Immunology 1991 **74** 432–438

Induction of interleukin-1 production by ligands binding to the scavenger receptor in human monocytes and the THP-1 cell line

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Accepted for publication 2 July 1991

SUMMARY

Foam cell formation via lipid accumulation through the scavenger receptor in human monocyte/macrophages is believed to be one of the earliest events in atherogenesis. In this study we demonstrate that stimulation of the scavenger receptor activates monocytes to produce interleukin-1 (IL-1). Polyinosinic acid (poly I) and fucoidan, both ligands known to bind to the scavenger receptor, induced IL-1β production in human monocytes. Polycytidylic acid, a structurally related compound to poly I, which does not bind to the scavenger receptor, was used as a negative control and had virtually no effect on IL-1 production. THP-1 cells, which normally do not express scavenger receptors, were almost unresponsive to poly I and fucoidan. PMA priming, which has been reported to up-regulate scavenger receptor expression in THP-1 cells, significantly enhanced IL-1 production by fucoidan and poly I. IL-1 produced by scavenger receptor stimulation was shown to be secreted extracellularly, and biologically active. Scavenger receptor-mediated IL-1 production was inhibited by H7, a protein kinase C inhibitor, and enhanced by IBMX, an inhibitor of cyclic AMP degradation, suggesting a synergistic effect of protein kinase C and cyclic AMP-mediated signal transduction pathways in scavenger receptor-mediated IL-1 production. Due to the potentially deleterious effects of IL-1 on the vessel wall, IL-1 produced by ligand binding to the scavenger receptor in human monocytes may play a role in the pathogenesis of atherosclerosis.

INTRODUCTION

Elevated levels of plasma low density lipoproteins (LDL) are associated with an increased incidence of atherosclerosis.[1] The first sign of atherosclerosis in the vessel wall is the formation of a fatty streak, which consists mainly of lipid-loaded monocyte/macrophages, the foam cells.[2,3] Monocytes express specific LDL receptors, which are regulated by the cholesterol needs of the cell, thus preventing excessive lipid accumulation.[4] By various modifications (oxidation, acetylation) LDL is converted to a form recognized by another monocyte/macrophage receptor, the scavenger receptor.[5] In contrast to native LDL, modified LDL can be taken up via the scavenger receptor in a non-regulated fashion, resulting in foam cell formation. Oxidative modification of LDL has been reported to occur *in vivo*, thus generating a physiological ligand to the scavenger receptor.[6]

Scavenger receptors were first discovered by their ability to bind and internalize acetylated LDL. Various reagents, such as oxidized or endothelial cell-modified LDL, maleyl-bovine serum albumin (maleyl-BSA) and the algal polysaccharide fucoidan, have also been reported to bind to the scavenger receptor.[5-8] The presence of a large number of negatively charged residues has been thought to be a common denominator of these compounds. However, a specific conformation determines the binding, since some closely related structures, such as different single-stranded polynucleotides, differ in their ability to bind to the scavenger receptor; polyinosinic acid (poly I) binds to it, but polycytidylic acid (poly C) does not.[7,9]

The cloning of cDNAs for two forms of scavenger receptors from bovine macrophages was reported first.[9,10] Recently, two forms of scavenger receptors from the human myeloid cell line THP-1, carrying 73% and 71% homology to their bovine counterparts, were also cloned.[11] The receptors are integral membrane proteins consisting of three polypeptide chains. Whether the existence of these two receptors and their possible heterotrimers accounts for all the scavenger activity in monocyte/macrophages is not yet known.

Activation of primed macrophages by ligands of the scavenger receptor has been reported previously. Maley-BSA and fucoidan trigger protease secretion, cytolytic activity and the expression of early proteins in primed murine macrophages.[12,13] However, maley-BSA has been reported to induce responses through sites distinct from the scavenger receptor.[14] Conflicting data exist on the ability of modified LDL to activate monocytes.[15,16] Due to the sensitivity of monocytes to trace amounts of LPS,[17] the possibility of contaminating LPS further complicates the analysis of lipoprotein effects on monocytes. Oxidized LDL has also been reported to exert direct toxic effects to cell cultures.[18]

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Activated monocytes are the main source of interleukin-1 (IL-1), a cytokine with a broad range of effects in inflammatory and immune responses (reviewed in refs 19 and 20). IL-1 has many potentially atherogenic effects. It increases monocyte adherence to the vessel wall by increasing ICAM-1 expression on the endothelial cells[21,22] and stimulates smooth muscle cell proliferation.[23] It also activates the endothelium to express increased procoagulant activity,[24] thus promoting clot formation.

In this report we investigated whether ligand binding to the scavenger receptor activates human monocytes and a monocytic cell line, THP-1 to produce IL-1.

MATERIALS AND METHODS

Reagents

Fucoidan, polyinosinic acid (poly I), polycytidylic acid (poly C), phorbol 12-myristate-13-acetate (PMA), isobutyl-methyl-xanthine (IBMX) and polymyxin B sulphate (PMB) were purchased from Sigma Chemical Company (St Louis, MO). Lipopolysaccharide (LPS; *Escherichia coli* 026:B6) was purchased from Difco Laboratories (Detroit, MI). Protein kinase C inhibitor H7 (1-[5-isoquinolone-sulphonyl]-2-methylpiperazine dihydrochloride) was purchased from Seikagaku Kogyo Co. (Tokyo, Japan).

Cell cultures

Leucocyte-enriched buffy coats were obtained from the Finnish Red Cross Blood Transfusion Service (Helsinki, Finland). Mononuclear cells were isolated from the buffy coats by Ficoll–Isopaque centrifugation (Pharmacia, Uppsala, Sweden). The cells were suspended at 10^7/ml RPMI-1640 medium (Flow Laboratories, Irvine, Renfrewshire, U.K.), supplemented with 10% human AB serum (Finnish Red Cross Blood Transfusion Service), 10 mM L-glutamine, and antibiotics (complete medium). The cell suspension was incubated in 75 cm^2 tissue culture flasks (Costar, Cambridge, MA) in 20-ml volume at 37°, 5% CO_2 for 1 hr. Non-adherent cells were then removed by washing the flasks twice with warm RPMI-1640 supplemented with HEPES, and adherent cells were collected by adding cold phosphate-buffered saline (PBS) ($Ca^{2+}Mg^2$ free; Orion Diagnostica, Espoo, Finland) to the cells and scraping with a rubber policeman. Adherent cells will be referred to as monocytes.

For RNA isolations, mononuclear cells from each buffy coat were divided directly to the experimental groups indicated in 90 mm Petri dishes, non-adherent cells were removed as described above and 10 ml of complete medium and the indicated stimulators were added. To avoid inter-individual variations between buffy coats, each experimental group consisted of cells derived from five to six buffy coats. At times indicated monocytes were harvested as described above.

The THP-1 cell line was obtained from the American Type Culture Collection (Rockville, MD). THP-1 cells were cultured in RPMI-1640 medium containing 10 mM HEPES, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 10% foetal calf serum (Flow Labs) and antibiotics (THP-1 medium). THP-1 cells were found to be mycoplasma negative in monthly testing. For PMA priming $0{\cdot}5 \times 10^6$ THP-1 cells/ml were treated with 1 ng/ml PMA for 20 hr, after which adherent cells were recovered and stimulated as described below.

IL-1 induction

10^6 monocytes in 1 ml complete medium, and 10^6 native or PMA-treated THP-1 cells in 1 ml THP-1 medium, were cultured with the indicated reagents in 24-well plates (Costar, Cambridge, MA) for 20 hr. After that cells were collected with the supernatants to get total IL-1 produced (i.e. intracellular, membrane-associated and secreted IL-1) or cells and supernatants were collected separately to determine the amount of secreted versus cell-associated IL-1. Cells were disrupted with three cycles of freezing and thawing and all the samples were stored at −20° before being assayed for their IL-1 content.

IL-1 assay

IL-1β contents of the samples was measured by IL-1β ELISA (Cistron, Pine Brook, NJ). The assays were performed according to the manufacturers's instructions. Each sample was tested in duplicate. Since the amounts of IL-1 produced by different donors varied greatly, the data are reported as a representative experiment from a single donor, along with the mean ± standard deviation and range of three experiments when IL-1 concentrations are presented. Biological IL-1 activity from the supernatants was determined by the thymocyte co-mitogenic assay as described by Mizel.[25] Each sample was tested at different dilutions in triplicate and the data shown are mean c.p.m. of the triplicate wells. The standard error of the mean was always less than 10%.

RNA isolation and analysis

At the times indicated after stimulation, adherent monocytes were harvested and total cellular RNA was isolated by guanidium isothiocyanate lysis and CsCl centrifugation.[26,27] The RNA isolated was quantified spectrophotometrically and 20 μg samples were size-fractionated on 1·2% agarose-formaldehyde gels, transferred to a nylon membrane (Pall, Glen Cove, NY), dried and baked at 80°. The IL-1β cDNA probe (HU-IL-1β, pcDSRα) used was provided by Dr Kari Varkila (DNAX Research Institute, Palo Alto, CA). The RNA levels on the nylon membranes were also quantified by using a constant probe, glyceraldehyde phosphate dehydrogenase (pRGAPDH-13). The latter was a gift from Dr Kari Alitalo (University of Helsinki, Finland). The IL-1β cDNA insert (*Bam*H1-digested fragments from the HU-IL-1β plasmid), in addition to the GAPDH insert (*Pst*1-digested fragments from pRGAPDH-13), were labelled with ^{32}P using a random-primed DNA labelling kit purchased from Boehringer Mannheim (Mannheim GmbH, Mannheim, Germany). Prehybridizations and hybridizations were performed in a solution containing 50% formamide, 5× Denhardt's solution, 5× SSPE and 0·5% SDS. Filters were washed in 1× SSC plus 0·1% SDS, twice for 30 min at room temperature and then at 60° for 30 min. Subsequently, the filters were exposed to Kodak AR X-Omat films at −70° with intensifying screens.

RESULTS

Induction of IL-1 production by ligands binding to the scavenger receptor in human monocytes

Monocytes were cultured with various concentrations of known scavenger receptor ligands poly I and fucoidan. Poly C, a structurally related compound to poly I, which is known not to

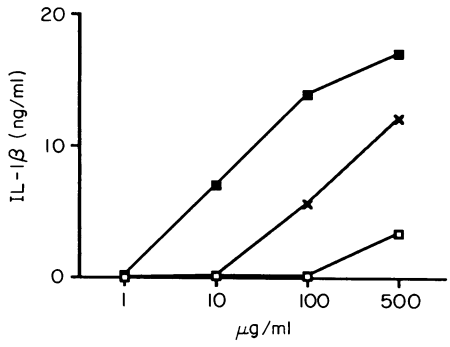

Figure 1. The effect of fucoidan (×), poly I (■) and poly C (□) on IL-1β production in human monocytes. 10^6 monocytes/1 ml complete medium were cultured with fucoidan, poly I or poly C at the concentrations indicated. After 20 hr supernatants and cells were harvested together and cells were disrupted by freezing and thawing. Samples were assayed for their IL-1β content in an IL-1β-specific ELISA. The data are reported as a representative experiment from a single donor ($n=4$).

bind to the scavenger receptor, was used as a negative control. After 20 hr supernatants and cells were harvested together, lysates were formed by freezing and thawing, and total IL-1α and IL-1β was measured using IL-1α- and IL-1β-specific ELISA kits. In all experiments fucoidan and poly I induced IL-1β production concentration dependently (Fig. 1). In cells derived from four different donors the IL-1β production (pg/ml) stimulated by 100 μg/ml poly I was 10,601 ± 4799 (mean ± SD)(range 4354–14,620), by 100 μg/ml poly C 33 ± 20 (range 20–63), and by 100 μg/ml fucoidan 3503 ± 2776 (range 1084–6120). Poly I stimulation was always higher than fucoidan, possibly reflecting the higher affinity of poly I to the scavenger receptor than fucoidan, which has been reported previously.[7] Poly C had a weak IL-1-inducing effect only in the highest concentration used (500 μg/ml).

Poly I and fucoidan also induced IL-1α protein production, the IL-1α levels being approximately 3–10 times lower than IL-1β levels (results not shown).

Up-regulation of scavenger receptor activity in THP-1 cells by PMA renders them sensitive to fucoidan and poly I-induced IL-1 production

The human monocytic cell-line THP-1 has been reported not to express scavenger receptor activity normally. Treatment with PMA induces both the expression of the scavenger receptor mRNA and scavenger receptor activity in this cell line.[9,11,28] To test whether fucoidan- and poly I-induced IL-1β production correlates with the scavenger receptor expression in THP-1 cells, native and PMA-primed THP-1 cells were cultured with the indicated stimulants and IL-1β levels were measured with an IL-1β-specific ELISA. Native THP-1 cells were only slightly responsive to poly I and fucoidan, but after PMA treatment both fucoidan- and poly I-induced IL-1β production was greatly enhanced (Table 1). Poly C had no effect on IL-1β production in native THP-1 cells, and in PMA-treated THP-1 cells only the highest poly C concentration used (500 μg/ml) had a slight IL-1β inducing effect.

Table 1. The effect of PMA-priming in the induction of IL-1β by ligands binding to the scavenger receptor in THP-1 cell line

Additions (μg/ml)	IL-1β (pg/ml)* THP-1	IL-1β (pg/ml)* PMA primed THP-1†
Media	<20	214
Fucoidan 500	57	19,383
Fucoidan 100	79	9212
Fucoidan 10	33	1368
Fucoidan 1	<20	311
Poly I 500	1157	43,482
Poly I 100	990	28,519
Poly I 10	94	6986
Poly I 1	21	481
Poly C 500	36	1789
Poly C 100	<20	335
Poly C 10	<20	245
Poly C 1	<20	231

* Determined by IL-1β-specific ELISA, representative of three experiments.

† $0{\cdot}5 \times 10^6$/ml THP-1 cells were cultured for 20 hr with 1 ng/ml PMA, after which adherent cells were collected. Native (THP-1) and PMA-treated cells (PMA-primed THP-1) were cultured at 10^6 cells/ml with the indicated reagents for 20 hr. After that cells and supernatants were harvested together and lysates were formed by three cycles of freezing and thawing.

Scavenger receptor-mediated IL-1 production is not abolished by polymyxin B

The possibility of contaminating LPS being responsible for the fucoidan- and poly I-induced IL-1β production was considered next. Polymyxin B (PMB) is an antibiotic capable of inhibiting IL-1 induction mediated by LPS.[29] PMB did not abolish IL-1 induced by optimal concentrations of scavenger receptor ligands, while it completely inhibited IL-1 production induced by optimal LPS concentrations (Fig. 2). The data are shown as the mean percentage of inhibition ($n=3$) induced by 5 μg/ml PMB on the indicated stimulants.

Kinetics of scavenger receptor-mediated IL-1α and IL-β mRNA expression

To further characterize scavenger receptor-mediated IL-1 production, the kinetics of IL-1α and β mRNA expression after scavenger receptor stimulation was studied. Adherent monocytes were stimulated with 100 μg/ml poly I, cells were harvested at the time points indicated and total cellular RNA isolated and analysed as described in the Materials and Methods. Stimulation with poly I induced a rapid increase in IL-1α and β mRNA steady-state levels, the peak being between 3 and 6 hr and mRNA levels decreasing by 24 hr (Fig. 3). Each experimental group consisted of cells derived from five to six blood donors and the experiment was repeated three times with similar results.

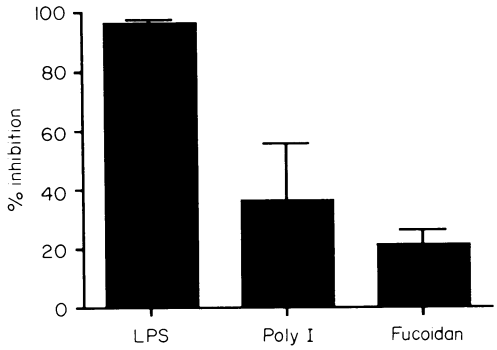

Figure 2. The effect of PMB on optimal LPS-, poly I- and fucoidan-induced IL-1 production. 10^6 monocytes/1 ml media were cultured with 100 ng/ml LPS, 100 μg/ml poly I or 1 mg/ml fucoidan with or without 5 μg/ml PMB. Cells and supernatants were collected together after 20 hr and assayed for their IL-1 content in an IL-1β-specific ELISA. The data shown are the mean percentage of inhibition ($n = 3$) induced by PMB on the indicated stimulants.

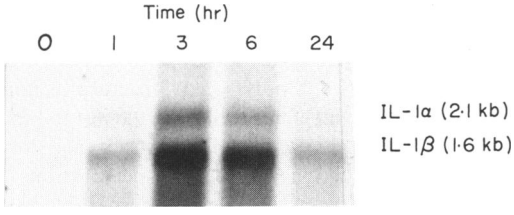

Figure 3. Kinetics of scavenger receptor stimulation-mediated IL-1α and IL-1β mRNA expression. Monocytes were stimulated with 100 μg/ml poly I and cells were harvested at the time-points indicated. Total cellular RNA was isolated and analysed as described in the Materials and Methods. The experiment was repeated three times with similar results.

Scavenger receptor-induced IL-1β is secreted to the extracellular compartment and is biologically active

Since poly I induced remarkably high IL-1β levels, the effect of poly I on IL-1β secretion versus cellular IL-1 production was tested. Monocytes were stimulated with various concentrations of poly I and cells (cellular IL-1) and supernatants (secreted IL-1) were collected separately and analysed in IL-1β ELISA. The concentration-dependent increase of IL-1β produced after poly I stimulation shown in Fig. 1 reflects an increase in secreted rather than the cellular levels of IL-1β (Fig. 4). The data are shown as IL-1β secreted versus cellular IL-1β produced from a single donor. The mean percentage of IL-1β secreted from total IL-1β produced was $58\% \pm 9$ with 100 μg/ml poly I and $36\% \pm 11$ with 10 μg/ml poly I ($n = 3$).

IL-1β is known to be first translated as a biologically inactive 31,000 MW precursor molecule.[30] This molecule is subsequently cleaved to a smaller, biologically active 17,500 form, which is secreted extracellularly through an unidentified mechanism. To test the biological activity of IL-1 secreted after scavenger receptor stimulation, monocytes were cultured as described above with 100 μg/ml poly I, 100 μg/ml poly C or 100 ng/ml LPS (which is an optimal IL-1-inducing concentration in human monocytes in our hands). At the end of the induction supernatants were collected, centrifuged to remove contaminating cells and tested for their activity in the thymocyte co-mitogenic assay, the classical test for biologically active IL-1. Supernatants from LPS and poly I-activated monocytes induced comparable levels of thymocyte proliferation, the effect of fucoidan was lower, while poly C-activated monocyte supernatants had no effect in the thymocyte assay (Fig. 5). The data are shown from a single donor as mean c.p.m. of each sample in triplicate. The experiment was repeated three times with similar results.

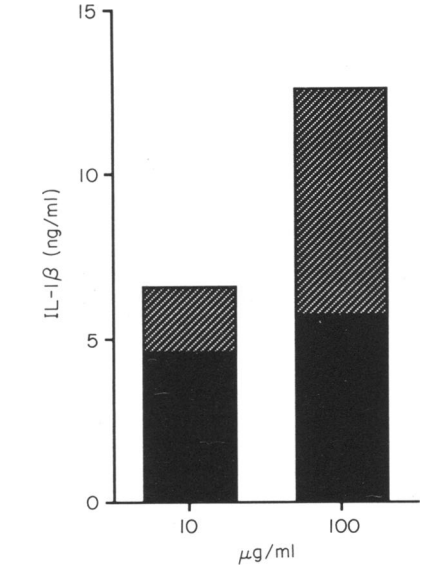

Figure 4. The effect of various concentrations of poly I on cellular (■) versus secreted (▨) IL1β production. Monocytes were stimulated with 10 or 100 μg ml poly I, after which cells (cellular IL-1β) and supernatants (secreted IL-1β) were collected separately and analysed for their IL-1β content. The data are shown as a representative experiment ($n = 3$) from a single donor.

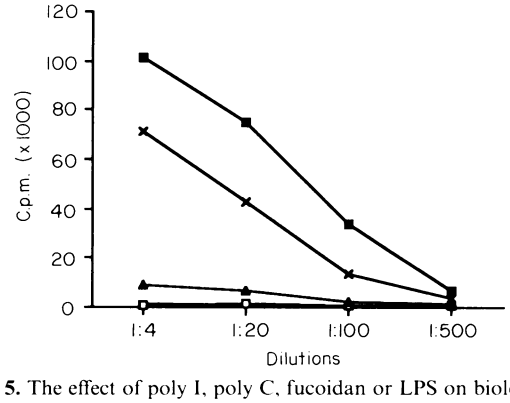

Figure 5. The effect of poly I, poly C, fucoidan or LPS on biologically active IL-1 secretion by human monocytes. 10^6 monocytes/1 ml complete medium were cultured with 100 μg/ml poly I (■) 100 μg/ml poly C (□), 100 μg/ml fucoidan (▲) or 100 ng/ml LPS (×). After 20 hr supernatants were collected and samples were assayed for their activity in the thymocyte co-mitogenic assay at the dilutions indicated. The data are shown from a single donor as mean c.p.m. of each sample in triplicate. The experiment was repeated three times with similar results.

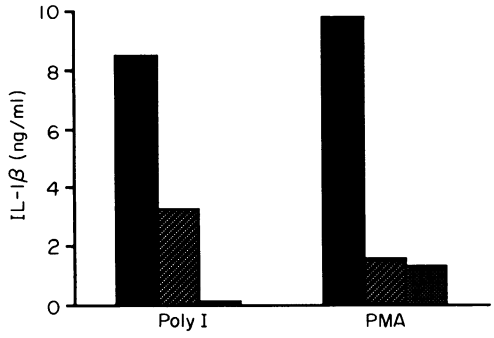

Figure 6. The effect of H7, a protein kinase C inhibitor on poly I- and PMA-induced IL-1β production. Monocytes were preincubated with medium (■), 12 μmol (■) or 25 μmol (▦) H7 for 1 hr, after which the cells were stimulated with 100 μg/ml poly I or 10 ng/ml PMA for 20 hr. IL-1β content of the cultures was measured by IL-1β-specific ELISA. The data are presented as a representative experiment ($n = 3$) from a single donor.

Scavenger receptor-mediated IL-1 production is inhibited by a protein kinase C (PKC) inhibitor

To examine the signal transduction pathways leading to scavenger receptor-mediated IL-1β production, the effect of H7, a preferential protein kinase C inhibitor, on poly I-mediated IL-1 production was tested. H7 concentration-dependently inhibited IL-1 induced by optimal poly I stimulation (Fig. 6), suggesting a protein kinase C-mediated stimulatory pathway in scavenger receptor-mediated IL-1 induction. The ability of H7 to inhibit IL-1 induced by PMA, a direct PKC activator, was used as a positive control. The data are presented as a representative experiment from a single donor. The mean percentage of inhibition induced by 25 μmol H7 was 70% ± 29 with 100 μg/ml poly I and 80% ± 12 with 10 ng/ml PMA ($n = 3$).

A synergistic effect of scavenger receptor stimulation on PMA-mediated IL-1 induction, a possible stimulatory role for cAMP-dependent signals

To further characterize the PKC-mediated IL-1 signal transduction pathway, the interaction of poly I- and PMA-induced signals was studied. Poly I and PMA had a synergistic effect on IL-1 induction (Table 2). The data from three different blood donors are shown. It has been demonstrated recently that cyclic AMP has an enhancing effect in PMA-mediated IL-1 production in human monocytes and myeloid cell lines.[31,32] The effect of poly I stimulation on intracellular levels of cAMP levels was then tested. Poly I induced a rise in intracellular levels of cAMP when measured with a commercial radioimmunoassay (results not shown). Cyclic AMP levels peaked in 15–20 min after poly I stimulation and returned to basal levels by 30 min. When poly I stimulation was performed in the presence of isobutyl-methyl-xanthine (IBMX), a cAMP phosphodiesterase inhibitor which inhibits the degradation of cAMP, IL-1 levels were also increased (Table 2). These results suggest a synergistic effect of PKC- and cAMP-dependent signal transduction pathways in poly I-mediated IL-1 induction.

Table 2. The effect of PMA and IBMX on poly I-mediated IL-1β production in human monocytes

Additions	IL-1β (pg/ml)*		
	Donor 1	Donor 2	Donor 3
—	32	20	19
Poly I 100 μg/ml	12,870	24,554	1587
PMA 10 ng/ml	1630	310	160
Poly I 100 + PMA 10 ng/ml	18,630	33,942	2019
IBMX 0·5 mM	< 20	71	ND
Poly I 100 + IBMX 0·5 mM	20,996	45,528	5979

*Monocytes were cultured with the indicated stimulants for 20 hr, after which total IL-1β was determined by an IL-1β-specific ELISA.
ND, not determined.

DISCUSSION

In the data presented here we show that fucoidan and poly I, both ligands known to bind to the scavenger receptor,[7,9] induced IL-1 production in human monocytes (Fig. 1). Since poly I and fucoidan may not exert all their effects through the scavenger receptor, as has been reported with another scavenger receptor ligand, maleylated-BSA,[14] poly C, a structurally analogous compound to poly I, which has been reported not to bind to the scavenger receptor,[7,9] was used as a negative control. Poly C had no effect on IL-1 production at the same concentrations (100 μg/ml) in which poly I induced IL-1β levels comparable to optimal LPS stimulation (Figs 1 and 5).

Poly I and fucoidan had a slight IL-1β-inducing effect on native THP-1 cells. Priming of THP-1 cells with PMA, which is known to induce scavenger receptor expression in this cell line,[9,11,28] and the subsequent stimulation with poly I or fucoidan greatly enhanced IL-1β production (Table 1).

Since monocytes are very sensitive to trace amounts of LPS,[17] the possibility of LPS contamination in the reagents was considered. The use of poly C as a negative control, as well as the levels of IL-1 produced after poly I stimulation, which are comparable to optimal LPS stimulation, seems to exclude significant LPS contamination in the polynucleotides used. Also, the inability of polymyxin B, an antibiotic capable of inhibiting LPS-mediated effects,[29] to abolish poly I- and fucoidan-induced IL-1 production, while it completely abolished LPS-mediated IL-1 production (Fig. 2), speaks against LPS contamination in the reagents used.

To further characterize scavenger receptor-mediated IL-1 production, the levels of intracellular and secreted IL-1 were determined. The concentration-dependent increase in IL-1 production by poly I reflects an increase in secreted rather than cellular IL-1 (Fig. 4). Secreted IL-1 was also shown to be biologically active (Fig. 5), suggesting that the extracellular IL-1β was of the 17,000 MW form, known to be biologically active,[30] and not the biologically inactive 31,000 MW precursor. It cannot be excluded that poly I- and fucoidan-mediated IL-1 induction would reflect their intracellular activation mechanism, and scavenger receptor would only mediate their delivery into the cells. However, with LPS it has previously been reported that the interaction of the LPS molecule with cell membrane is

required for IL-1 release.[31] The ability of poly I to mediate IL-1 secretion seems to indicate a signal-transducing role for the scavenger receptor in this process.

Since it has been reported previously that different stimulators can induce IL-1β gene expression via distinct pathways,[32] the signalling mechanisms of scavenger receptor-mediated IL-1 production were studied. The kinetics of poly I-induced IL-1β gene expression was determined first. Poly I-induced IL-1α and IL-1β mRNA levels peaked at 3–6 hr and decreased after 24 hr.

To further characterize scavenger receptor-mediated signal transduction pathways, the effect of a protein kinase C inhibitor, H7, on poly I-mediated IL-1 production was determined. H7 concentration-dependently abolished poly I-mediated IL-1β production (Fig. 6), suggesting a PKC-mediated signal transduction mechanism in scavenger receptor stimulation. The interaction of poly I and PMA, a direct PKC activator, had a synergistic effect on IL-1β production (Table 2). It has previously been reported that cyclic AMP has an enhancing effect on PMA-mediated IL-1 induction in human monocytes and myeloid cell lines.[33,34] Poly I was shown to increase intracellular levels of cAMP (results not shown), and IBMX, an inhibitor of cAMP degradation, had an enhancing effect on poly I-mediated IL-1 production (Table 2). It would thus seem that a synergistic interaction of PKC- and cyclic AMP-mediated signal transduction pathways is operative in scavenger receptor-mediated IL-1 production.

Inflammatory mediators have been suggested to play a role in the pathogenesis of atherosclerosis. Expression of IL-1β, TGF-β1 and platelet derived growth factor-B (PDGF-B) has been demonstrated in advanced lesions of atherosclerosis in non-human primates.[35] IL-1 has various potentially atherogenic effects on the vessel wall.[21–24] Considering the central role of the scavenger receptor in foam cell formation, IL-1 production through ligand binding to the scavenger receptor could serve as a mediator in the pathogenesis of atherosclerosis. The role of modified lipoproteins, the putative physiological ligands of scavenger receptors *in vivo*, in monocyte activation remains controversial. As monocyte activation[36] and lipid accumulation[5] seem to be involved in foam cell formation, it seems possible that modified lipoproteins would play an activating role at the initiation of foam cell formation. Conflicting data on the activating and suppressing roles of modified lipoproteins exist. Oxidized LDL has been reported to exert toxic effect on cells cultured *in vitro*[18] and to suppress tumour necrosis factor-alpha (TNF-α) expression in activated murine peritoneal macrophages in a non-scavenger receptor-mediated fashion.[15] Malondialdehyde-LDL, another ligand for the scavenger receptor, was reported not to induce inflammatory gene expression in murine peritoneal macrophages.[37] However, oxidized LDL has been reported to stimulate chemotaxis[38] and production of arachidonic acid metabolites in monocytes,[39] and recently acetylated LDL was reported to induce TNF expression in human monocytes.[16] Our own experience in the use of lipoproteins in cell culture has also been highly controversial. When testing the effect of modified lipoproteins on IL-1 production, conflicting results were obtained with different lots of modified lipoproteins. This could reflect differences in the degree of modification of the lipoproteins. In contrast to the difficulties in using modified lipoproteins, the ligands used in this work provide a highly reproducible model of studying scavenger receptor-mediated functions *in vitro*. Whether these results may be extended to reflect the physiological versus pathological functions of this receptor *in vivo* remains to be elucidated.

ACKNOWLEDGMENTS

This work was supported by grants from The Finnish Cancer Society, Finnish Cultural Foundation, Paulo Foundation and Orion Corporation Research Foundation. The author would like to thank Dr Mikko Hurme for reviewing the manuscript, Drs Christian Ehnholm and Risto Renkonen for valuable discussions, and Ms Anita Saarinen for technical assistance.

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