Poly(ADP-ribose) Polymerase is a Regulator of Chemokine Production: Relevance for the Pathogenesis of Shock and Inflammation

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Abstract

Background: Chemokines are key regulators of leukocyte traffic in various forms of inflammation and reperfusion injury. There is emerging evidence that the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) importantly contributes to the up-regulation of a variety of proinflammatory signal transduction pathways and associated genes.

Materials and Methods: We tested whether the expression of the chemokines macrophage inflammatory protein (MIP)-1α and MIP-2 are under the control of PARP during inflammation.

Results: Pharmacologic inhibition of PARP and genetic deletion of PARP suppressed the expression of MIP-1α and MIP-2 protein and mRNA in immunostimulated cultured murine macrophages and fibroblasts. PARP inhibition also suppressed the activation of NF-κB, a key transcription factor known to be involved in the generation of chemokines in immunostimulated cells. In vivo, in various models of local and systemic inflammation, including dextran sodium sulfate–induced colitis and endotoxic shock, pharmacologic inhibition of PARP suppressed the expression of MIP-1α and MIP-2. These effects were associated with a marked suppression of the inflammatory response, including an attenuation of neutrophil infiltration into inflamed organs.

Conclusions: A combination approach of pharmacologic inhibition and genetic deletion revealed that the major isoform of PARP (PARP-1) plays a predominant, but not exclusive, role in the regulation of chemokine production in vivo. Suppression of chemokine expression may be a novel mode of anti-inflammatory action of PARP inhibition.

Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1), a monomeric nuclear enzyme present in eukaryotes, is the major isoform of an expanding family of poly(ADP-ribosyl)ating enzymes designated as PARPs 1–6 (1–3). PARP-1 primarily functions as a DNA damage sensor. Upon binding to damaged DNA, mainly through the second zinc finger domain, PARP-1 forms homodimers and catalyzes the cleavage of NAD^+ into nicotinamide and ADP-ribose and uses the latter to synthesize branched nucleic acid-like polymers poly(ADP-ribose) covalently attached to nuclear acceptor proteins. The biological role of PARP-1 is complex and includes the regulation of DNA repair and maintenance of genomic integrity (1–3).

PARP-1 has been implicated in a variety of pathophysiologic processes. PARP-1 overactivation—in response to severe oxidant-induced DNA damage—has been shown to promote cell dysfunction culminating in necrosis (4–6). PARP-1–mediated cell necrosis has been implicated as a principal form of cell and organ damage in various forms of reperfusion injury including stroke and myocardial infarction (7–10).

Multiple reports indicate the importance of PARP-1 in promoting cell recruitment and thereby inducing organ injury in various forms of inflammation (11–14). The molecular mechanism of this function is not fully understood. Because chemokines are principal regulators of mononuclear and polymorphonuclear cell trafficking in various forms of inflammation (15), we investigated whether PARP modulates chemokine production, and whether this activity of the enzyme may regulate the course of the inflammatory process.

Methods

Culture and Treatment of Cells

The mouse macrophage cell line J774.1 and pulmonary fibroblasts from wild-type and PARP-1–deficient mice (14) were grown in RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2. Cells were cultured in 96-well plates (200 μl medium per well) until 80% confluence. For chemokine assays, the cells were pretreated with the PARP inhibitor PJ-34 (1–30 μM) for 30 min before stimulation with bacterial lipopolysaccharide (LPS, 10 μg/ml). Supernatants for chemokine determination were obtained 24 hr after stimulation with LPS. Levels of MIP-1α and MIP-2 were measured using specific murine

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ELISA kits. Plates were read at 450 nm by a Spectramax 250 microplate reader from Molecular Devices (Sunnyvale, CA, USA). Cell viability was monitored using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method as described (4) and was unaffected by any of the treatments.

Western Blot Analysis

J774 cells in 6-well plates were pretreated with PJ-34 (30 μM) or vehicle (medium) and 30 min later the cells were stimulated with LPS (10 μg/ml) for 15 min. After washing with PBS, the cells were lysed by the addition of modified radioimmunoprecipitation (RIPA) buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.25% Na-deoxycholate, 1% NP-40, 1 μg/ml pepstatin, 1 μg/ml leupeptin, 1 mM PMSF, 1 mM Na_3VO_4). The lysates were transferred to Eppendorf tubes, centrifuged at 15,000 $\times$g and the supernatant was recovered. Protein concentrations were determined using a Bio-Rad Protein Assay (Bio-Rad, Hercules, CA, USA). Ten milligrams of sample was separated on an 8–16% Tris-Glycine gel (Novex, San Diego, CA, USA) and transferred to a nitrocellulose membrane. The membranes were probed with antiphospho–mitogen-activated protein kinase (MAPK; p42/p44), antiphospho–*c*-Jun N-terminal protein kinase (JNK, Promega, Madison, WI, USA), anti–phospho-p38 MAPK (p38 MAPK, New England Biolabs, Beverly, MA, USA) and subsequently incubated with a secondary horseradish peroxidase-conjugated donkey anti-rabbit antibody (Boehringer, Indianapolis, IN, USA). Bands were detected using ECL Western Blotting Detection Reagent (Amersham Life Science, Arlington Heights, IL, USA) (16).

RNA Isolation and RT-PCR

J774 cells were pretreated with PJ-34 (30 μM) or its vehicle for 30 min, which was followed by a 3-hr exposure to LPS (10 μg/ml). At the end of the incubation period, total cellular RNA was extracted and chemokine mRNA levels were determined by semiquantitative RT-PCR. Total RNA was isolated from J774 cells using TRIzol Reagent (Life Technologies, Grand Island, NY, USA). Reverse transcription of the RNA was performed using MuLV reverse transcriptase from Perkin Elmer (50 U/μl, Foster City, CA, USA). RNA (5 μg) was transcribed in a 20-μl reaction containing 10.7 ml RNA, 2 μl 10$\times$ PCR buffer, 2 μl 10 mM dNTP mix, 2 μl 25 mM $MgCl_2$, 2 μl 100 mM DTT, 0.5 μl RNase inhibitor (Perkin Elmer, 20 U/μl), 0.5 μl 50 mM oligo d(T), and 0.3 ml reverse transcriptase. The reaction mix was incubated at 42°C for 15 min for reverse transcription. Thereafter, the reverse transcriptase was inactivated at 99°C for 5 min. RT-generated DNA (1–5 μl) was amplified using Expand High Fidelity PCR System (Boehringer Mannheim). The reaction buffer (25 μl) contained 1–5 μl cDNA, water, 2.5 μl PCR buffer, 1.5 μl 25 mM

$MgCl_2$, 1 μl 10 mM dNTP mix, 0.5 μl 10 mM oligonucleotide primer (each), and 0.2 μl enzyme. cDNA was amplified using the following primers and conditions: MIP-1α (17)-5-ATGAAGGTCTCCAC-CACTGCCCTTGC-3 (sense) and 5-TTAGTCAGG-AAAATGACACCTGGCTGGG-3 (antisense); MIP-2 (17)-5-ATGGCCCCTCCCACCTGCCGCTCC-3 (sense) and 5-TCAGTTAGCCTTGCCTTTGTTCAGTATC-3 (anti-sense), and β-actin (18)-5 -GAGACCTTCAA-CACCC-3 (sense) and 5-GTGGTGGTGAAGCTG-TAGCC-3 (anti-sense), an initial denaturation at 94°C $\times$ 5 min, 18, 23, and 24 cycles of 94°C $\times$ 30 sec for MIP-1α MIP-2, and β-actin, respectively, 58°C $\times$ 45 sec, 72°C $\times$ 45 sec; a final dwell at 72°C $\times$ 7 min. The expected PCR products were MIP-1α 200 bp, MIP-2, 302 bp, and β-actin, 230 bp. PCR products were resolved on a 1.5% agarose gel and stained with ethidium bromide (19).

NF-κB Electromobility Shift Assay

J774 cells were stimulated with LPS (10 μg/ml) for 45 min and nuclear protein extracts were prepared as described previously (20). To determine the effect of PARP inhibition, cells were pretreated with PJ-34 (30 μM) or its vehicle 30 min before stimulation. All nuclear extraction procedures were performed on ice with ice-cold reagents. Cells were washed twice with PBS and harvested by scraping into 1 ml of PBS and pelleted at 6000 rpm for 5 min. The pellet was resuspended in one packed cell volume of lysis buffer (10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1.5 mM $MgCl_2$, 0.2% v/v Nonidet P-40, 1 mM DTT, and 0.1 mM PMSF) and incubated for 5 min with occasional vortexing. After centrifugation at 6000 rpm, 1 cell pellet volume of extraction buffer (20 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1.5 mM $MgCl_2$, 25% v/v glycerol, 1 mM DTT, and 0.5 mM PMSF) was added to the nuclear pellet and incubated on ice for 15 min with occasional vortexing. Nuclear proteins were isolated by centrifugation at 14,000 $\times$g for 15 min. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad). Nuclear extracts were stored at −70°C until used for EMSA. The oligonucleotide probe used for the EMSA was purchased from Promega. Oligonucleotide probes were labeled with γ-[^{32}P] ATP using T4 polynucleotide kinase (Gibco, BRL) and purified in Bio Spin chromatography columns (BioRad). For the EMSA analysis, 10 μg of nuclear proteins were preincubated with EMSA buffer (12 mM HEPES pH 7.9, 4 mM Tris-HCl pH 7.9, 25 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 50 ng/ml poly[(d-I)(d-C)], 12% glycerol v/v, and 0.2 mM PMSF) on ice for 10 min before addition of the radiolabeled oligonucleotide for an additional 25 min. The specificities of the binding reactions were tested by incubating duplicate samples with 100-fold molar excess of the unlabeled oligonucleotide probe. Protein–nucleic acid complexes were resolved using a nondenaturing polyacrylamide gel consisting of 5%

acrylamide (29:1 ratio of acrylamide:bisacrylamide) and run in $0.5\times$ TBE (45 mM Tris-HCl , 45 mM boric aid, 1 mM EDTA) for 1 hr at constant current (30 mA). Gels were transferred to Whatman 3M paper, dried under vacuum at 80°C for 1 hr, and exposed to photographic film at −70°C with an intensifying screen.

Transient Transfection and Luciferase Activity

For transient transfections, 3×10^5 RAW 264.7 cells were seeded per well of a 24-well tissue culture dish 1 day prior to transient transfection. Cells were transfected with 10 μl/ml of Lipofectamine 2000 (Life Technologies, Rockville, MD) and 8 μg/ml of DNA containing a NF-κB luciferase promoter construct (Clontech) dissolved in RPMI 1640 medium without FBS. This pNF-κB-Luc vector contains four tandem copies of the NF-κB consensus sequence fused to a TATA-like promoter region from the Herpes simplex virus thymidine kinase promoter. Cells were incubated for 5 hr in 5% CO_2 at 37°C before the addition of 0.5 ml of regular medium with 10% FBS. Transfected cells were allowed to recover at 37°C for 20 hr. The cells were then pretreated with PJ-34 (30 μM) or its vehicle (10% DMSO) for 30 min, which was followed by stimulation with LPS (2 μg/ml) for 4 hr. Luciferase activity was measured by the Luciferase Reporter Assay System (Promega, Madison, WI) and normalized relative to μg of protein (21).

Endotoxic Shock Model

To induce endotoxic shock, wild-type and PARP-1 deficient mice (22) were injected with *E. coli* bacterial lipopolysaccharide (LPS) at a dose of 10 mg/kg IP. To inhibit PARP, animals were pretreated with PJ34 at a dose of 10 mg/kg IP 30 min prior to the injection of LPS. At 1, 3, and 6 hr after LPS injection, groups of mice were killed and plasma levels of chemokines were analyzed by ELISA (see above.)

Colitis Model

Colonic inflammation was induced by the administration of dextran sodium sulfate (DSS) in the drinking water (23). The animals were exposed to 5% DSS (molecular weight of 40–44 kDa) ad libitum. To inhibit PARP, mice were treated via oral gavage with PJ34 (10 mg/kg/day, administered in two divided doses) or vehicle (saline) starting on day 1 and continuing throughout the study. The parameters recorded included mortality, body weight, colon length, colon histology, myeloperoxidase (MPO) levels, and malon dialdehyde (MDA) levels as well as the measurement by ELISA of chemokine levels from colonic homogenates (24).

Materials

All materials, unless otherwise specified, were obtained from Sigma/Aldrich (St. Louis, MO, USA). The phenanthridinone derivative PARP inhibitor, PJ34—

the hydrochloride salt of N-(-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide—was synthesized as described (25). This compound is a potent, bioavailable inhibitor of PARP, which lacks antioxidant effects and thus is suitable for mechanistic investigations into the regulatory roles of PARP (25).

Data Analysis and Presentation

For the in vitro studies, all values in the figures and text are expressed as mean ± standard error of the mean of n observations, where n represents the number of wells studied (six to nine– wells from two to three independent experiments). Histologic scores are expressed as median values. Numerical data sets were examined by analysis of variance and Bonferroni's *t*-test. A *p*-value less than 0.05 was considered statistically significant.

Results

PARP Deficiency and Pharmacologic Inhibition of PARP Inhibit Chemokine Production in Immunostimulated Cells

Immunostimulation of wild-type fibroblasts and vehicle-treated J774 macrophages induced a significant production of MIP-1α and MIP-2 (Fig. 1). In the absence of functional PARP-1, the production of chemokines was abolished (Fig. 1a). Likewise, the PARP inhibitor PJ34 elicited a dose-dependent suppression of chemokine production (Fig. 1b). PARP regulates the production of chemokines on a transcriptional level, as PARP inhibition suppressed the production of MIP-1α and MIP-2 mRNA (Fig. 2). Because the expression of chemokines is known to be controlled by a variety of signal transduction
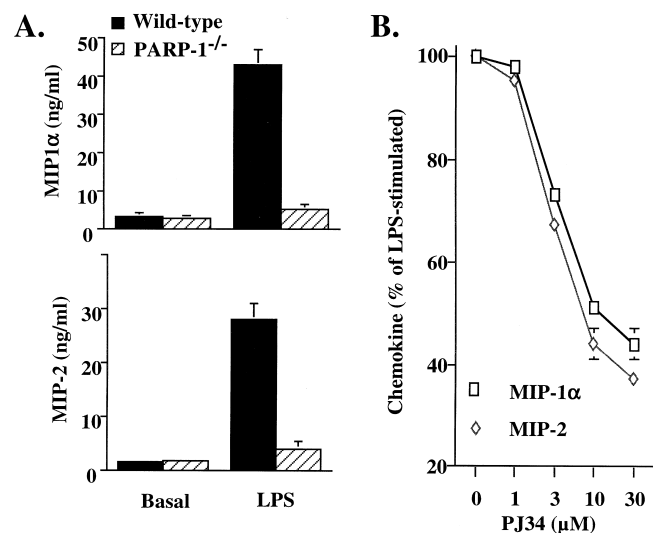

Fig. 1.   Inhibition of LPS-induced chemokine production by PARP-1 deficiency of by inhibition of the catalytic activity of PARP by PJ-34. (A) PARP-1–deficient fibroblasts fail to produce MIP-1α and MIP-2 in response to 10 μg/ml LPS stimulation; n = 6 per group. (B) Dose-dependent suppression by PJ34 of MIP-1α and MIP-2 production in J774 macrophages stimulated with LPS (10 μg/ml), n = 6 per group.
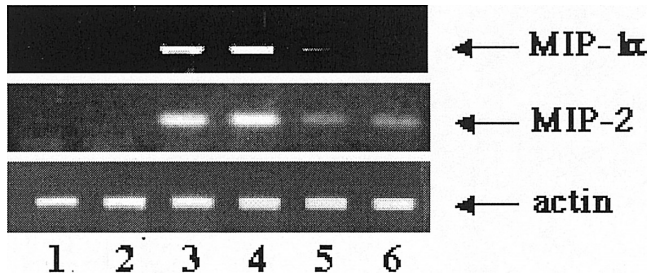
Fig. 2. PARP inhibition by PJ-34 pretreatment (30 μM) inhibits LPS-induced MIP-1α and MIP-2 mRNA accumulation in J774 cells. Lanes 1 and 2, no stimulation; lanes 3 and 4, 10 μg/ml LPS; lanes 5 and 6, PJ-34 pretreatment 30 min before LPS. β-Actin mRNA levels were unaffected by LPS or PJ-34 treatment. MIP-1α, MIP-2, and β-actin mRNA levels were quantitated using semiquantitative RT-PCR. This figure is representative of three separate experiments.

pathways, we tested whether PARP inhibition affects the activation of MAPK, JNK, and p38 MAPK. PARP inhibition did not affect the activation of any of these kinases (Fig. 3). However, PARP inhibition suppressed the activation of the transcription factor nuclear factor kappa B (NF-κB), as shown both by an EMSA (Fig. 4a) as well as by a transient transfection method utilizing a NF-κB luciferase promoter construct (Fig. 4b).

PARP Deficiency and Pharmacologic Inhibition of PARP Inhibit Chemokine Production in Response to Immunostimulation In Vivo

In wild-type mice, intraperitoneal injection of bacterial LPS to mice induced a marked elevation of MIP-1α and MIP-2 in the plasma. This response was markedly blunted in PARP-1–deficient mice (Fig. 5). At 1 hr post-LPS, pharmacologic inhibition of PARP with PJ34 elicited a more marked inhibitory effect on chemokine production than the genetic ablation of PARP-1. Furthermore, PJ34 treatment of
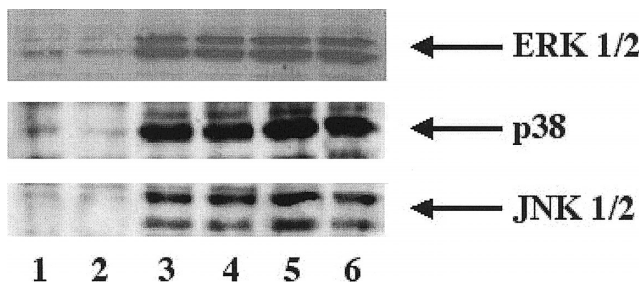

Fig. 3. PARP inhibition by PJ-34 (30 μM) fails to affect p42/44 MAPK (ERK1/2) and JNK activation or the activation of p38 in immunostimulated J774 cells. Lanes 1 and 2, no stimulation; lanes 3 and 4, 10 μg/ml LPS; lanes 5 and 6: PJ-34 pretreatment 30 min before LPS. The activation of p42/44, JNK, and p38 was determined with Western blotting using antibodies raised against the phosphorylated (active) forms of p42/44, JNK, and p38. Cellular extracts were obtained 15 min after stimulation with LPS. This figure is representative of two different experiments.
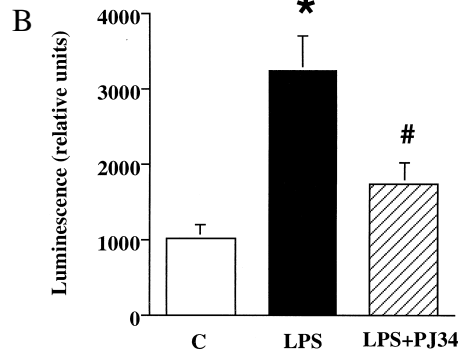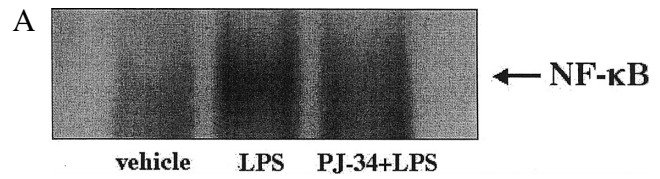

Fig. 4. PARP inhibition by PJ34 inhibits NF-κB activation in immunostimulated macrophages. (A) PJ-34 pretreatment (30 μM) inhibits LPS-induced NF-κB DNA binding in J774 cells. Nuclear extracts were prepared 45 min after LPS (10 μg/ml) challenge and NF-κB DNA binding was assessed using the gel mobility shift assay. **(B)** Luciferase activity in RAW macrophages transiently transfected with an NF-κB promoter construct. PJ-34 pretreatment (30 μM) inhibited LPS-induced increases in luciferase activity. Data represent mean $\pm$ SEM values of $n = 9$ wells from three experimental determinations. *$p < 0.05$ represents significant increase in luciferase activity after LPS and in #$p < 0.05$ represents significant inhibition by PJ34.

PARP-1–deficient mice induced an additional suppression of chemokine production at 1 hr post-LPS, and reduced the plasma level of chemokines to the level seen in wild-type mice treated with PJ34 (Fig. 5). In contrast to 1 hr post-LPS, at 3 and 6 hr post-LPS, pharmacologic PARP inhibition and PARP-1 deficiency elicited comparable degrees of inhibition of chemokine production (Fig. 5). The most likely interpretation of these findings is that
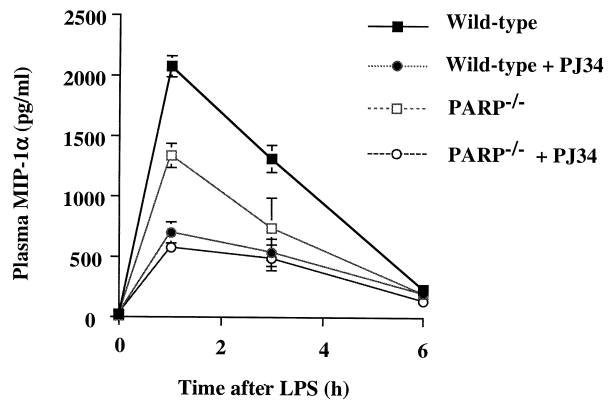

Fig. 5. Plasma MIP-1α levels at 1, 3, and 6 hr after LPS (10 mg/kg) injection in mice in the presence or absence of pretreatment with the PARP inhibitor PJ34 (10 mg/kg). Each point represents data from $n = 6$ animals.
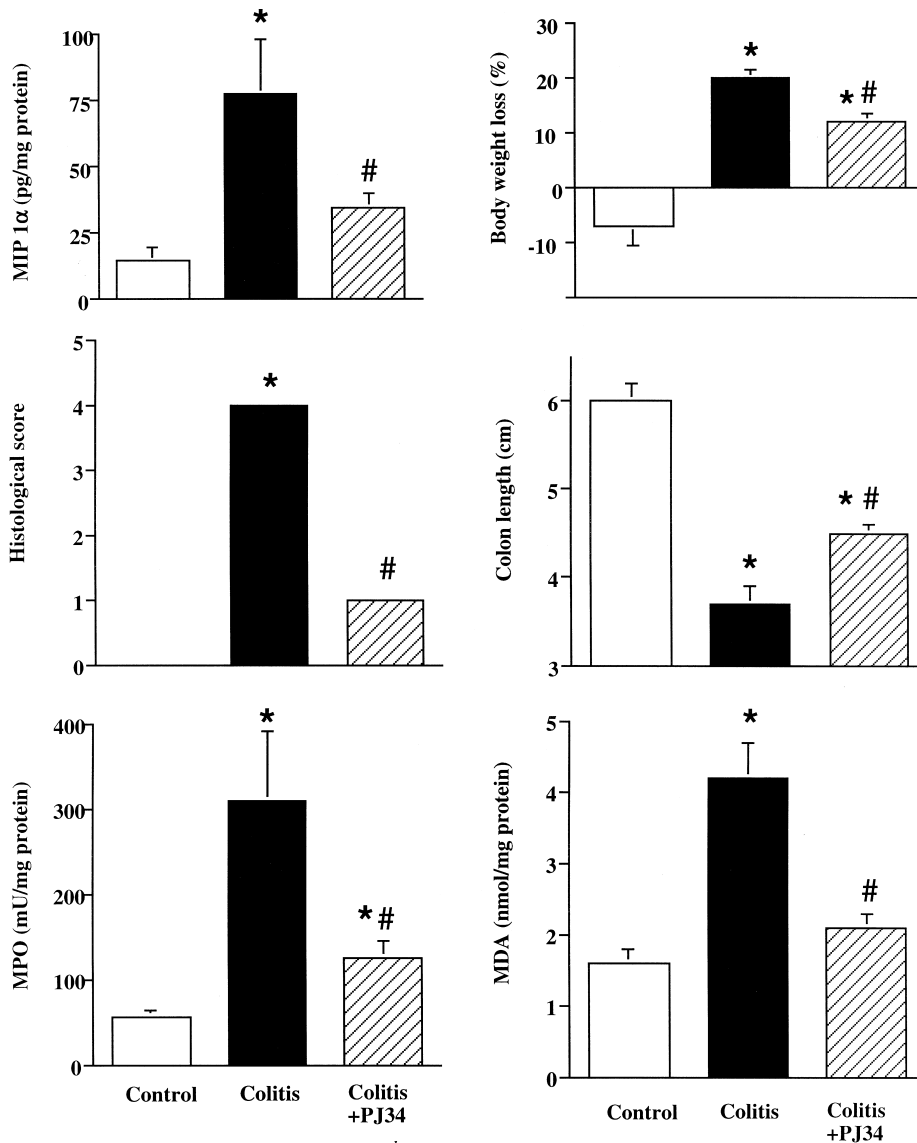
Fig. 6. Colonic MIP-1α levels, body weight loss, histologic severity score, colon length, colonic MPO and MDA levels in control and DSS-colitic mice in the presence or absence of treatment with the PARP inhibitor PJ34 (10 mg/kg/day). $*p < 0.05$ represents significant increase in the various parameters after DSS colitis and in $^{#}p < 0.05$ represents significant inhibition by PJ34 during colitis. Each bar represents data from $n = 7 - 9$ animals.

although among the PARP isoforms PARP-1 is the principal facilitator of chemokine production in endotoxemia, other poly(ADP-ribos)ylating enzymes may also contribute to this response, especially in the early phase of the inflammation.

PARP Inhibition Suppresses Chemokine Production and the Inflammatory Response in Colitis

Treatment of colitic mice with the PARP inhibitor PJ34 markedly reduced the degree of chemokine production in the colonic homogenates (Fig. 6). Accordingly, PARP inhibition reduced the infiltration of neutrophils into the colon, as measured by the quantification of MPO levels in colonic homogenates (Fig. 6). PARP inhibition also resulted in a marked protection against weight loss, improvement of colonic histology, preservation of colonic length, and reduction in oxidative tissue damage, as evaluated by the measurement of colonic MDA levels (Fig. 6).

Discussion

The current study demonstrates that the catalytic activity of PARP-1 regulates the production of the proinflammatory chemokines MIP-1α and MIP-2 in immunostimulated cells in vitro and in systemic and local inflammation models in vivo. Based on the in vitro data we present here in macrophages, we conclude that the regulation occurs at the transcriptional level, and may be related to the regulation by PARP of the activation of an NF-κB–dependent gene transcription system. Based on the findings that in PARP-1 deficient cells the production of the chemokines was largely abolished, we conclude that from the various PARP isoforms PARP-1 is the most likely contributor to the regulation of chemokine production. In contrast to the in vitro data, the in vivo results in endotoxic shock demonstrated that PJ34 was more effective than PARP-1 deficiency in suppressing chemokine formation, at least at earlier time points of endotoxemia.

Because phenanthridinones such as PJ34 inhibit the various PARP isoforms in a nonisoform-selective fashion (26), this finding may suggest that—in addition to PARP-1—some of the minor isoforms of PARP can play an additional role in the regulation of chemokine production.

An alternative explanation to the observed discrepancy between the results of the in vitro and in vivo studies (PJ34 versus PARP deficiency) may be that PARP-1 is major activator in macrophages (the cell type in which their in vitro studies were done), but perhaps in vivo the cellular sources of the chemokines in question (MIP-1α and MIP-2) may also include other cell types in which other isoforms of PARP may play a regulatory role.

The fact that PARP can regulate the expression of inflammatory mediators has been subject of much research interest. Suppression of the expression of iNOS, TNF-α and ICAM-1 has been reported in PARP-deficient mice and in the presence of pharmacologic inhibition of PARP (9,13). Whether it is the catalytic activity of PARP or the actual presence of PARP that is important in the regulation of inflammatory gene expression is a controversial subject. For example, in endotoxic shock, Oliver et al. (27) found that PARP deficiency but not pharmacologic inhibition of PARP was able to suppress the activation of NF-κB. Similarly, in endothelial cells exposed to high glucose to mimic diabetic vascular complications, we observed that PARP-1 deficiency but not PARP inhibition with PJ34 suppressed the activation of NF-κB (25). On the other hand, both PARP inhibition as well as deficiency were found to inhibit the expression of iNOS and the expression of ICAM-1 in various experimental systems (9,14). The activation of the HIV-LTR promoter and an NF-κB–dependent artificial promoter has been shown to be drastically reduced in PARP deficient cells. Furthermore, NF-κB–dependent gene activation could be restored by the expression of PARP in PARP-deficient cells. In one series of studies, it appeared that NF-κB and PARP formed a stable immunoprecipitable nuclear complex, and this interaction does not need DNA binding (28). Hassa et al. (29) demonstrated that a PARP-1 mutant lacking the enzymatic and DNA binding activity interacted comparably to the wild-type PARP-1 with p65 or p50, concluding that the enzymatic activity of the enzyme is not essential for its interaction with NF-κB (29). In contrast, Chang and Alvarez-Gonzalez concluded that NF-κB–p50 DNA binding was dependent on the presence of NAD; DNA binding by NF-κB–p50 was not efficient in the absence of NAD, and was blocked in the presence of pharmacologic inhibitors of PARP, allowing the conclusion that NF-κB–p50 DNA binding is protein-poly(ADP-ribosyl)ation dependent (30). It appears that the relative role of the presence versus catalytic activity of PARP in the regulation of NF-κB activation may depend on the experimental system used, the stimulus of induction,

and possibly the cell type involved. In our current work, both in vitro and in vivo demonstrates that the regulation of the expression of chemokines by PARP-1 is dependent on the catalytic activity of the enzyme. The possibility that PARP-1 regulates chemokine production via modulating the activity of a chemokine-inducing pathway other than NF-κB appears unlikely under the current experimental conditions, as we have demonstrated here that the activation of none of the other signal transduction pathways studied (such as mitogen-activated protein kinase, c-Jun N-terminal protein kinase, and p38 MAPK) were affected in the absence of functional PARP.

A recent study by Ha and Snyder clarified some of the issues related to the role of PARP (catalytic activity and physical presence) in the regulation of proinflammatory mediator production (31). It was demonstrated in immunostimulated glial cells that a whole host of transcription factors, including NF-κB, AP-1, SP-1, Oct-1, YY-1, and Stat-1 were down-regulated in the absence of PARP, and a host of proinflammatory mediators were reduced. Parallel experiments with an isoquinolinones PARP inhibitor demonstrated that the activation of NF-κB does not require catalytic activity of PARP, but the production of some of the proinflammatory mediators (such as IL-1β and iNOS-derived NO) is dependent on the catalytic activity of the enzyme (31).

In many models of inflammation and reperfusion injury, PARP inhibition and PARP deficiency have been shown to be associated with reduced infiltration of polymorphonuclear granulocytes, and a reduction in associated oxidative stress (9–14). Previously, there was no plausible explanation for this finding; in traditional schemes, PARP activation was thought to lay downstream from the processes of neutrophil infiltration and oxidative stress. Based on the current results, we propose that the regulation of chemokine production may be responsible for some of these effects.

Because chemokines are crucial in regulating cell adhesion and cell trafficking in a variety of pathophysiologic conditions including asthma, tumor cell metastasis, and AIDS (31–33), the current results may open new directions for the basic research on the roles of PARP, as well as for the preclinical development and testing of potent pharmacologic inhibitors of PARP.

Acknowledgments

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