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Review

The functional role of poly(ADP-ribose)polymerase 1 as novel coactivator of NF-κB in inflammatory disorders

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Abstract. Mammalian poly(ADP-ribose)polymerase 1 (PARP-1) is an abundant nuclear chromatin-associated protein and belongs to a large family of enzymes that catalyzes the transfer of ADP-ribose units from its substrate β-nicotinamide adenine dinucleotide (NAD^+) covalently to itself and other nuclear chromatin-associated proteins. PARP-1 knockout mice are protected against myocardial infarction, streptozotocin-induced diabetes, lipopolysaccharide-induced septic shock, and zymosan-induced multiple organ failure, indicating that PARP-1 is involved in the regulation of the pathogenesis of these disorders. PARP-1 and nuclear factor kappa B (NF-κB) have both been suggested to play a crucial role in inflammatory disorders. NF-κB encompasses a family of inducible transcription factors which play a crucial role in the regulation of genes involved in immune and inflammatory responses. Recent reports have shown that PARP-1 can act as a coactivator of NF-κB. These findings might provide new insights into the pathophysiology of different diseases such as type I diabetes and septic shock. The purpose of this review is to give a short overview of the current knowledge about PARP-1 and its functional and biochemical interactions with NF-κB. A more precise role for PARP-1 in NF-κB-dependent gene regulation and cellular metabolism during development of pathophysiological processes is discussed. Special considerations is given to the pathophysiological significance of these findings in terms of inflammatory disorders.

Key words. PARP-1; NF-κB; coactivator; positive cofactor; inflammatory response; endotoxin; septic shock; diabetes; peroxynitrite.

Diversity of the poly(ADP-ribose)polymerase family

Until recently, only one type of poly(ADP-ribose)polymerase (PARP) was thought to exist: PARP-1. However, the development of mice deficient for the PARP-1 gene has completely changed this view. The observation of poly(ADP-ribose) formation following DNA damage in different cells of PARP-1 knockout (PARP-1–/–) mice at levels between 2.9–105.8% of wild-type values, dependent on the tissue and cell type, strongly suggested the existence of additional PARP types [1, 2; reviewed in refs 3, 4]. For example, poly(ADP-ribose) formation is drastically reduced in PARP-1–/– brain, pancreas, liver, small intestine, colon, and testis (3–14%), but is not reduced in PARP-1-skeletal muscle or eye [2]. Lower levels of residual poly(ADP-ribose) formation (20–60%) can be observed in PARP-1–/– stomach, bladder, thymus, heart, lung, kidney, and spleen [2]. Thus, other forms of PARP presumably mediate the formation of poly(ADP-ribose) in these tissues. To date, more than 14 novel poly(ATP-ribose)polymerases have been identified in various species [reviewed in refs 5–7]. Moreover, more than 16 new PARP family members can be found in the human genome [7]. These new PARPs are structurally distinct from the 'classical' 114-kDa PARP-1 enzyme, and can be

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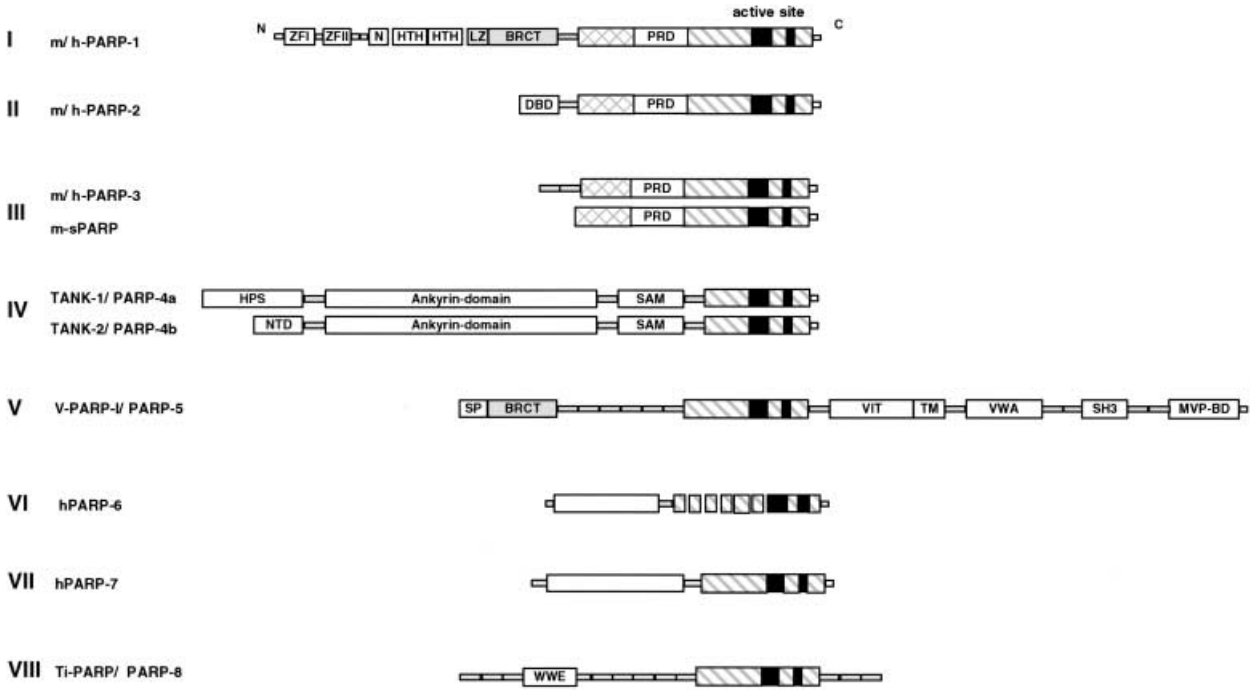

Figure 1. The structures of the PARP subclasses I–VIII. Abbreviations: h, human; m, mice; TANK, tankyrase; V-PARP, vault PARP; Ti-PARP, BRCT; BRCA-C-terminal domain; PRD, PARP regulatory domain; MVP-BD, major vault binding domain; SAM, sterile α module; DBD, DNA-binding domain; HPS, His-Pro-Ser-rich domain; TM, transmembrane domain; ZF, zinc finger; HTH, helix-turn-helix domain; WWE domain, named after three of its conserved residues and is predicted to mediate specific protein-protein interactions in ubiquitin and ADP-ribose conjugation systems; VWA, von Willebrand factor type A domain; VIT, vault protein inter-alpha-trypsin domain; LZ, leucine zipper; SH3, sarc homology domain 3; N, nuclear localization signal.

classified together with PARP-1 according to their structures and sizes into at least eight subgroups. Figure 1 shows the schematic comparison of protein structures and a proposed classification of the mammalian PARP multigene family based on the literature. Only limited data are available regarding the physiological roles of these non-classical PARP family members. The novel PARP family members seem to be involved in specific functions requiring limited levels of poly(ADP-ribosyl)ation [5]. For example, PARP members of class II and III, including h/mPARP-2 and h/mPARP-3 bear the strongest resemblance to PARP-1 [8, 9; and reviewed in ref 5]. Experimental data suggest that PARP-2 plays an important role in the response to DNA damage [8]. Less is known about class III including human and mouse PARP-3 and the short PARP-1 (sPARP-1) [9, 10]. Surprisingly, in contrast to PARP-1 and 2, the PARP activity of sPARP-1 is not dependent on DNA strand breaks [10]. Recently, two other mammalian proteins with PARP activity, tankyrase-1 and 2/3 (TANK-1 and 2) belonging to class IV, were identified as components of the telomeric complex [11–13]. Both, TANK-1 and TANK-2/3 were proposed to regulate the length of telomeres by modulating the activity of TRF1, a negative regulator of telomere length [11–13]. Kaminker and colleagues [12] have shown that over-expression of TANK-2/3, in contrast to TANK-1, caused rapid necrotic cell death in the absence of DNA damage. This effect could be prevented by the general non-specific PARP inhibitor, 3-aminobenzamide (3-AB), suggesting that the rapid necrotic cell death was due to the PARP activity of TANK-2 [12]. The functions of the other PARP family members of classes V–VIII, such as the vault PARP (VPARP)/PARP-5 [14], the predicted members PARP-6 and PARP-7 [7], as well as the 2,3,7,8-tetra-chloro-dibenzo-p-dioxin (TCDD)-inducible (TiPARP)/PARP-8 [15] are not yet known. Interestingly, a recent report showed that CEO1, a plant homologue of TiPARP, a member of class XII seems to be involved in protection against oxidative damage [15, 16].

In the following sections, we focus on PARP-1, the most relevant member of the PARP family.

Poly(ADP-ribose)polymerase 1

Mammalian PARP-1, a 114-kDa abundant nuclear chromatin-associated protein, belongs to a large family of enzymes that catalyzes the transfer of ADP-ribose units from β-nicotinamide adenine dinucleotide (NAD^+) onto glutamic acid residues of nuclear protein acceptors. The existence of a PARP enzyme was first reported nearly 40 years ago [reviewed in ref. 17]. PARP-1 is one of the best-

characterized examples of this family. The activity of PARP-1 is strongly stimulated by the presence of nicks and strand breaks in DNA [reviewed in ref. 18]. These observations have contributed to the idea that PARP mediates stress-induced signaling and functions in an NAD^+-dependent manner in certain cellular processes [reviewed in refs 19, 20]. Since then, the biological significance of PARP has been reported in many cellular processes [reviewed in refs 17, 21, 22]. However, the physiological function of PARP-1 is still under heavy debate. Earlier studies using inhibitors of PARP enzymatic activity such as 3-AB and nicotinamide suggested that PARP-1 plays a crucial role in DNA replication, DNA base excision repair (BER), V(D)J recombination, as well as regulation of telomere length [reviewed in refs 17, 22]. Other functions proposed for PARP-1 include gene expression, chromatin organization, proliferation and differentiation, cellular NAD^+ metabolism, and apoptosis [reviewed in refs 21, 22]. PARP-1 also serves as a marker for the onset of apoptosis, after which it is cleaved by caspases into DNA-binding and catalytic fragments [23, 24].

Structure of PARP-1

PARP-1 is found in all multicellular lower and higher eukaryotes studied so far [17]. The structure of the 'classical' type 1 PARP has been extensively characterized (figs. 1, 2). PARP-1 is a highly conserved multifunctional enzyme consisting of three domains: a DNA-binding domain (DBD) containing a bipartite nuclear localization signal (NLS) which is interrupted by a caspase cleavage site, an automodification domain, and a catalytic domain. The catalytic domain is the most highly conserved region of the PARP molecule [reviewed in refs 17, 25].

The N-terminal DBD of human PARP-1 spans residues 1–373 and has a molecular mass of approximately 42 kDa. This domain contains two zinc fingers (FI and FII) and two helix-turn-helix (HTH) motifs [26, 27]. Two studies have shown that these two zinc fingers, FI and FII, are the main structures responsible for binding to double-strand breaks (DSBs) or single-strand breaks (SSBs) and for activation of PARP-1 enzyme activity [26, 27]. Site-directed mutagenesis of residues 21, 125, 138, or 162 drastically decreased its ability to bind to SSBs or DSBs [26, 28]. The moderate non-specific association of PARP-1 with non-damaged DNA has been proposed to depend most probably on the HTH motifs [29]. Moreover, the zinc fingers can also act as an interface with various protein partners [reviewed in ref. 22].

The automodification domain of human PARP-1 extends from residues 374 to 525 bearing a leucine zipper (LZ) motif in the N-terminal part and a BRCA1 carboxyl-terminal (BRCT) protein interaction domain from residues 384 to 479 in the C-terminal part [30]. Both the LZs and the BRCT domain are well known to be involved in protein-protein interactions [31, 32]. Experimental data suggested that the LZs might be responsible for homodimerization of PARP-1 [30, 33]. In addition, the automodification domain contains possible auto-poly(ADP-ribosyl)ation sites implicated in the negative regulation of interactions between PARP-1 and DNA [34–36]. The majority of the 25–30 glutamic acid residues believed to be covalently auto-poly(ADP-ribosyl)ated by PARP-1 are located within the automodification domain [34, 35].

The catalytic domain of hPARP-1 is located in the C-terminal part of the enzyme between residues 526–1014. The minimal pADPr transferase domain has been further mapped to a 40-kDa fragment at the very C terminus of human PARP-1 which can be subdivided into two domains [37, 38]. The N-terminal domain (residues 656–794), which comprises the highly conserved PARP regulatory domain, consists of five α helices and one 3_{10}-helix [39]. The C-terminal domain (residues 795–1014) shares several structural features with mono(ADP-ribosyl)transferases including an evolutionarily conserved region, called the 'PARP signature,' spanning residues 859–908 [37]. Indeed, site-directed mutagenesis of the evolutionarily very conserved Glu988 in human PARP-1 decreases elongation of the (ADP-ribosyl)dimers into polymers 2000-fold and also reduces the initiation of new chains of pADPr [38, 39]. In addition, mutagenesis of residues 890 or 908, as well as the loss of the last 45 amino acids (residues 969–1014) at the C-terminal end, completely abolishes enzyme activity [39, 40]. PARP-1 is a special member of the (ADP-ribosyl)transferase (AR-

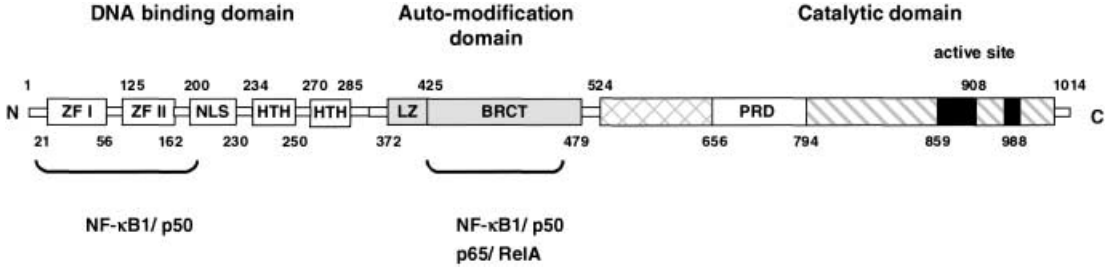

Figure 2. Schematic representation of the NF-κB/PARP-1 interaction map. Abbreviations: BRCT, BRCA-C-terminal domain; ZF, zinc finger; HTH, helix-turn-helix domain; LZ, leucine zipper; NLS, nuclear localization signal; PRD, PARP regulatory domain.

Tase) superfamily since it mono(ADP-ribosyl)ates a protein like the other ARTases, and then elongates this modification to a polymer. The 15-kDa region, spanning residues 525–656 found between the automodification and the minimal catalytic domain has not been extensively characterized and its function is still unknown [17].

Tissue distribution, expression levels, and subcellular localization of PARP-1

The tissue distribution of PARP-1 and its enzymatic activity have been examined in several rat and mouse organs [41, 42]. Northern blot analysis and in situ hybridization have revealed that the PARP-1 gene is constitutively expressed in testis, spleen, brain, thymus, intestine, colon, and nasal cavities [41, 42]. Very high levels of PARP-1 were found in lymphoid organs, especially the thymus, in the germinal centers of the spleen, and in the Peyer's patches in the ileum, while only very low levels of PARP-1 expression were found in organs such as liver, kidney, and heart [41–43]. In the central nervous system (CNS), PARP-1 is highly expressed in regions with a high neuronal cell density such as hypocampal neurons of the regions CA1 and 3, granule cells of the dentate gyrus, Purkinje cells of the cerebrellar cortex, as well as microglia and astrocytes in several regions [43, 44]. Interestingly, for non-neuronal cell types, a direct correlation could be observed between cell proliferation and high expression levels of PARP-1. Several studies have shown that an increase in PARP-1 mRNA levels is observed during thymocyte proliferation and upon activation of lymphocytes and peripheral blood mononuclear cells [45, 46]. Moreover, the PARP-1 mRNA level reaches its peak either in the G1 or the S phases [47]. The tissue-, cell- and cell cycle-specific expression pattern of PARP-1 suggests strongly not only that PARP-1 is critical to major cellular functions but also that its expression is modulated through complex transcriptional regulation.

Observation of several different tissues and cell lines using conventional fluorescence microscopy revealed that PARP-1 is exclusively localized to the nucleus [48]. Subsequent studies using either confocal laser scanning microscopy, electron microscopy, or cell fractionation experiments showed that PARP-1 is not homogeneously distributed in the nucleus [48]. PARP-1 was shown to be associated with nuclear matrix regions and localized to centromeres during metaphase [49], while other studies indicated that PARP-1 is found preferentially in nucleoli and defined nuclear bodies [48, 50]. Interestingly, PARP-1 was also shown to be associated with actively transcribing nucleolar regions and nuclear bodies [50]. Treatment of cells with RNA synthesis inhibitors caused PARP-1 immunofluorescence to become evenly distributed throughout the nucleus [50]. The association of PARP-1 with actively transcribed regions in the chromatin strongly implies a role for PARP-1 in transcription. Surprisingly, treatment with DNA synthesis inhibitors did not change the distribution of PARP-1 in the nucleus [50].

Poly(ADP-ribosyl)ation responses

Activation of PARP-1 was proposed to be one of the earliest responses of mammalian cells to genotoxic stress [reviewed in refs 51, 52]. The enzymatic activity of PARP-1 is strongly stimulated in vitro and increased by 10- to 500-fold in the presence of nicks and DSBs in DNA [reviewed in ref. 17]. These observations have led to the idea that PARP-1 might act as a 'molecular nick sensor,' thereby mediating stress-induced signaling in the presence of DNA lesions in an NAD^+-dependent manner to downstream effectors involved in coordinating the cellular response to DNA damage [20]. The 'molecular nick sensor' signaling model proposes that PARP-1 recognizes and rapidly binds to DNA strand breaks through its zinc fingers and, in turn, the catalytic domain of PARP-1 is allosterically activated and starts to synthesize complex branched poly(ADP-ribose) chains, resulting in automodifications of PARP-1 itself and probably to extensive modification of histones at sites of DNA strand breaks. Modification of chromatin proteins and PARP-1 itself might then subsequently act as a strong signal that may rapidly recruit other DNA damage-signaling molecules [20].

More than 40 nuclear chromatin-associated proteins have been implicated to function as a substrate for PARP-1 and to be modified by poly(ADP-ribose) chains in vivo [reviewed in ref. 17]. Target proteins include topoisomerase I and II, histones, p53, and high-mobility group proteins [53–55 and reviewed in ref. 56]. In intact organisms, PARP-1 itself is the predominant acceptor of poly(ADP-ribose) [reviewed in ref. 17]. Except for PARP-1 itself, data about modifications of proteins by PARP-1 in vivo should, however, be very cautiously interpreted. Despite intense studies in the last 30 years, neither specific glutamic acid residues functioning as poly(ADP-ribose) acceptor sites nor any specific poly(ADP-ribosyl)ation motifs could be identified in vitro. Moreover, only a few of the proposed substrates of PARP-1, such as p53, topoisomerase I, and histone 1, have been shown to directly interact with PARP-1 [57–59]. One has also to stress that the physiological consequences of poly(ADP-ribosyl)ation of the substrates are in most cases unknown. Lindahl and colleagues [52] have even proposed that the minor degree of modification of poly(ADP-ribose) acceptor proteins could be explained as an artificial side reaction in vitro.

PARP-1–/– mice

In recent years, several laboratories developed mice deficient for the PARP-1 gene. The three different knockout mice were created by interruption of either exon 2, exon 4, or exon 1 of the PARP-1 gene in mice [60–62]. Surprisingly, PARP-1–/– mice from all three different laboratories are viable and fertile. Furthermore, they did not show any phenotypic abnormalities such as organ failures as one would have clearly expected from the data obtained using inhibitors of PARP enzyme activity [60–62] and taking into account that knockouts of genes like XRCC1, DNA polymerase-*β,* or APE, which play a crucial role in the BER pathway are lethal [63–65]. Indeed, carefully designed studies with PARP-1–/– cells clearly demonstrated that PARP-1 is dispensable and not essential for replication, repair of DNA damage, or apoptosis in vitro or in vivo [66, 67]. Interestingly, recent studies using PARP-1–/– mice showed that they were protected against lipopolysaccharide (LPS)-induced septic shock, collagen-induced arthritis, streptozotocin-induced diabetes, hemorrhagic shock, and neuronal damage induced by transient middle cerebral artery occlusion (MCAO) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), indicating that PARP-1 plays a crucial role in inflammatory and neurodegenerative disorders and is involved in the pathogenesis of these events [68–73].

PARP-1 and necrotic cell death

Recent studies using DNA-damaging agents such as 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), hydrogen peroxide (H_2O_2), and peroxynitrite, which are well known to induce necrosis at high concentrations, showed that several cell types from PARP-1–/– mice were protected against necrotic cell death [72, 74, 75, and reviewed in ref. 4]. These studies clearly suggested that PARP-1 plays an important and essential role in necrotic cell death, which is in sharp contrast to its putative and negligible functions in apoptosis. The induction of PARP-1 enzyme activity by DNA strand breaks or by other stimuli results in synthesis of poly(ADP-ribose)polymers by consuming NAD^+ as a substrate. With every catalytic step, PARP-1 consumes 50–200 molecules of NAD^+ [17]. Thus, the enzyme activity of PARP-1 might be expected to affect cellular energy levels. Nathan Berger and colleagues [76] were the first to suggest (in 1983) that this process of poly(ADP-ribosyl)ation might be linked to necrotic cell death. According to the 'PARP-1 suicide' model, massive levels of DNA damage lead to over-activation of PARP-1 which may rapidly deplete intracellular NAD^+ levels, thereby reducing the rate of glycolysis and electron transport in the mitochondria and abolishing ATP formation, which results in cellular dysfunction and, finally, in necrotic cell death. This hypothesis could be confirmed using novel PARP inhibitors or cells from PARP-1 –/– mice. Inhibition of PARP-1 enzyme activity or complete absence of PARP-1 significantly improved cellular energetics and cell viability after exposure to necrosis-inducing agents [66, 74, 77–79, and reviewed in refs 3, 4]. Since cellular energy dynamics and necrotic cell death play an important role in inflammatory and neurodegenerative disorders, 5 years ago, PARP-1 was suggested to play a key role in these processes [72].

PARP-1 and cancer

Initial analysis of cells isolated from PARP-1–/– mice revealed a measurable genomic instability, although without consequences for the mice, since PARP-1–/– mice did not show any particular predisposition to develop spontaneous tumors [60–62]. However, several reports showed that PARP-1 might have an indirect role in tumorigenesis [80–83]. Sugimura and colleagues [81] showed that the susceptibility to carcinogenicity induced by *N*-nitroso-bis-(2-hydroxypropyl)amine (BHP) is increased in PARP-1–/– compared with wild-type mice. They proposed that the elevated tumorigenicity observed in PARP-1–/– mice might be at least in part due to the absence of PARP-1 in transcriptional regulation of genes involved in cell differentiation or maintenance of genomic integrity. In fact, Simbulan-Rosenthal and colleagues [84] reported that the expression of several genes involved in regulation of cell cycle progression and differentiation was significantly downregulated in PARP-1–/– cells, ex vivo. On the other hand, they showed that loss of PARP-1 moderately upregulates the expression of extracellular matrix or cytoskeletal proteins which are implicated in cancer initiation or progression [84]. Interestingly, a number of transcription factors involved in cell cycle control, cell proliferation, and differentiation, such as p53, YY1, E2F-1, or AP2, are also controlled by PARP-1 [85–88]. Two recent studies investigated the functional interaction of PARP-1 and p53 during tumor development using mice deficient in PARP-1 and p53 and obtained different results [82, 83]. The first study by Wang and colleagues [82] demonstrated that PARP-1 deficiency significantly increased the frequency of various tumor types such as carcinomas, lymphomas, and sarcomas in p53–/– mice, implicating PARP-1 as a cofactor for suppressing tumorigenesis. However, studies by Conde and colleagues [83] showed that loss of PARP-1 caused a significant delay in development of spontaneous tumors in p53–/– mice, resulting in increased survival of PARP-1/p53 double-knockout mice. The latter results suggest a role for PARP-1 in tumor progression. These discrepancies could be explained by the different genetic backgrounds used [82, 83]. Several earlier studies showed that the genetic back-

ground plays an important role in tumor incidence and spectrum [89, 90, and reviewed in ref. 91]. One can also not rule out that PARP-1 could negatively or positively cooperate with tumor suppressors other than p53 to promote or inhibit tumor formation, dependent on the tumor type and genetic background. Further careful analysis using mice of different genetic background and including transcriptosome and mutation analysis will help to elucidate the exact role of PARP-1 in tumorigenesis.

The role of PARP-1 in inflammatory disorders

The regulation of immune and inflammatory responses is a complex physiological process that is of profound importance to both homeostasis and ultimate survival of an organism. Without inflammation and activation of the immune system, an organism could not survive the insult of injury or would rapidly succumb to invading pathogens. The inflammatory response is composed of an elaborate cascade of inflammatory mediators. Their regulation must be tightly coordinated to maintain appropriate and timely immune reactions without an over-reaction that can cause damage to the host. Yet, without mechanisms that shut down prolonged, inappropriate, or excessive immune response and inflammation, the organism would die from damage caused by these physiological responses. Therefore, both pro- and anti-inflammatory mechanisms must be activated and balanced for an organism to survive in the face of environmental stimuli that elicit an immune response.

A pathophysiological role for PARP-1 has been demonstrated in a number of diseases and animal models, including streptozotocin-induced diabetes [69, 77, 92], zymosan-induced vascular failure, a non-septic model of multiple organ dysfunction [68], LPS-induced septic shock, and carrageenan-induced pleurisy [70, 93–95], as well as collagen-induced arthritis (CIA), a model for chronic inflammation [95, 96]. A partial list of inflammatory disease models in which PARP-1 is involved is given in table 1. In the following sections, the pathogenesis of type I diabetes, zymosan-induced multiple organ failure (MOF) and septic shock will be briefly described.

Type I diabetes

Type I diabetes is an autoimmune disease characterized by selective T lymphocyte-mediated destruction of the insulin-secreting β cells in the pancreatic islets of Langerhans, which is thought to be the determining event in the pathogenesis of type I diabetes [reviewed in refs 97, 98]. Environmental factors (pathogens, drugs, and diet) and the genetic background [major histocompatibility complex (MHC) and non-MHC genes] are critical for the initiation of the autoimmune response against the pancreatic β cells [reviewed in refs. 99, 100]. The presence of β cell-destructive Th1 and β cell-regenerative Th2 cytokine subsets in the pancreas, and the balance between them, has been proposed to be the determining factor in diabetes development [101, 102]. Th1 and Th2 cytokines are reciprocally regulated, with Th1 cytokines modulating the actions of Th2 cytokines and vice versa [101, 102]. Chronic hyperglycemia mainly caused by the destruction of the insulin-secreting β cells can lead to the development of diabetes-specific microvascular pathology in the retina, renal glomerulus, and peripheral nerve. As a consequence of its microvascular pathology, diabetes is a leading cause of blindness, end-stage renal disease, and a variety of debilitating neuropathies [reviewed in ref. 103]. There are two streptozotocin-induced animal models which mimic at least in part several biochemical and pathological hallmarks of type I diabetes in non-human animals [104,

Table 1. Partial list of inflammatory disease models in which PARP-1 is involved.

Type of disease	Common phenotype of PARP-1–/– mice	References
LPS-induced septic shock	full resistance to endotoxic shock	70, 93
Acute respiratory distress syndrome	reduction of high-permeability pulmonary edema and flooding of alveolar spaces by neutrophilic leukocytes, due to reduced recruitment of polymorphonuclear neutrophils	94
2,4,6-trinitro-benzene-sulfonic acid-induced mucosal injury in murine colitis	reduction of mucosal injury due to resolution of colonic damage and reduction of neutrophil infiltration; reduction of necrosis in endothelial cells of intestine	204
Streptozotocin (SZT)-induced diabetes	normoglycemic, reduced sensitivity to SZT and normal function of endothelial and pancreatic β cells	77, 107, 108, 119
Myocardial postischemic injury	reduction of myocardial infarct size	205, 20
Hemorrhagic shock	increased survival advantage due to protection from cardiovascular decompensation, vascular hypocontractility, gut barrier failure, and lung neutrophil recruitment,	73
Zymosan-induced inflammation and multiple organ failure (MOF)	resistance against zymosan-induced inflammation and MOF due to reduced neutrophil recruitment and organ injury.	68

105]. Streptozotocin is a specific β cell toxin taken up by β cells through the glucose transporter Glut-2 [106]. Administration of a single large dose of streptozotocin induces diabetes within 48 h by directly destroying the β cells, while multiple low-dose streptozotocin administration can cause β cell damage resulting in an immune cell response directed toward the β cells [107, 108]. Streptozotocin-induced and human autoimmune type I diabetes are both characterized by a progressive hyperglycemia and insulitis, associated with drastic upregulation of the inducible isoform of nitrite oxide (NO) synthase (iNOS) in β cells and islet-infiltrating immune cells [96, 109, 110]. Stimulation of β cells by polymorphonuclear neutrophil (PMN)/macrophage/T cell-generated cytokines induces the expression of inflammatory mediators, such as iNOS [111] and subsequent massive production of NO and related free radical species such as peroxynitrite [111, 112]. Peroxynitrite is a potent and cytotoxic oxidant formed by the reaction of NO with the superoxide radical and oxygen, generated by NADPH oxidase, respectively [reviewed in ref. 113]. The formation of free radical species in β cells inhibits insulin secretion [114], leads to extensive DNA damage and thereby activates the PARP-1 'suicide pathway' which finally results in cellular energy depletion and necrotic β cell death [77, 92, 115, 116]. Type I diabetes is also associated with an increase in advanced glycation end product (AGE) formation in diabetic retinal vessels and renal glomeruli [reviewed in ref. 97]. The activity of AGEs influences two different events: first, they modify extracellular matrix components and intracellular proteins thereby altering their function and, second, certain of these modified plasma proteins bind to AGE receptors on endothelial cells, mesangial cells or macrophages, thereby inducing the activation of the pleiotropic transcription factor NF-κB [reviewed in refs 97, 117] causing pathological changes in gene expression of pro-inflammatory mediators [118]. In the last decade, several independent groups have shown that not only do novel inhibitors of PARP enzyme activity provide a markedly increased protection but also PARP-1–/– mice of different genetic background were nearly completely protected from streptozotocin-induced diabetes [77, 107–109, 119]. PARP-1–/– mice remained normoglycemic and their β cells showed preserved structure and function concomitant with a drastic reduction in peroxynitrite formation, neutrophil recruitment, and endothelial dysfunction [77, 107–109, 119].

Zymosan-induced multiple organ failure (MOF)

Administration of zymosan, a cell wall derivative of yeast, *Saccharomyces cerevisiae*, is used as non-septic model of acute peritonitis and MOF in experimental animals [reviewed in ref. 120]. The human and experimental MOF syndrome is invariably characterized by serious inflammation that leads to a well-defined sequence of organ failures, starting with the lungs, progressing to the clotting system, subsequently involving the bowel and kidney, and ultimately resulting in liver failure [reviewed in refs 120, 121]. The mortality risk increases with the progression of each organ failure [reviewed in refs 120, 121]. Zymosan, composed of α-mannan and β-glucan, is a particulate ligand for different receptors leading to cellular activation and production of inflammatory mediators through activation of several transcription factors including AP-1 and NF-κB [122–124]. Phagocytosis of zymosan by macrophages is mediated through different receptors such as the mannose receptor, complement receptor 3, Toll-like receptor 2, as well as dectin-1 [125–129]. The zymosan-induced inflammatory products include cytokines [e.g., tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8], hydrogen peroxide, and arachidonic acid [122, 124, 130]. A broad range of cell types, including macrophages, PMNs, and natural killer cells, can be activated by zymosan [reviewed in refs 120, 131]. The interaction of zymosan with macrophages is thought to be the first step in the initiation of the immune response [120, 127, 131]. Activation and accumulation of PMNs are the initial events of tissue injury caused by release of oxygen free radicals, arachidonic acid metabolites, and lysosomal proteases [reviewed in ref. 121]. PMNs become activated once in the inflammatory sites to secrete a variety of substances, such as growth factors, chemokines, cytokines, complement components, proteases, NO, reactive oxygen metabolites, and peroxynitrite, which are important mediators of tissue injury [reviewed in refs 120, 121]. Recent reports suggested that zymosan induces vascular dysfunction mainly through peroxynitrite production and subsequent over-activation of the PARP-1 'suicide pathway' [68, 120, 132]. Szabo and coworkers [68] demonstrated that PARP-1–/– mice were resistant to zymosan-induced inflammation and MOF when compared with wild-type mice. This effect was likely related to the fact that loss of PARP-1 prevented PMN recruitment and reduced organ injury [68].

Septic shock

Septic shock is the most common cause of death in intensive care units, with a high mortality rate, often as a result of a systemic Gram-negative bacterial infection. It is defined as an acute circulatory failure or dysfunction of a number of organ systems associated with severe sepsis, persisting despite adequate fluid resuscitation, causing a shock-like state and leading to death [reviewed in refs 133–136]. Septic shock can be mimicked by intravenous injection of microbial products such as bacterial LPS [reviewed in refs 137, 138]. LPSs activate a complex signaling cascade, enabling the expression of many crucial

genes involved in the pathogenesis of septic shock, such as cytokines (e.g., TNF-α, IL-1β,) adhesion molecules (e.g., ICAM, VCAMs), and iNOS [reviewed in refs 134, 135]. One of the striking features of septic shock is the increased production of peroxynitrite which can induce massive levels of DNA SSBs and activation of the PARP-1 'suicide-pathway' resulting in necrotic cell death and endothelial dysfunction [139–141]. In 1997, Szabo and coworkers [140] showed that PARP inhibitors can strongly reduce tissue damage caused by high doses of endotoxin. Moreover, recent studies from different groups reported that PARP-1–/– mice were extremely resistant to LPS-induced lethality [70, 93]. The production of peroxynitrite and neutrophil recruitment during endotoxic shock as well as local and systemic inflammation were drastically reduced in the absence of PARP-1 [70, 93].

The switch from homeostasis to disorders

Several cellular signaling pathways have been identified as the main regulators of immunity and inflammation: NF-κB, interferon response factors (IRFs), STATs, and glucocorticoid receptor-mediated signal transduction cascades [reviewed in refs 142, 143]. NF-κB, IRFs, and STATs belong to different families of inducible transcription factors which mediate the transcriptional activation of pro-inflammatory mediators, whereas glucocorticoid receptors upregulate anti-inflammatory mediators and negatively impact many aspects of immune and inflammatory responses [reviewed in refs 144, 145]. The balance between these mediators often determines the outcome after inflammatory insults [146]. Excessive inflammatory responses likely involve a significant shifting of the cytokine balance toward the side of pro-inflammatory cytokine production and signaling [146, 147].

In inflammatory disorders there is often unregulated production of pro-inflammatory mediators that can lead to MOF [reviewed in refs 148–150]. In the last decade, much progress has been made regarding the etiology, pathogenesis, and mechanisms of these disorders. However, the exact molecular basis behind this shift in the balance of inflammatory mediators remains to be determined. PARP-1 might, therefore, be an obvious research target for the analysis of inflammatory responses in instances where these processes become chronic or dysregulated.

Nuclear transcription factor kappa B

NF-κB is a widely expressed, inducible transcription factor of particular importance to cells of the immune system [reviewed in ref. 151]. NF-κB plays a crucial role in the regulation of gene expression of many genes involved in mammalian immune and inflammatory responses, including cytokines, cell adhesion molecules, complement factors, and a variety of immune receptors [reviewed in ref. 152]. NF-κB has additionally been implicated as an important regulator of cellular events such as apoptosis, cell proliferation, and differentiation [reviewed in ref. 153]. The Rel family members include p65 (Rel A), Rel B, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2), which form homo- and heterodimers. These proteins share a conserved 300-amino acid region within their amino termini, termed the Rel homology domain (RHD) [153]. This domain is responsible for DNA binding, dimerization, nuclear translocation, and interaction with heterologous transcription factors. Although all Rel family members bind to DNA, only p65 (RelA), c-Rel, and RelB contain a transactivation domain. The prototypical and most studied form, NF-κB, is a 'classical' heterodimer consisting of the two subunits p50 (NF-κB1) and p65 (RelA). When these two subunits are coexpressed at comparable levels in the cell, their affinity for each other is higher than the affinity of either homodimer, and therefore the 'classical' NF-κB p65/p50 heterodimer is preferentially formed. This heterodimer has a high affinity for the consensus NF-κB DNA sequence 5′-GGGRNNYYCC-3′ and is generally considered to be the predominant, inducible form of NF-κB in most cells [reviewed in ref. 151]. In unstimulated cells, NF-κB is sequestered in the cytoplasm as an inactive transcription factor complex by its physical association with one of the several inhibitors of NF-κB (IκB). This family of IκBs includes IκBα, IκBβ, IκBγ, IκBε p105/p50 (C terminus), p100/p52 (C terminus), IκB-R, and Bcl-3 [reviewed in refs 142, 154]. IκBα and IκBβ preferentially interact with dimers containing p65 and have been shown to be the main functional modulators of the 'classical' NF-κB p65/p50 heterodimer [reviewed in refs 142, 154]. Treatment of cells with extracellular stimuli, including cytokines such as IL-1 or TNF-α, viral proteins, bacterial LPS, phorbol esters, UV and γ-irradiation or potent oxidants leads to the rapid phosphorylation of IκB at serines 32 and 36 by a high molecular weight IκB kinase complex [reviewed in ref. 142; for a detailed list of NF-κB-inducers, see ref. 152]. There are two different but related Ser/Thr kinases, identified by several groups, termed IKK-α and IKK-β, which are responsible for the inducible phosphorylation of IκB [reviewed in ref. 142]. Phosphorylation of IκB leads to the ubiquitination of IκB at lysines 21 and 22, and subsequent degradation by the 26S proteasome pathway. Consequently, dissociation of NF-κB unmasks nuclear localization sequences of p65 and p50, which leads to nuclear translocation and binding of NF-κB to specific κB consensus sequences in the chromatin and activation of specific subsets of genes [142, 152]. Remarkably, one consequence of NF-κB activation is the upregulation of IκBα gene expression, caused by a κB consensus sequence within the IκBα promoter [155].

Several lines of evidence suggest that newly synthesized IκBα, alone or as a complex of IκBα and p50, enters the nucleus, strips non-chromatin-associated subunits of NF-κB, and mediates their export from the nucleus, thereby reestablishing a cytoplasmic pool of inhibited complexes [156]. Retention of NF-κB in the cytoplasm and attenuation of NF-κB-mediated transcriptional activation therefore provides a feedback mechanism for modulating the extent and duration of inflammatory responses by the cell.

Dysregulation of NF-κB in disease

During the last decade, excessive activation or inappropriate regulation of immune and inflammation cascades have clearly been demonstrated to cause tissue and cellular damage which may lead to cellular dysfunction and death [157, 158]. NF-κB-mediated transcription was thought to contribute exclusively to survival pathways. Thus, NF-κB was implicated as a classical anti-apoptotic transcription factor [reviewed in ref. 159]. However, the extensive literature on the ubiquitous involvement of NF-κB in regulating inflammatory processes clearly suggests that although NF-κB activation can prevent apoptosis of the cell in which NF-κB is activated, it might directly or indirectly lead to cell death of other/surrounding cells by promoting the production of cytotoxic agents such as peroxynitrite [reviewed in refs 160, 161]. Remarkably, the relative contribution of NF-κB-mediated transcription to cell death pathways seems to be associated with its dysregulation, concomitant with a persistent nuclear activity of NF-κB and over-production of pro-inflammatory cytokines [160, 161]. In contrast, transient activation of NF-κB seems to be responsible for the pro-survival functions of NF-κB [reviewed in ref. 161]. Indeed, there has been increasing experimental evidence suggesting that abnormalities in the regulation of NF-κB activity are tightly linked to the pathogenesis of inflammatory disorders including toxic/septic shock, radiation damage, myocardial infarction, acute-phase reactions, and diabetes, as well as chronic inflammatory diseases such as asthma, rheumatoid arthritis, and inflammatory bowel disease [reviewed in refs 146, 162]. Moreover, a number of drugs used for the treatment of certain chronic inflammatory diseases, such as glucocorticoids and non-steroidal anti-inflammatory drugs, were recognized to be inhibitors of the NF-κB pathway [reviewed in refs 152, 163]. Studies directly on the level of gene expression might give us profound insights into most detrimental NF-κB-dependent processes.

A role for PARP-1 as a novel coactivator of NF-κB

There are striking similarities between the expression pattern of PARP-1 and the detrimental transcriptional activity of NF-κB. In most tissues and cell types associated with high PARP-1 expression, dysregulated NF-κB activity seems to contribute to cellular dysfunction and necrotic cell death during inflammatory disorders. The relative contribution of NF-κB transcriptional activity to either a 'good' inflammation, manifested by cell survival and tissue regeneration, or a 'bad' inflammation, causing cell death and tissue destruction, rather depends on the kinetics of activation and intrinsic metabolic differences between different cell types or within the same cell and on the nature as well as intensity of the activating stimulus [reviewed in refs 161, 164]. Conceivably not only does the simultaneous activation of other transcription factors such as AP-1, ETS, C/EBPβ, STAT-1, and p53 influence the spectrum of induced NF-κB-dependent genes but the availability of different coactivators and cofactors of NF-κB does so too, thereby determining whether activation of NF-κB leads to cell survival, necrotic cell death, or apoptosis. The strongest indication for a direct role of PARP-1 in NF-κB-dependent transcription was the impaired expression of NF-κB-dependent pro-inflammatory mediators in PARP-1–/– mice [reviewed in ref. 22]. The upregulation of several inflammatory response genes such as TNF-α, IL-6, interferon (IFN)-γ, VCAM, ICAM, P-selectin, and iNOS was shown to be drastically reduced (60–90%) in PARP-1–/– mice after treatment with inflammatory stimuli such as LPS or streptozotocin [68, 94, 96, 109, 140, 165, 166, and reviewed in refs 4, 22]. Table 2 lists genes involved in inflammation which are affected in PARP-1–/– mice or PARP-1-deficient cells. Furthermore, growing experimental evidence suggests that PARP-1 can function as a coactivator of transcription factors. PARP-1 has been shown to increase the transcriptional activity of several transcription factors including HTLV-TAX, bMYB, TEF1/Max, and AP-2 [87, 167–169]. PARP-1 has been identified as an interaction partner for over two dozen transcription factors, classical coactivators, and

Table 2. Genes downregulated in PARP-1 –/– mice or PARP-1-deficient cells.

Cytokines	TNF-α, IL-1β, IL-6, IL-8, IL-10, IFN-γ	[68, 70, 94, 107, 165, 207]
Chemokines	MIP-1α and MIP-2	[94]
Adhesion molecules	P-selectin, E-selectin, VCAM and ICAM, CD-11α	[165, 206]
Enzymes	iNOS	[70, 83, 165, 208]

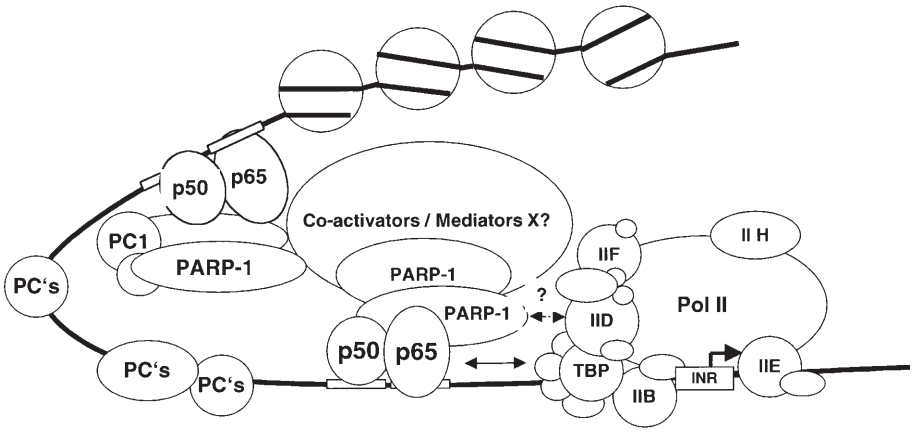

Figure 3. Model of the PARP-1/NF-κB complex. Abbreviations: PC-1, positive cofactor 1; PC's, positive cofactors 2–6 [PC-2 (mediator-like complex), PC-3 (Dr2/topoisomerase I), PC-4 (ssDNA-binding protein), PC-5 and PC-6]; IIA–H, general transcription factors; Pol II, RNA polymerase II; INR, transcription initiation region; TBP, TATA box-binding protein

general cofactors including p53, RXRα, bMYB, TEF-1, Oct-1, AP-2, YY-1, TRBP, and PC-3 (Dr2/topoisomerase I) [57, 59, 86, 87, 170, 171].

Recently, several reports showed that PARP-1 is required for specific NF-κB-dependent gene activation and can act as a coactivator for NF-κB in vivo [70, 172, 173]. PARP-1 was shown to be required and sufficient for specific transcriptional activation of NF-κB in response to pro-inflammatory stimuli and genotoxic stress. Neither the nuclear translocation nor the DNA-binding ability of NF-κB were affected in PARP-1–/– cells [70, 172, 173]. However, PARP-1 directly interacted through different domains with both subunits of NF-κB (p65 and p50) in vitro [173] (depicted in fig. 3) and formed a stable immunoprecipitable nuclear complex together with p50 and p65 in vivo, which was independent of DNA [172–175]. Interestingly, a recent report showed that microglial migration is strongly controlled in living brain tissue by expression of the integrin CD11α,which was in turn regulated by the PARP-1/NF-κB-protein complex [174]. Downregulation of PARP-1 or CD11α by transfection with anti-sense DNA almost completely abrogated microglial migration and protected neurons from secondary damage [174]. Surprisingly, neither the enzymatic activity of PARP-1 nor its DNA-binding activity were required for full activation of NF-κB in response to various stimuli in vivo [165, 173]. Finally, PARP-1 could activate the HIV-LTR, as well as the natural iNOS and P-selectin promoters upon stimulation of the cells with inflammatory stimuli in an NF-κB-dependent manner [172, 173]. However, the exact molecular mechanisms of coactivation remain to be elucidated.

Meisterernst and colleagues [176] identified in 1997 human PARP-1 as one active component of the upstream stimulatory activity (USA)-derived positive cofactor complex PC-1 [176]. Earlier studies showed that the USA complex is essential for transcriptional activity of transcription factors such as NF-κB, SP1, and Oct-1 in the presence of a complete set of general transcription factors [177, and reviewed in ref. 178]. Subsequent fractionation studies of the crude precursor USA fraction in the last decade led to the discovery of at least six independent subfractions of positive cofactors: PC-1/PARP-1, PC-2 (Mediator-like complex), PC-3 (Dr2/topoisomerase I), PC4 (ssDNA-binding protein), PC-5, PC-6, and HMG2 [reviewed in refs 178, 179]. PC-1/PARP-1 was thought to provide a structural/architectural function together with the other USA-derived positive cofactors PC-3/Dr2/topoisomerase I, PC-4/ssDNA-binding protein, PC52 and HMG2 in assembling and stabilizing the pre-initiation complex [178, 179]. In addition, since PC-1/PARP-1, PC-3, and PC-4 are all non-sequence-specific DNA-binding proteins, these cofactors were suggested to function by affecting the accessibility of RNA polymerase II to chromatin [179]. PARP-1 might also function synergistically with other USA-derived positive cofactor complexes in stabilizing the interaction between NF-κB and the basal transcription machinery, thereby facilitating the formation and subsequent activation of the pre-initiation complex in vivo. Indeed, PC-2, PC-3 (Dr2/topoisomerase I), and PC-4 (ssDNA-binding protein) have been reported to synergistically enhance NF-κB-dependent transcription in a reconstituted cell-free transcription system [177, 180, 181]. Interestingly, Malik and colleagues [181] identified PC-2 as a 500-kDa Mediator-like complex that is a submodule of the larger TRAP/SMCC-Mediator complex [181]. NF-κB activation occurs in virtually all cell types, in combination with a variety of coactivators [reviewed in ref. 182]. The genes activated by NF-κB will also vary depending on the cellular context. Thus, in terms of process specificity and given that transcription functions at the level of chromatin in vivo, PARP-1 quite possibly not only

functions in terms of an interplay between the PC-1 complex and other positive cofactor complexes but also synergistically during the pathogenesis of inflammatory disorders with cell- and stimuli-specific types of Mediator and NF-κB-associated chromatin-remodeling complexes including SWI/SNF-like or GCN5-like histone acetyltransferase (HAT) complexes [183, and reviewed in ref. 184]. Surprisingly, PARP-1 was recently identified as a direct interaction partner of the thyroid hormone receptor-binding protein (TRBP), a novel general coactivator which was suggested, based on its structure, to be either a part of a new Mediator complex or to represent an unidentified member of the TRAP/DRIP-Mediator complex [171]. However, the fact that PARP-1–/– mice do not show the same phenotype as RelA/p65–/– animals indicates that there are only subsets of NF-κB-dependent genes also dependent on PARP-1, such as iNOS or TNF-α. Indeed, p65 can interact directly with the basal transcription machinery as well as with distinct sets of cofactors and coactivators, such as p300/CBP [177, 185]. Thus, in certain instances, the requirement for PARP-1 is conceivably bypassed through other specific coactivators and cofactors, most probably in a cell type- and stimuli-dependent manner.

Poly(ADP-ribosyl)ation activity and NF-κB

An interesting aspect is the possible contribution of a poly(ADP-ribosyl)ation activity in NF-κB-dependent gene expression.

Recently, two studies published by independent groups suggested that the enzymatic activity of PARP-1 might directly influence NF-κB-dependent transcription [175, 186]. The first study by Kameoka and coworkers [186] showed that poly(ADP-ribosyl)ation can markedly suppress the DNA-binding activity of NF-κB and provided evidence that PARP-1 can poly(ADP-ribosyl)ate both subunits of NF-κB, p50, and p65 in vitro, implying that poly(ADP-ribosyl)ation might even inhibit NF-κB-dependent transcription. Several reports demonstrated that poly(ADP-ribosyl)ation can inhibit the activity of transcription factors in transcription assays in vitro [187–189]. Althaus and coworkers [190] showed that even a low concentration, free poly(ADP-ribose) polymers can completely inhibit the DNA-binding activity of p53. A second study was reported by Chang and Alvarez-Gonzalez [175], namely that the DNA-binding activity of NF-κB1/p50 is reversibly regulated only by the automodification reaction of PARP-1, in vitro. They showed that when PARP-1 was not autopoly(ADP-ribosyl)ated, it inhibited the sequence-specific binding of NF-κB to its cognate oligonucleotide in vitro, whereas automodification of PARP-1 completely reversed this effect. In addition, they demonstrated clearly that p50, the small subunit of NF-κB, was not a target for poly(ADP-ribosyl)ation [175]. The effect observed by Chang and Alvarez-Gonzalez might, however, be due to nonspecific binding of PARP-1 to the ends of the oligos, thereby sterically competing NF-κB1/p50. Unfortunately, neither group confirmed their results by using in vivo or in vitro transcription assays or including enzymatic or DNA-binding mutants of PARP-1 in their in vitro assays.

In sharp contrast to these two findings, different groups have reported that poly(ADP-ribosyl)ation affects neither the DNA-binding activity of NF-κB, nor is it required for NF-κB-dependent gene expression [108, 165, 173]. At first glance, this seems to be incompatible with reports describing an inhibitory effect (15–30%) of inhibitors of PARPs and mono(ADP-ribosyl)transferases on the expression of inflammatory mediators in mice [68, 94–96, 109, 140, 165, 166, and reviewed in refs 4, 22]. However, the observations might be explained in two ways. First, 3-AB and nicotinamide as well as novel types of PARP inhibitors can inhibit not only the enzyme activity of PARP-1 but also of other PARPs such as PARP-2 or TANK-2 and even mono(ADP-ribosyl)transferases [6, 12, 107, 191, 192]. Indeed, poly(ADP-ribose) formation is drastically reduced only in PARP-1–/– brain, pancreas, liver, small intestine, colon, and testis, whereas still moderate levels of residual poly(ADP-ribose) formation can be observed in PARP-1–/– stomach, bladder, thymus, heart, lung, kidney, and spleen [2]. Additionally, several reports suggested a functional role of mono(ADP-ribosyl)transferases in inflammatory response pathways [193, 194]. Second, the enzymatic activity of PARP-1 might be required for transcriptional activity of other transcription factors involved in these inflammatory processes. Several groups have shown that cooperative activities between transcription factors such as AP-1, STAT-1, or IRF-1 in the enhanceosomes of NF-κB-dependent genes including iNOS, I-CAM, COX, and IFN-β and IFN-γ are required for full synergistic activation of these genes [195–197]. Indeed, in human endothelial cells, the PARP inhibitor 3-AB reduces oxidant-induced binding activity of AP-1 to the promoter of ICAM-1 [198]. We cannot exclude that NF-κB might be poly(ADP-ribosyl)ated under certain conditions, such as the last steps of necrotic cell death, in vivo. However, poly(ADP-ribosyl)ation is unlikely to be directly required for repression or stimulation of the transcriptional activity of NF-κB under physiological conditions in vivo.

Model for the function of the transcriptional NF-κB/PARP-1 complex in inflammatory disorders

Combining these data, we propose a new pathophysiological model for the dual function of PARP-1 in inflammatory disorders. In the original models proposed by

Szabo, PARP-1 was thought to act only in parallel or downstream of NF-κB activation and induction of iNOS [120, 199]. However, results of different groups clearly suggest that PARP-1 acts as a coactivator of NF-κB upstream of the synthesis of iNOS, P-selectin and other pro-inflammatory mediators during the inflammation processes, independent of the enzymatic and DNA-binding activity of PARP-1 [70, 108, 165, 172, 173]. These results also explain why PARP-1–/– mice are extremely resistant to streptozotocin-induced diabetes and lethality induced by LPS. Moreover, since NF-κB is involved in most diseases from which PARP-1–/– mice are largely protected, we propose that the PARP-1 coactivator function of NF-κB is a common mechanism and a critical molecular event involved in the pathogenesis of different diseases such as septic shock, type-1 diabetes, and arthritis.

Although the proximal events and cellular inflammatory networks in several inflammatory diseases are quite different [reviewed in refs 97, 134, 135, 150], they all have some common features characterized by the activation of NF-κB, the production of pro-inflammatory mediators and oxygen free radicals, as well as subsequent culmination in activation of the PARP-1 'suicide pathway'. Thus, PARP-1 functions in these pathophysiological processes at two different levels, first through its coactivator function for NF-κB, and second by depleting intracellular NAD^+ and ATP levels which results in necrotic cell death and tissue damage. Pharmacological inhibition of PARP-1 improves the adverse clinical effects in different pathologies associated with inflammation after cell death [78, 79, 95, 96, 107–109, 166, and reviewed in ref. 6]. Since the enzymatic and DNA-binding activity is not required for NF-κB-dependent transcriptional activation after treatment of cells with pro-inflammatory or genotoxic substances, we propose that the observed anti-inflammatory effects of the PARP-1 inhibitors do not influence PARP-1 coactivator function but only inhibit the NAD^+ and ATP depletion and subsequently also necrotic cell death and tissue damage. A strongly simplified model (depicted in fig. 4) is described below. In this section, we will only focus on the proposed NF-κB/PARP-1-dependent pathways. For detailed descriptions of all different cellular networks of inflammation see the sections above and recent reviews [97–100, 102, 121, 134, 135, 147, 148, 162, 163, 199].

LPS-induced septic shock

When LPSs are present in the bloodstream, as a result of a systemic Gram-negative bacterial infection, they are immediately captured by LPS-binding protein and then transferred to their cognate extracellular receptor complex composed of CD-14, MD-2, and Toll-like receptor 4 (TLR-4) [reviewed in refs 200, 201]. Binding of LPS to the CD-14/MD-2/TLR-4 receptor complex in target cells, such as monocytes and residential macrophage populations, results via TLR-4-associated MYD88 complexes in activation of a diverse set of complex signaling cascades including the TRAF6/TAK1/IKK-α/IKK-β-IκB-NF-κB pathway [reviewed in refs 200, 202]. In macrophages, NF-κB might then upregulate in concert with its coactivator PARP-1 the expression of specific sets of pro-inflammatory mediators involved in the pathogenesis of septic shock: cytokines (TNF-α, MIF, and IFN-γ) and interleukins (IL-1β, IL-6, and IL-8). The massive production and release of cytokines by macrophages might then in turn activate in target cells, such as monocyte/macrophage populations and epithelial and endothelial cells, the NF-κB/PARP-1 complex in concert with other transcription factors, including STAT-1 and AP-1, which results in the repeated upregulation of the expression of inflammatory cytokines and chemokines (e.g., TNF-α, IFN-γ IL-1β IL-6, IL-8, MIP-1α, and MIP-2), endothelial adhesion molecules including intercellular adhesion molecules (e.g., ICAMs), endothelial leukocyte cell adhesion molecules (e.g., ELAMs or E-selectin), platelet endothelial cell adhesion molecule-1 (P-selectin), and vascular cell adhesion molecules. The increased expression of these genes in effector cells (monocytes/macrophages) and target cells (epithelial and endothelial cells) results in the recruitment and activation of PMNs and macrophages. Secretion of pro-inflammatory cytokines by recruited PMNs and macrophages leads to continued stimulation of epithelial and endothelial cells that in turn might activate the NF-κB/PARP-1 complexes. Thus, a positive autoregulatory loop might be established that can amplify the inflammatory response and increase the duration of chronic inflammation resulting in a persistent activation of the NF-κB/PARP-1 complexes characterized by over-expression of pro-inflammatory mediators and massive recruitment of PMNs and macrophages. At the same time, TNF-α, IL-1β, and IFN-γ stimulate endothelial cells, PMNs and macrophages to drastically upregulate the expression of iNOS, most probably through the NF-κB/PARP-1 complexes. Cellular iNOS induction in effector (PMNs and macrophages) and target (endothelial and epithelial) cells results in a massive increase in the concentration of NO. NO can diffuse from PMNs and macrophages to endothelial and epithelial target cells where it is converted into a cytotoxic derivative, peroxynitrite. Rapid DNA single-stranded breaks are induced by the high concentration of peroxynitrite in effector cells, leading to excessive activation of PARP-1, depletion of cellular energy, and necrotic cell death. Necrosis of endothelial cells then in turn might result again in a massive recruitment and activation of PMNs and macrophages as a positive feedback loop that finally leads to enhanced systemic inflammation, endothelial dysfunction, and organ failure.

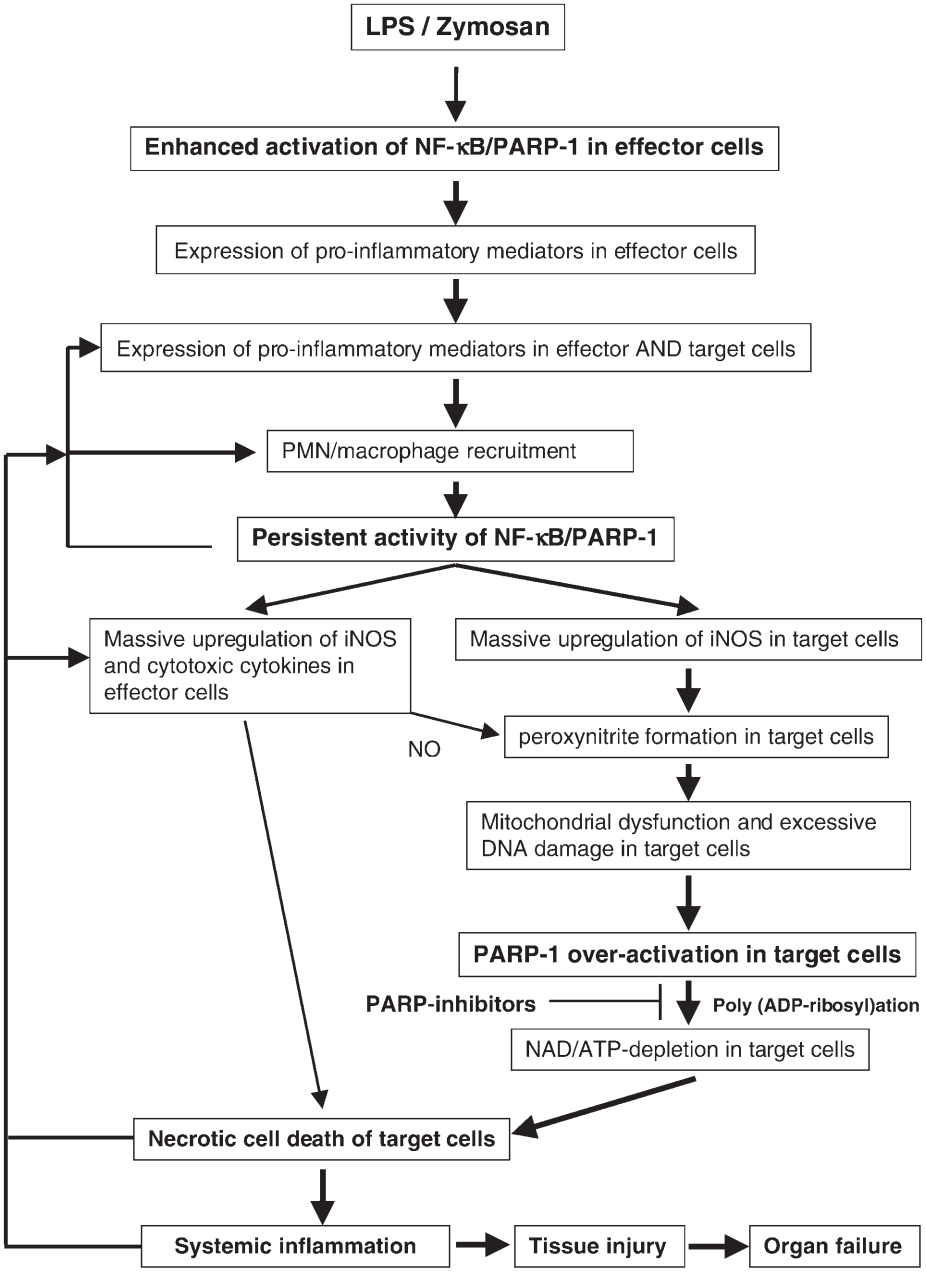

Figure 4. Proposed model for function of the transcriptional NF-κB/PARP-1 complex in inflammatory disorders.

Type 1 diabetes

Although the cellular inflammatory networks in autoimmune type I diabetes are quite different and more complex than those in LPS-induced septic shock, NF-κB in concert with its coactivator PARP-1 might play, at least in part, a similar role in autoimmune type I diabetes. Although, it is rather unlikely that the activities of the NF-κB/Rel family members and other transcription factors are dependent on PARP-1 during the activation of diabetogenic or protective/regulatory T cells and subsequent triggering of the autoimmune process such as epitope spreading and sustaining the autoimmune responses by continuous antigen stimulation, we propose that NF-κB/PARP-1 might play an important role in the recruitment of PMNs/macrophages and in disturbing the reciprocally regulated balance between Th1 and Th2 cytokines, as well as in upregulation of cytotoxic inflammatory mediators in islet-infiltrating immune cells, islet capillary endothelial cells, and in insulin-secreting β cells themselves, thus being directly involved in the destruction of the β cells, as described in previous sections.

Perspectives

Although the proposed role of PARP-1 as coactivator of NF-κB during the pathogenesis of inflammatory disorders seems to be quite convincing, much remains to be elucidated about the roles of NF-κB and PARP-1 in these processes. What is the molecular basis for the disruption of the regulatory mechanisms that control the specificity and extent of NF-κB activity in the nucleus, thereby determining the switch from an anti-apoptotic to pro-necrotic role for NF-κB? Is the aberrant NF-κB activity dependent on the nature and intensity of the activating stimulus or on the availability of coactivators as well as simultaneous activation of other transcription factors?

The impaired NF-κB activity and reduced susceptibility to excitotoxic stimuli in PARP-1–/– mice strongly suggest that not only the nature and intensity of the activating stimulus and simultaneous activation of other transcription factors, such as AP-1, C/EBPβ, and STAT-1, but also the availability of coactivators such as PARP-1 might lead to extended activation and persistent nuclear activity of NF-κB, thereby determining whether its activity leads to necrotic cell death of surrounding cells. Future studies of the NF-κB/PARP-1 transcriptosome, using DNA-oligonucleotide microarray analysis, will therefore be important for our understanding of how the processes of pathogenesis are regulated at the level of transcription. The NF-κB/PARP-1-dependent target genes activated will vary depending upon the cellular context, which is achieved through a combination of regulatory mechanisms. The selective activation of different NF-κB hetero- and homodimer complexes with subtly different DNA-binding specificities results in the targeting of different promoters and enhancers [182]. It is therefore important to elucidate whether other subunits of NF-κB beside p50 and p65 are involved in these processes. In addition, cooperative recruitment of coactivators by the NF-κB/PARP-1 complex and promoter-specific interactions with other heterologous transcription factors might enhance different targeting of promoters and result in synergistic transactivation. The cooperative interplay of interactions integrates the NF-κB response with other signaling pathways [182]. Changes in the transcription factors that function cooperatively with NF-κB/PARP-1 at different promoters might switch its activity from one set of genes to another. It will therefore also be interesting to investigate whether PARP-1/PC-1 might also cooperatively interact and function synergistically with other heterologous transcription factors such as AP-1, IRFs, HIFs, C/EBPβ, and STAT-1. Moreover, investigation of the interplay between PARP-1/PC-1 and other positive cofactor complexes as well as cell- and stimuli-specific types of Mediator and NF-κB-associated chromatin-remodeling complexes during the process of pathogenesis will be of great interest.

One of the most interesting questions of all is the functional role PARP-1 might play under normal physiological conditions. For example, whether PARP-1 is also required for the transcriptional activity of NF-κB during 'good' inflammation responses, manifested by cell survival and tissue regeneration, or only in 'bad' inflammation, causing cell death and tissue destruction. There are no reports in the literature demonstrating that PARP-1–/– mice were prone to bacterial or fungal infections, at least under normal culture conditions, implying that basal bacterial clearance is not impaired in PARP-1–/– mice. Thus, it is probably safe to say that PARP-1 does not play an important role in baseline immune processes.

In addition, it is important to note that the functions of other PARP family members, especially PARP-2 and PARP-3, in disorders as well as NF-κB-dependent processes are yet completely unknown and have to be addressed. Indeed, there is at least one report which indicates a functional role of other PARP family members in inflammatory disorders [107]. PARP–/– mice treated with 5-iodo-6-aminobenzopyrone, a novel potent PARP inhibitor, showed an increase in sensitivity to streptozotocin in the animal MDLS model, characterized by increased blood glucose and incidence of diabetes comparable to untreated PARP–/– mice [107]. These results suggest that other PARP family members may have opposite functions in inflammatory disorders.

Conclusion

One of the major challenges in understanding the role of PARP-1 in the pathogenesis of inflammatory disorders was the elucidation of its NF-κB coactivator function. DeMurcia and colleagues [70] proposed that this finding might help to design new therapeutic strategies for treatment of inflammatory disorders, based on the combined pharmacological inhibition of NF-κB and PARP-1. To date a variety of widely used anti-inflammatory agents inhibit the NF-κB pathway, resulting in a decrease in NF-κB-dependent transcription [reviewed in refs 152, 162, 203]. However, blocking the NF-κB pathway for prolonged periods is not feasible, since NF-κB plays an important role in the maintenance of host defense responses. Moreover, since PARP inhibitors are not only able to inhibit the enzymatic activity of PARP-1 but also other PARPs, complete long-term inhibition of the enzymatic activity of PARP-1 might lead to severe side effects. Therefore, the NF-κB/PARP-1 interface might be an obvious target for the development of new types of drugs disrupting specific protein-protein interactions. Disrupting the physical interaction of PARP-1 and NF-κB might inhibit the abnormal transcriptional activity of NF-κB, thereby reducing the inflammatory response at the level of transcription in instances where this process becomes

chronic or dysregulated. NF-κB/PARP-1-mediated transcriptional processes are, per se, not the only ones playing a role in inflammatory disorders but are surely a critical molecular event in these inflammatory networks. Thus, only careful designed combinations of drugs which not only inhibit NF-κB/PARP-1-mediated transcription but also the enzymatic activity of PARP-1 as well as PARP-1- and NF-κB-independent processes might lead to a complete downregulation of these detrimental processes in inflammatory disorders.

Taken together, future investigation of the various roles of PARP-1 in transcription and signaling under pathophysiological conditions in vivo, will certainly represent an intense and exciting new field of research.

Since we have focused on the NF-κB/PARP-1-dependent pathways and did not address other functions of PARP-1 in this review, we highly recommend to readers the recent review books on the subject of PARP and poly(ADP-ribosyl)ation reactions: *Cell Death: The Role of PARP*, edited by C. Szabo [CRC Press, 2000]; *From DNA Damage and Stress Signaling to Cell Death-Poly(ADP-Ribosyl)ation Reactions,* edited by G. deMurcia and S. Shall [Oxford University Press, 2000], and *PARP as a Therapeutic Target,* edited by J. Zhang [CRC Press, 2002].

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