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The Tryptophan Catabolite Picolinic Acid Selectively Induces the Chemokines Macrophage Inflammatory Protein-1α and -1β in Macrophages

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We previously found that the tryptophan catabolite picolinic acid (PA) is a costimulus for the activation of macrophage effector functions. In this study, we have investigated the ability of PA to modulate the expression of chemokines in macrophages. We demonstrate that PA is a potent activator of the inflammatory chemokines MIP (macrophage inflammatory protein)-1α and MIP-1β (MIPs) mRNA expression in mouse macrophages in a dose- and time-dependent fashion and through a de novo protein synthesis-dependent process. The induction by PA occurred within 3 h of treatment and reached a peak in 12 h. The stimulatory effects of PA were selective for MIPs because other chemokines, including monocyte chemoattractant protein-1, RANTES, IFN-γ-inducible protein-10, MIP-2, and macrophage-derived chemokine, were not induced under the same experimental conditions and were not an epiphenomenon of macrophage activation because IFN-γ did not affect MIPs expression. Induction of both MIP-1α and MIP-1β by PA was associated with transcriptional activation and mRNA stabilization, suggesting a dual molecular mechanism of control. Iron chelation could be involved in MIPs induction by PA because iron sulfate inhibited the process and the iron-chelating agent, desferrioxamine, induced MIPs expression. We propose the existence of a new pathway leading to inflammation initiated by tryptophan catabolism that can communicate with the immune system through the production of PA, followed by secretion of chemokines by macrophages. These results establish the importance of PA as an activator of macrophage proinflammatory functions, providing the first evidence that this molecule can be biologically active without the need for a costimulatory agent. The Journal of Immunology, 2000, 164: 3283–3291.

The recruitment of inflammatory cell populations to sites of injury or infection is driven by the local secretion of appropriate chemotactic signals (1). In the past few years, a rapidly expanding group of small chemotactic proteins, referred to as the chemokine superfamily, has been identified and characterized (2). The chemokine superfamily has been subdivided into four distinct classes, which differ with respect to the number and arrangement of the conserved cysteine residues at the N terminus of the primary amino acid sequence (CXC or α, CC or β, C or γ, and CX3C or δ) and are encoded by different sets of genes clustered on separate chromosomes (2–4). Chemokines are produced by both immune and nonimmune cells in response to inflammatory stimuli or tissue damage (5–11) and are involved in an impressive array of immunoregulatory and inflammatory functions, including leukocyte migration and activation (12–14), myelopoiesis, and neangiogenesis (3, 4).

A primary source for chemokines is activated mononuclear phagocytes, which are important mediators of cellular immunity against infections and tumors (15, 16), acting directly through the release of effector molecules (15) or indirectly by recruiting T lymphocytes and NK cells to target tissues through the release of chemokines (10, 17). The extent and magnitude of a local macrophage response are regulated by an interplay of stimulatory and inhibitor signals of various nature that include cytokines and metabolites produced by surrounding cells (16), microbial products present in an inflammatory environment (10, 18), or tissue-selective factors, such as changes in O2 tension and pH (19, 20). However, the contribution of stimuli not directly derived from the immune system to the induction of chemokines has not been fully elucidated.

Recent studies have suggested a role for amino acid catabolites as important signals for mononuclear phagocyte physiology. The metabolism of two essential amino acids, L-tryptophan (L-TRP) and L-arginine, for instance, has been associated with the tumoricidal and microbicidal activities of murine macrophages (21). Enhanced breakdown of L-TRP has been demonstrated in inflammatory reactions and implicated in antimicrobial activity, T cell tolerance, and in some of the biological effects of IFN-γ (22–25), and increased expression of indoleamine 2,3-dioxygenase (IDO), the inducible enzyme controlling L-TRP catabolic pathway in extrahepatic tissues (26), was detected in inflammatory lesions and placenta (21, 23, 24, 27). L-TRP catabolism, initiated either by IDO or epatic enzymes, leads to the production of metabolites, some of which are biologically active molecules (21). Among

3 Abbreviations used in this paper: L-TRP, L-tryptophan; ActD, actinomycin D; CHX, cycloheximide; DFX, desferrioxamine; IDO, indoleamine 2,3-dioxygenase; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; PA, picolinic acid; RPA, RNase protection assay.

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them, PA, an end-product of L-TRP degradation (28) detected in human milk, pancreatic juice, and intestine (29, 30), is endowed with important immunomodulatory properties involving activation of mononuclear phagocyte effector functions (31). This molecule is a potent stimulus for induction of macrophage-mediated cytotoxicity, able to inhibit tumor growth in tumor-bearing mice through the stimulation of macrophage tumoricidal activity (32, 33). PA may also contribute to the microbialid activity of macrophages in vivo. Intrapertoneal and intracerebral administration of this metabolite protects mice against a lethal intracerebral challenge with the opportunistic pathogen Candida albicans (34). Moreover, PA, in combination with IFN-γ, inhibits retrovirus expression in macrophages, both in vitro (35) and in vivo (31), and triggers the transcriptional activation of the inducible isofrom of the NO synthase gene, stimulating production of NO (36), a major effector molecule implicated in the expression of macrophage tumoricidal and microbialid activities (37).

Despite the growing body of evidence demonstrating the importance of PA for the activation of macrophage lytic activities, there is currently little information on its effects on mononuclear phagocyte proinflammatory functions. Because increased catabolism of tryptophan appears to be correlated to the inflammatory response, the present study was designed to determine whether PA could trigger macrophage expression of chemokines and thus contribute to the onset of inflammation. To address this issue, we analyzed the expression pattern of several α- and β-chemokine mRNA in both mouse peritoneal macrophages and in the murine macrophage cell line ANA-1 in response to PA stimulation. The data presented in this study demonstrate for the first time that PA alone can potently and selectively induce the coordinated expression of the CC-chemokines macrophage inflammatory protein-1α (MIP-1α) and MIP-1β (MIPs) mRNA in murine macrophages.

Materials and Methods

Cells and culture conditions

The mouse macrophage cell line ANA-1 was established by infecting bone marrow-derived cells from C57BL/6 mice with the J2 recombinant retrovirus, carrying the v-raf/v-myc oncogenes (38), and was shown to display the phenotypic and functional features and the morphology of well-differentiated macrophages (36, 38). ANA-1 macrophages were cultured in DMEM (ICN Biomedicals, Aurora, OH) supplemented with 10% heat-inactivated FCS (HyClone Laboratories, Logan, UT), 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma, St. Louis, MO). Peritoneal macrophages were obtained from C57BL/6 mice injected i.p. with 1 ml of 3% thioglycolate broth (Sigma, St. Louis, MO). After 4 days, the peritoneal exudate cells were collected by lavage of the peritoneal cavity with 10 ml of sterile PBS (ICN Biomedicals). Cells were washed, resuspended, and plated in RPMI 1640 (ICN Biomedicals) supplemented with 10% heat-inactivated FCS (HyClone Laboratories, Logan, UT), 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma, St. Louis, MO). After 4 days, the peritoneal exudate cells were collected by lavage of the peritoneal cavity with 10 ml of sterile PBS (ICN Biomedicals). Cells were washed, resuspended, and plated in RPMI 1640 (ICN Biomedicals) supplemented as described above. Macrophages were isolated by adherence to tissue culture dishes, and their purity was about 94%, as assessed by morphology as described above. Special care was taken to ensure LPS-free conditions in all the experiments.

Reagents

Mouse IFN-γ (sp. act. ≥ 10^7 U/mg) was purchased from Life Technologies (Gaithersburg, MD). LPS (from Escherichia coli serotype 011:B4) was purchased from Sigma. PA, desferrioxamine, and ferrous sulfate were from Sigma. During the course of experiments, several batches of PA were used, and all of them gave consistent and reproducible results. PA was dissolved in PBS, and the pH was adjusted to 7.4. The stock solution was then passed through a 0.2-μm filter, aliquoted, and stored at −20°C. Actinomycin D (ActD; Calbiochem-Novabiochem, La Jolla, CA) was dissolved in ethanol at 1 mg/ml and used at a final concentration of 5 μg/ml for the times specified in the text. Cycloheximide (Sigma) was used at 7.5 μg/ml final concentration. The content of endotoxin, as determined by assay with a chromogenic Limulus amebocyte lysate test (QCL-1000; BioWhittaker, Walkersville, MD), was below the detection limit of 6 pg/ml in all of the reagents used.

RNase protection assay (RPA)

Total RNA was extracted from ANA-1 macrophages using the Trizol isolation reagent (Life Technologies) and subjected to RPA analysis using the RiboQuant MultiProbe RPA System from PharMingen (San Diego, CA), according to the manufacturer’s instruction. Briefly, a 32P-labeled antisense RNA probe set specific for different mouse α/β-chemokines (Mouse Chemokine Template Set, mCK-5) was hybridized in excess to 10 μg of total RNA from each sample in solution, after which free probe and other ss-RNA were digested with RNases. The remaining RNase-protected probes, annealed to homologous sequences in the sample RNA, were purified by ethanol precipitation and resolved on denaturing PAGE. Following separation by PAGE, protected 32P-labeled probe fragments were visualized by film autoradiography (Kodak XAR-5 films; Eastman Kodak, Rochester, NY): the presence of the target mRNA in the sample was revealed by the appearance of an appropriately sized fragment of the probe. For quantification, autoradiographs were scanned using a light densitometer (PhosphorImager; Molecular Dynamics, Sunnyvale, CA).
**Northern blot analysis**

Total cellular RNA was purified from ANA-1 macrophages and from thioglycolate-elicited peritoneal macrophages using the Trizol RNA reagent (Life Technologies), according to the manufacturer’s instructions. A total of 20 μg of RNA from each sample was electrophoresed under denaturing conditions on a 1.2% agarose gel containing 2.2 M formaldehyde, transferred onto Nytran membranes (Schleicher & Schuell, Keene, NH), and cross-linked by UV irradiation. Filters were hybridized with 32P-labeled probes and autoradiographed, as previously described (36). Different times of exposure were used to obtain comparable levels of band intensities with different probes. For MIP-1α and MIP-1β detection, the mouse MIP-1α and MIP-1β full-length cDNAs from the pBR322 vector, kindly provided by Dr. Antonio Sica (Istituto Mario Negri, Milan, Italy), were used. For MCP-1 detection, the full-length JE cDNA from the pUC19 vector, obtained from Dr. Antonio Sica, was used. The pBluescript II SK+ vector containing the mouse MDC cDNA was gently provided by Dr. Silvano Sozzani (Istituto di Ricerche Farmacologiche “Mario Negri,” Milan, Italy). The pEMBL-8 vector containing the β-actin cDNA was kindly provided by Dr. Cecilia Garre (Istituto di Biologia e Genetica, Facoltà di Medicina e Chirurgia, Università di Genova, Italy).

**Nuclear run-on experiments**

Nuclear run-on experiments were performed, as previously described (36). Briefly, nuclei were isolated from 10 × 10⁶ cells/sample by cell lysis and collected by centrifugation. In vitro RNA elongation was performed by adding 2× transcription buffer and 100 μCi of 800 Ci/mmol [α-32P]uridine triphosphate (NEL, Boston, MA) to the nuclei suspension, incubating at the mixture at 29°C for 30 min, and for additional 10 min at 30°C after addition of CaCl₂ and RNA-free DNase I (Promega, Gaithersburg, MD). Nuclei were lysed with 1 ml Trizol, and total RNA was isolated according to the manufacturer’s procedure. Equal amounts of labeled elongated transcripts were added in Hybrisol solution to Nytran membranes, on which denatured pBR322 plasmid containing the full-length MIP-1α cDNA or the full-length MIP-1β cDNA, denatured pEMBL plasmid containing the full-length β-actin, and EcoRI-linearized pBR322 were immobilized using a dot-blot apparatus (Bio-Rad Laboratories, Hercules, CA). Hybridization was performed at 42°C for 48 h, and filters were then washed as described for Northern analysis. When needed, the autoradiographs were scanned using a light densitometer.

**Results**

PA is a potent and selective inducer of MIP-1α and MIP-1β mRNA expression in mouse macrophages

Initial experiments were designed to study the effects of PA in regulating the expression pattern of several α- and β-chemokine mRNA in the murine macrophage cell line ANA-1. Cells were cultured for 18 h in medium alone, supplemented with PA, or LPS as a positive control (2, 10). MultiProbe RPA was then performed on total RNA preparations to analyze the expression of distinct chemokine transcripts (Fig. 1). We found little or no constitutive expression of the mRNA for the β-chemokines monocyte chemotactic protein (MCP-1/JE), MIP-1α, MIP-1β, and RANTES, and the α-chemokines IFN-γ-inducible protein-10 and macrophage inflammatory protein-2 (MIP-2). PA caused a significant up-regulation of MIP-1α and MIP-1β (MIPs) mRNA without affecting the levels of the other chemokine mRNAs, providing the first suggestion that PA alone can induce gene expression in macrophages and differentially modulate the expression of different chemokine mRNA. As previously reported (2, 10), LPS strongly induced the expression of the message for all of the chemokines tested.

To strengthen these conclusions, Northern blot analysis was performed on total RNA isolated from ANA-1 macrophages stimulated for 18 h with medium alone or containing 4 mM PA. As shown in Fig. 2A, low constitutive levels of MIPs mRNA were detectable, although MIP-1β seemed less expressed than MIP-1α because longer blot exposure was required to obtain bands of comparable intensity. PA treatment caused a major enhancement of MIP-1α and MIP-1β mRNAs. MIPs mRNA induction, ranging from 8- to 15-fold, was consistently observed in five independent experiments performed, although slight fluctuations in the baseline levels were detected. Under the same conditions, PA did not affect the constitutive expression of MCP-1 mRNA (Fig. 2A). To determine whether this response was a general consequence of macrophage activation, the effects of other macrophage activators, IFN-γ (100 IU/ml) or LPS (10 ng/ml), on MIPs mRNA levels were also investigated. IFN-γ failed to increase MIPs expression, while potently inducing MCP-1 mRNA accumulation, whereas LPS greatly up-regulated the expression of all three chemokines tested (Fig. 2A).

The selective stimulatory activity of PA on MIPs was further confirmed by the demonstration that PA did not affect the low constitutive mRNA expression of the murine macrophage-derived

![FIGURE 2](http://www.jimmunol.org/) PA specifically up-regulates the constitutive expression of MIP-1α and MIP-1β in mouse macrophages. A, Total RNA was isolated from ANA-1 cells treated for 18 h with 4 mM PA, 100 IU/ml IFN-γ, or 10 ng/ml LPS, and analyzed by Northern blotting. B, Total RNA from ANA-1 cells treated for 18 h with 4 mM PA, or 10 ng/ml LPS was analyzed for MDC expression. C, Thioglycolate-elicited mouse peritoneal macrophages were stimulated for 18 h with medium alone or supplemented with 4 mM PA, and Northern blot analysis was performed on total RNA. The blots were sequentially hybridized with the cDNAs for the indicated chemokines. β-Actin levels were determined to ensure that comparable amounts of RNA were loaded in each lane. Blots hybridized with MIP-1α and MIP-1β cDNAs were exposed for 4 and 24 h, respectively, to obtain comparable band intensities.
chemokine, MDC, a CC chemokine typically produced by mature macrophages (39, 40), that was up-regulated by LPS (Fig. 2B).

Thioglycolate-elicited peritoneal exudate macrophages from C57BL/6 mice were analyzed to extend the results obtained with ANA-1 cells to primary macrophage cultures. Control macrophages expressed low constitutive levels of MIPs mRNA (Fig. 2C). Stimulation with PA increased MIP-1α and MIP-1β mRNA accumulation, although to a lesser extent than in ANA-1 cells. A similar pattern of results was consistently observed in three independent experiments, with the induction ranging from 4- to 8-fold over control.

The kinetics of MIP-1α and MIP-1β mRNA induction by PA is shown in Fig. 3A. MIP-1α and MIP-1β transcripts were detectable as early as 3 and 6 h after treatment, respectively, reached plateau levels at 12–24 h, and declined thereafter.

To determine the dose dependence of PA effects, MIP-1α and MIP-1β mRNA expression was assessed after stimulation for 12 h with increasing amounts of PA (Fig. 3B). Plateau levels of expression were obtained with 4 mM PA, a dose previously shown to be optimal for mouse macrophage activation (36). At the concentrations used, PA did not affect cell viability, as determined by the trypan blue dye exclusion test (data not shown).

These results provide the first evidence that PA is a potent and selective inducer of MIP-1α and MIP-1β mRNA expression in murine macrophages.

PA augments the transcriptional activity of MIP-1α and MIP-1β genes and enhances the mRNA stability of both chemokines

Run-on experiments were conducted on nuclei isolated from ANA-1 cells stimulated for 4 h (Fig. 4A) or 6 h (Fig. 4B) with medium alone, PA, or LPS, to study the mechanism responsible for MIP-1α and MIP-1β mRNA induction. MIP-1α gene was transcriptionally active in medium-treated cells, and susceptible to augmentation in response to LPS. A 2.6-fold increase in the rate of MIP-1α gene transcription was observed in cells stimulated with PA for 4 h (A) or 6 h (B). In contrast, constitutive transcription of
MIP-1\(\beta\) gene was not detected, and induction of transcription was not observed after 4 h (A), but only after 6 h (B) of treatment with PA. In conclusion, the up-regulation of MIPs mRNA expression by PA was associated with the transcriptional activation of the genes, although the kinetics of MIP-1\(\beta\) induction was delayed compared with that of MIP-1\(\alpha\).

To measure the stability of MIP-1\(\alpha/\beta\) mRNA induced by PA, ANA-1 cells were incubated in the presence or absence of PA for 12 h, and mRNA expression was tested immediately or after addition of 5 \(\mu\)g/ml of ActD for the indicated lengths of time to block further RNA transcription. As indicated in Fig. 5, MIPs mRNAs decayed with different kinetics in untreated and PA-treated cells. The levels of MIP-1\(\alpha\) and MIP-1\(\beta\) transcripts in unstimulated macrophages decreased by 50% (\(t_{1/2}\)) after 2.5 and 1.2 h, respectively. PA-treated cells displayed a greater MIPs mRNA stability. The \(t_{1/2}\) of MIP-1\(\alpha\) mRNA was increased to more than 8 h, whereas that of MIP-1\(\beta\) was increased to 2 h by PA. In addition, 30% of MIP-1\(\beta\) mRNA was still detectable after 8 h of exposure to ActD in PA-treated cells, whereas in control cells it became undetectable after 4 h of exposure to the drug. We concluded that PA stabilized MIP-1\(\alpha\) and to a lesser extent MIP-1\(\beta\) mRNAs.

To determine whether de novo protein synthesis was required for PA-induced expression of MIPs mRNA, ANA-1 cells were treated for 12 h with PA in the presence or absence of 7.5 \(\mu\)g/ml of the protein synthesis inhibitor cycloheximide (CHX). Under these experimental conditions, CHX inhibited protein synthesis by more than 90% (data not shown). The addition of CHX to PA-treated cells completely prevented the up-regulation of both MIP-1\(\alpha\) and MIP-1\(\beta\) mRNA expression (Fig. 6). Similar results were observed in three independent experiments.

Taken together, these results indicate that PA-dependent up-regulation of MIP-1\(\alpha\) and MIP-1\(\beta\) mRNA in ANA-1 macrophages is controlled through a dual molecular mechanism involving both the transcriptional activation of the genes and the posttranscriptional stabilization of the messages, and that it requires active protein synthesis.

Iron chelation is involved in PA-dependent induction of MIPs gene expression

It has been previously reported that the biological effects of PA involved chelation of iron (41, 42). To determine the requirement for iron chelation in PA-dependent induction of MIPs expression, ANA-1 cells were stimulated for 12 h with PA, alone or in the presence of increasing concentrations of FeSO\(_4\), and the expression of MIP-1\(\alpha\) and MIP-1\(\beta\) mRNA was analyzed. One representative experiment of three performed is depicted in Fig. 7A. The addition of FeSO\(_4\) caused a major and dose-dependent suppression of MIPs mRNAs in PA-stimulated macrophages, detectable at 100 \(\mu\)M and maximal at 300 \(\mu\)M. FeSO\(_4\) alone did not have any effect on the expression of MIPs mRNA at any of the concentrations tested (Fig. 7A). We conclude that chelation of iron was part of the mechanism of action of PA.

To further investigate the role of iron chelation in MIPs induction, we studied the effects of a synthetic iron-chelating drug, desferrioxamine (DFX), on MIP-1\(\alpha\) and MIP-1\(\beta\) expression. ANA-1 macrophages were treated with DFX, and the expression of MIPs mRNA was then tested (Fig. 7B). DFX selectively increased MIP-1\(\alpha\) and MIP-1\(\beta\), but not MCP-1, mRNA expression (10-
15-fold above the baseline), and addition of FeSO₄ caused a reduction in DFX-mediated MIPs up-regulation greater than 90%.

These results show that DFX is a stimulus for the induction of MIP-1α and MIP-1β in macrophages and that FeSO₄ inhibits the activating properties of PA and DFX, indicating that iron chelation is involved in the regulation of MIPs expression in macrophages.

**Discussion**

The central role of macrophage-derived chemokines in the regulation of host immune and inflammatory responses is well established (2–4, 10, 14). However, the network of stimuli that control their production has not been fully characterized, and it extends beyond the classical signals derived from the immune system. In an attempt to identify alternative physiopathological stimuli regulating chemokine expression, we have investigated the response of mouse macrophages to PA, a l-TRP catabolite with macrophage costimulatory properties (21, 31, 33–35, 43). We found that PA is a potent and selective inducer of MIP-1α and MIP-1β mRNA in murine macrophages.

The murine macrophage cell line ANA-1 expressed low constitutive levels of MIP-1α and MIP-1β mRNA, and PA caused a major up-regulation of their expression. MIP-1α increased earlier than MIP-1β, but both chemokines reached plateau levels 12 h after PA stimulation. Slight fluctuations in the baseline levels of MIPs mRNA were observed, probably due to cell adherence to plastic (16). However, the constitutive levels of MIP-1β were lower than those of MIP-1α, even if PA induced a comparable up-regulation of the two transcripts. Similar results were observed using fresh murine peritoneal exudate macrophages, excluding an idiosyncratic behavior of the ANA-1 cell line. Interestingly, PA stimulatory activity was selective for MIP-1α and MIP-1β because it was not exerted on several other β- and α-chemokines, including MCP-1, RANTES, IFN-γ-inducible protein-10, MIP-2, and MDC. Furthermore, the augmentation of MIPs mRNA expression by PA was not a general response to macrophage stimulation. In fact, IFN-γ, a potent activator of mononuclear phagocyte functions (16, 36), did not increase MIP-1α or MIP-1β mRNA, despite its ability to induce MCP-1 expression. We can rule out the possibility that endotoxin contamination contributed to the activation of MIPs expression, because PA preparations did not contain any detectable levels of LPS, and addition of polymyxin B sulfate, which binds to and neutralizes LPS (44), did not reduce PA stimulatory activity. 
We found that MIP-1 expression in macrophages has been previously reported (3, 19, 48). The consensus sequence in their 3’ untranslated region (3, 49), present in the 3’ UTR of many cytokine genes, and controlling mRNA stability and translation (50–52). Posttranscriptional regulation of MIP-1α and MIP-1β mRNA expression in macrophages has been previously shown following stimulation by bacterial endotoxin (49), in response to oxidative stress (19) or IL-10 (47).

PA-dependent increase of MIPs mRNA was abolished by addition of protein-synthesis inhibitors, demonstrating a requirement for active protein synthesis and suggesting that MIPs expression may be controlled by a de novo synthesized transcriptional enhancer(s) and/or by a factor(s) with a short t1/2 involved in the regulation of mRNA stability.

The mechanism of action of PA is not known, although it has been reported that this agent interferes with several biochemical pathways (31). PA binds iron and interferes with iron uptake (31, 41), and iron chelation may contribute to PA activities (41, 42). In this study, we show that iron sulfate suppressed PA-induced MIP-1α and MIP-1β mRNA up-regulation, suggesting a role for iron chelation in the induction of MIPs. This conclusion is supported by the demonstration that DFX, a synthetic iron chelator (53, 54), is similar to PA in selectively inducing MIP-1α and MIP-1β mRNA, and that this effect is abrogated by iron sulfate. Induction of MIP-1α and MIP-1β by DFX may have practical clinical implications because this drug is used for the treatment of several pathological conditions, including iron overload (54), cancer (55), and Alzheimer’s disease (53), and, interestingly, several lines of evidence indicate that chemokines might contribute to the pathogenesis of these diseases (3).

In conclusion, the results presented in this study identify a novel connection between L-TRP degradation, its end-product PA, and the expression of MIP-1α and MIP-1β chemokines in macrophages, extending previous findings on the important role of L-TRP catabolism in regulating host immune mechanisms and inflammatory processes (21, 22, 25, 27, 56, 57). Expression and activation of IDO, a key enzyme in the degradation of L-TRP along the kynurenine pathway (26), in placenta and human macrophages have been associated with inhibition of T cell immune responses (24, 58). To which extent IDO activation leads to PA production and whether such pathway is relevant in mouse macrophages are currently being investigated. A recent report demonstrating that PA significantly decreases IDO expression and activity in mouse macrophages (59) suggests a role for this catabolite as an inhibitor of IDO-mediated T cell immunosuppression in local tissue microenvironment, and is consistent with our results showing the importance of PA in the activation of macrophage proinflammatory functions.

The levels of PA detected in vivo in biological fluids varies from 3 (30) to 300 μM (29), and we have measured micromolar concentrations of PA (from 10 to 80 μM) in the serum of patients with chronic liver diseases (Dazzi, et al., submitted manuscript), although the concentrations of PA in tissues are still unknown. The stimulatory effects of PA in vitro require concentrations of about 2–4 mM (36 and this study), and our unpublished observations indicate that millimolar concentrations of PA are necessary in vitro to achieve detectable intracellular quantities of PA in ANA-1 macrophages (detection limit 100 pM). Levels of PA higher than those detected in human serum may be achieved in vivo in the intercellular space or could be locally produced during inflammatory responses, as suggested by elevation of L-TRP catabolites in localized compartments under pathologic conditions (60). Moreover, it is possible that the in vivo environment may alter the accessibility of PA to the cell, decreasing the biologically active concentration. In fact, the association of PA with serum proteins and/or with divalent cations (41) may modify the permeability of the compound. Finally, the possibility exists that PA may act endogenously in the producing cells and that its biologic activity may not correlate with the circulating levels.

MIP-1α and MIP-1β are important mediators of the inflammatory reactions (3, 12, 13) and potent T cell chemoattractants, able to regulate T cell trafficking during an immune response in vivo (3, 4, 14, 61). The modulation of MIPs expression is a crucial set point for the control of the kinetics and composition of the cellular infiltrate in target tissues (3, 17). The demonstration that PA can induce MIP-1α and MIP-1β expression indicates that this molecule can regulate the inflammatory response at multiple levels. In a simplistic view, PA alone elicits MIPs chemokine expression in macrophages and, consequently, T cell recruitment, and, in conjunction with T cell products such as IFN-γ, triggers the fully activated phenotype.

It was recently demonstrated that MIP-1α and MIP-1β can inhibit HIV-1 infection by binding to their receptor CCR5, which functions as a cell membrane fusion cofactor with CD4 for the virus gp120 envelope protein, and, thus, by blocking HIV-1 entry into the cells (62, 63). Schmidtmayrova et al. (64) showed elevated levels of MIP-1α/β expression in HIV-1-infected monocytes and in microglia and astrocytes from the brain tissue of patients with HIV encephalitis. On the other hand, decreased levels of circulating L-TRP and accumulation of L-TRP catabolites, such as kynurenine and quinolinic acid, have been detected in the cerebrospinal fluid of patients infected with HIV-1, particularly in those with neurologic deficits and AIDS dementia complex (25, 65). These results raise questions on the role of L-TRP catabolites in AIDS, particularly of PA that, by up-regulating MIPs expression, could exert protective effects on the host. Evaluation of PA levels in the fluids of AIDS patients is currently underway.

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* C. Dazzi, G. Candiotti, A. Ponzaletto, and L. Varesio. A new HPLC method for the detection of picolinic acid, a catabolite of L-tryptophan, in biological fluids. Submitted for publication.


