IL-4.

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