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Beyond DNA repair, the immunological role of PARP-1 and its siblings

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Summary

ADP-ribosylation is the addition of one or more (up to some hundreds) ADP-ribose moieties to acceptor proteins. There are two major families of enzymes that catalyse this reaction: extracellular ADP-ribosyl-transferases (ARTs), which are bound to the cell membrane by a glycosylphosphatidylinositol anchor or are secreted, and poly(ADP-ribose)-polymerases (PARPs), which are present in the cell nucleus and/or cytoplasm. Recent findings revealed a wide immunological role for ADP-ribosylating enzymes. ARTs, by sensing extracellular NAD concentration, can act as danger detectors. PARP-1, the prototypical representative of the PARP family, known to protect cells from genomic instability, is involved in the development of inflammatory responses and several forms of cell death. PARP-1 also plays a role in adaptive immunity by modulating the ability of dendritic cells to stimulate T cells or by directly affecting the differentiation and functions of T and B cells. Both PARP-1 and PARP-14 (CoaSt6) knockout mice were described to display reduced T helper type 2 cell differentiation and allergic responses. Our recent findings showed that PARP-1 is involved in the differentiation of Foxp3$^+$ regulatory T (Treg) cells, suggesting a role for PARP-1 in tolerance induction. Also ARTs regulate Treg cell homeostasis by promoting Treg cell apoptosis during inflammatory responses. PARP inhibitors ameliorate immune-mediated diseases in several experimental models, including rheumatoid arthritis, colitis, experimental autoimmune encephalomyelitis and allergy. Together these findings show that ADP-ribosylating enzymes, in particular PARP-1, play a pivotal role in the regulation of immune responses and may represent a good target for new therapeutic approaches in immune-mediated diseases.

Keywords: autoimmunity; immunotherapeutics; inflammation.

Introduction

Post-translational modifications largely contribute to cell physiology by regulating protein stability, localization and functions. Many of these modifications occur in response to and regulate the interaction with environmental cues, these features being particularly relevant to immune cell functions.

ADP-ribosylation is the post-translational addition of ADP-ribose moieties from NAD to target proteins. ADP-ribosylating enzymes control a wide array of cellular processes, including DNA repair, chromatin structure, transcriptional regulation, mitochondrial functions, apoptosis, necrosis, cell division and differentiation. In the last decade, a wealth of findings have highlighted the role of ADP-ribosylating enzymes in inflammation and immune functions.[1]

The first molecule shown to have ADP-ribosylating activity was the diphtheria toxin which, by targeting the elongation factor-2 in eukaryotic cells, inhibits protein synthesis.[2] However, ADP-ribosylating enzymes are present in almost all organisms, indicating that this reaction is a phylogenetically ancient post-translational modification. ADP-ribosylation is catalysed by extracellular ADP-ribosyl-transferases (ARTs) or by nuclear/cytoplasmic poly(ADP-ribose)-polymerases (PARPs). Based on sequence, structural homologies and similarity of reactions catalysed, a unification of all ADP-ribose transferases (ARTs and PARPs) in one large protein family has been recently proposed.[3]

Among the ARTs (four expressed in humans; six in mice), ART1 (or ARTC1, ADP-ribosyl-transferase C2 and C3-like 1) and ART2 (ARTC2) are the best characterized family members. These cell membrane glycosylphosphatidylinositol-anchored receptors covalently bind single ADP-ribose moieties to extracellular or cell surface proteins using the extracellular NAD as substrate. As discussed below, by sensing NAD extracellular concentration, the level of which is very low under physiological conditions,[4] ARTs act as danger sensors and play a relevant role in inflammatory/immune responses.

PARPs (or ARTDs, ADP-ribosyl-transferase diphtheria toxin-like) constitute a large group of proteins (17 in humans; 16 in mice) sharing a highly conserved sequence within the active site, defined as the PARP signature. PARPs synthesize and bind branched polymers of ADP-ribose to acceptor proteins, such as PARPs themselves, histones, DNA repair proteins, transcription factors and chromatin regulators.[1] Actually, not all PARPs synthesize polymers.[5] PARP-1 accounts for the majority of the poly (ADP-ribose) (PAR) polymer synthesis. Although characterized for a long time as a DNA damage sensor and as a key factor in DNA repair systems, the structural basis for DNA damage-dependent PARylation by PARP-1 has been elucidated only recently.[6] PARylation also plays an important role in the regulation of gene transcription independently from DNA damage.[5,7,8] The activation of PARP-1 and other family members is regulated in response to signal pathways and modulates the activity of many transcription factors, as already reviewed.[7,9,10]

Due to its negative charge, the ADP-ribose polymer drastically affects protein functions. As also DNA is negatively charged, ADP-ribosylated histones are repelled by DNA, relaxing nucleosomal structure and allowing DNA repair enzymes as well as transcription factors to access DNA.[11] The half-life of ADP-ribose polymers is very short (minutes) because they are quickly detached from acceptor proteins and/or hydrolysed to mono(ADP-ribose) by poly(ADP-ribose)-glycohydrolase (PARG) and PAR hydrolase (ARH).[12] Free or protein-bound ADP-ribose polymers also work as signal transducers by interacting with other proteins through their PAR recognition motifs.[13] The dynamic equilibrium between polymer synthesis and degradation and the role of PARs in signal transduction are particularly important during stress responses requiring fast adaptation to environmental changes.[14] PARylation is therefore a post-translational modification that plays an important role in epigenetic regulation and gene expression under physiological conditions, also when DNA integrity is maintained.

Recently, several aspects of PARylation and PARP enzymes, with particular emphasis on PARP1, have been reviewed: members and family organization,[15] new nomenclature,[3] nuclear functions and molecular mechanisms,[1] chromatin structure and gene expression,[7,11] stress responses,[14] role in cellular signalling,[9,16] therapeutic potential of PARP inhibitors.[17,18]

Here, we summarize and discuss the recently uncovered immunological role of ADP-ribosylating enzymes from the PARP and ART families.

ADP-ribosylation at the crossroads of inflammation, cell death and innate immunity

Both ARTs and PARPs are involved in several inflammatory processes. The role of PARPs in inflammation has been investigated with pharmacological inhibitors and confirmed and/or better focused with gene-specific deficient mice.[19–22] As shown in several experimental models, PARP inhibitors display protective effects in acute and chronic inflammatory diseases (see below).[23–27]

PARP-1 sustains the expression of cytokines, chemokines and other inflammatory mediators such as tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, interferon-γ (IFN-γ), CCL3 and inducible nitric-oxide synthase (iNOS). PARP-1 is required for or increases the expression of several adhesion molecules (intercellular adhesion molecule 1, vascular cell adhesion molecule, P-selectin, E-selectin and mucosal addressin cell adhesion molecule 1), chemoattractant chemokines (IL-8, macrophage inflammatory proteins 1 and 2, monocyte chemoattractant protein 1) and matrix metalloproteinase 9. Consistently, PARP enzymatic inhibition or PARP-1 gene knock down results in inhibition of cell migration to inflammatory sites.[28–30]

A common set of inflammatory mediators (including iNOS, IL-1β, TNF-α) is regulated by both PARP-1 and PARP-2, indicating that these two enzymes modulate inflammation through partially overlapping mechanisms.[31] As many of the enzymatic inhibitors are not acting exclusively on PARP-1 or PARP-2, caution should be applied when attributing the effects of PARP enzymatic inhibition to either family member. PARP-1 is involved in gene expression and activation of neutrophils, macrophages, dendritic cells, microglia and other cell types.[19,20,32] PARP-1/2-mediated pro-inflammatory responses are not limited to cells of the innate immune system but play a relevant role also in other cell types such as endothelial cells and fibroblasts.[28,33,34] PARP-1, -2 and -3 cooperatively regulate the activation (IL-1, TNF-α and CCL2 expression) of astrocytes, cells contributing not only to central nervous system homeostasis but also to the local innate immune responses [35] (Fig. 1).

As PARG degrades the PAR polymers synthesized by PARPs, one could expect that PARG activity might result in anti-inflammatory effects. Conversely, as PARP-1 activity is blocked by extensive auto-PARylation, PARG inhibition results in PARP-1 hindrance. Indeed PARG inhibition, with gallotannin or with more recently developed non-tannin small molecules, protects astrocytes

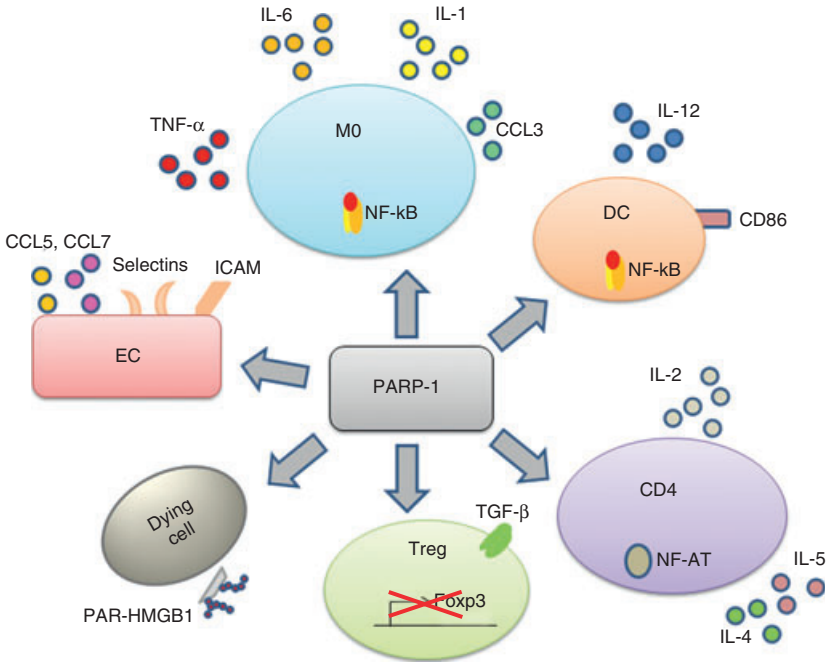

Figure 1. Poly(ADP-ribose)-polymerase 1 (PARP-1) is expressed in almost all cells and plays a central role in inflammation and immunity. PARP-1 regulates gene transcription through several mechanisms including epigenetic modifications and interactions with and modification of transcription factors. PARP-1 activates nuclear factor-κB (NF-κB; as shown in macrophages and dendritic cells) and NF-AT (T cells), increases the expression of co-stimulatory molecules (CD86), pro-inflammatory cytokines, chemokines and adhesion molecules in several cell types. In T cells, PARP-1 increases the expression of interleukin-2 (IL-2) and T helper type 2 (Th2) cytokines as well as cell proliferation, whereas it negatively controls the expression of Foxp3. PARP-1 by poly-ADP-ribosylating HMGB1 strengthens the danger signals associated with cell death. CD4, CD4 T cells; DC, dendritic cells; EC, endothelial cells; M0, macrophages; PAR-HMGB1, poly-ADP-ribosylated-HMGB1; Treg, regulatory T cells.

from oxidative stress and reduces infarct volume in brain ischaemia/reperfusion injury models.[12,36] A control of the inflammatory response with PARG inhibitors was obtained also in an experimental spinal cord trauma model, resulting in neuroprotection.[37]

Central to the role of PARP-1 in inflammation is its involvement in nuclear factor-κB (NF-κB) activation. Two recent studies demonstrated that PARP-1 is required to trigger IKKγ (NEMO, NF-κB essential modulator) SUMOylation and monoubiquitination, which in turn allow IKK and NF-κB activation.[38,39] Previous findings showed that PARP-1 interacts with elements of the NF-κB family and, acting as a docking molecule, favours the formation of the transcription complex, independently of its enzymatic activity.[40] Another study demonstrated that PARylation sustains p65 NF-κB nuclear retention by decreasing its interaction with Crm1 (a nuclear exporting protein).[41] The importance of these pathways is further strengthened by the resistance of PARP-1 knockout mice to lipopolysaccharide-induced septic shock. In macrophages from these animals, NF-κB-dependent TNF-α and iNOS expressions are compromised and the positive loop between oxidative stress and sustained PARP-1/PARP-2/NF-κB activation is compromised. As a consequence, the cytokine storm that would lead to death by septic shock is strongly reduced.[42]

PARP-1 promotes inflammation also by PARylating the high mobility group box 1 protein (HMGB1) and consequently inducing its translocation to the cytosol, where HMGB1 may leak out of necrotic cells and act as a danger pro-inflammatory factor.[43] PARylated HMGB1 released by dying cells inhibits efferocytosis, reducing the clearance of apoptotic cells from inflammatory foci and further increasing inflammation.[44] Interestingly, such a mechanism is targeted by a protease of the intracellular pathogen *Chlamydia*, which, by degrading HMGB1 and PARP-1, reduces the inflammatory response to membrane-damaged cells and prevents pathogen clearance.[45]

PARP-1 plays a relevant role in different forms of cell death.[46] PARP-1 over-activation induced by severe genomic stress or intense inflammation results in intracellular NAD and ATP depletion and consequent necrosis due to energy failure.[47] PARP-1 is also involved in apoptosis-inducing factor-mediated caspase-independent and caspase-dependent apoptosis.[48–50] Recent findings revealed that also the PAR polymer itself can act as a cell death effector downstream of PARP-1, activating a form of cell death named parthanatos.[46] Hence, PARP-1 is involved in the induction of several

forms of cell death, and the prevailing pathway depends on the balance among the degree of PARP-1 activation, the metabolic status of the cell and other intervening factors.

Tissue damage due to direct injury or inflammation induces cell death and increases NAD^+ concentration in extracellular compartments. A rise in extracellular NAD activates ART2, which ADP-ribosylates the P2X7 purinergic receptor leading to Ca^{2+} flux, pore formation and cell death. Hence, ARTs can function as danger sensors and regulate the response by amplifying the inflammatory process.

All of these findings show that (poly)ADP-ribosylation, as catalysed by ARTs and PARPs, is involved in several aspects of inflammation and contributes to the up-regulation of danger signals, creating the conditions to initiate and sustain the (innate) immune response.

(Poly)-ADP-ribosylation in T-cell development, differentiation and activation

T-cell development and function are regulated by PARylation. PARP-1 and PARP-2 expression are particularly high in the cortex and sub-capsular areas of the thymus, where immature lymphocytes actively proliferate. *Parp-2* gene inactivation (not *Parp-1*) drastically reduces the thymus size and the number of $CD4^+$ $CD8^+$ double-positive (DP) thymocytes. *Parp-2*-deficient DP thymocytes display increased apoptosis, reduced T-cell receptor (TCR) expression and skewed TCR-α chain repertoire toward 50 Jα segments.[51] Apoptosis can be reversed by the removal of p53,[52] suggesting that cell death is induced by unresolved DNA damage. These findings indicate that PARP-2 is important for T-cell survival during thymopoiesis by preventing the activation of DNA damage-dependent apoptotic response during TCR-α rearrangements.

PARP-1 plays a role also in peripheral T cells. PARP-1 knockout T cells stimulated in the absence of antigen-presenting cells display a lower cell proliferation and a similar susceptibility to apoptosis compared with wild-type cells.[53] A reduced proliferation, associated with an increased frequency of CD25/Foxp3 Treg cells, was also observed in purified PARP-1 knockout CD4 cells. Indeed, CD25-depleted CD4 cells from PARP-1 knockout mice proliferate at a rate comparable with or slightly higher than wild-type cells.[54]

During T-cell activation several transcription factors are activated through different signal pathways. PARP-1 contributes to this process by binding to and ADP-ribosylating NF-ATc1 and NF-ATc2.[55,56] According to Valdor *et al.*,[56] PARP-1 negatively regulates NF-AT as PARP inhibition increases the NF-AT-dependent transactivation and delays NF-AT nuclear export through an unidentified mechanism. At variance, according to Olabisi *et al.* [55] PARylation increases NF-AT DNA binding and PARP-1 knockout T cells exhibit reduced expression of IL-2 and IL-4. Further studies will allow us to better understand how PARP-1 modulates NF-AT activation and function.

In T cells, PARP-1 regulates positively or negatively the expression of a large number of genes including genes coding for chemokines and cytokines.[53] In PARP-1 knockout T cells, the expression of T helper type 1 (Th1) cytokine (IFN-γ) and chemokines (Xcl1, Ccl4 and Ccl9) are increased while IL-4 production is reduced.[53] Our group found that naive CD4 cells from PARP-1 knockout mice generate less IL-4- and IL-5-secreting cells and express GATA-3 mRNA at lower levels than wild-type cells, even when stimulated under Th2-polarizing conditions (unpublished observations). Lack or enzymatic inhibition of PARP-1 exposes signal transducer and activator of transcription 6 (STAT6), which is required for IL-4 signalling, to calpain-mediated degradation, with consequent reduction in GATA-3 and IL-5 mRNA expression.[57] Interestingly, STAT6-dependent activity is regulated also by PARP-14 (CoaSt6). Upon IL-4 stimulation, PARP-14 ADP-ribosylates the histone deacetylases HDACs present at the Gata3 promoter and favours STAT6 binding.[58,59] Hence, GATA-3 mRNA expression and therefore Th2 cell differentiation are regulated at different levels by multiple members of the PARP family.

Recently, our group described an increased number but normal phenotype and function of $Foxp3^+$ Treg cells in central (thymus) and peripheral lymphatic organs from PARP-1 knockout mice.[54] PARP-1 knockout naive CD4 cells stimulated in the presence of transforming growth factor-β displayed a higher rate of conversion to inducible $Foxp3^+$ Treg cells, associated with a higher Foxp3 mRNA expression. No differences in apoptosis susceptibility were observed under Treg-inducing conditions [54] (Table 1).

Also, ARTs regulate Treg cell homeostasis. Treg cells express P2X7 at high levels rendering them more suscepto NAD-induced ART2·2-mediated cell death compared with other T cells.[60] CD38 is an NAD-glycohydrolase that, by hydrolysing extracellular NAD^+, limits its availability for ART2.[61] Consistently, lower Treg cell numbers are found in mice deficient for CD38 indicating that extracellular NAD^+ regulates Treg cell homeostasis.[62] In the context of tissue damage and inflammation, when effector cells have to carry out their functions, the number of Treg cells could be limited by the ART2–P2X7 pathway [62] while differentiation of naive CD4 cells to inducible Treg cells could be inhibited by PARP-1.[54] These findings suggest that NAD and ADP-ribosylation play an important role in (local) inflammatory/immune reactions.[4]

Although Treg and Th17 cells represent two developmentally related populations of CD4 T cells with opposite functions, naive CD4 from PARP-1 knockout mice generate similar numbers of IL-17-producing retinoic acid receptor-related orphan receptor γt-expressing CD4 cells

Table 1. Immunological characteristics of poly(ADP-ribose)-polymerase-deficient mice

Knock down gene	General features	Cytokine/immunoglobulin production	T-cell and B-cell compartments
PARP-1	Knockout mice resistant to: Lipopolysaccharide-induced septic shock Streptozotomycin-induced diabetes Peroxynitrite-induced arthritis Oxazolone-induced contact hypersensitivity Reduced airway inflammation	↓ NF-κB, NF-AT and AP-1 activation ↓ IL-1β and TNF-α production ↓ iNOS expression ↓ selectins, integrins and ICAMs expression ↓ IL-2 production ↓ Th2 cytokines (IL-4/IL-5/IL-13) production ↓ T-cell proliferation ↑/= IFN-γ production = IL-17 production ↑ Xcl1, Ccl4 and Ccl9 expression ↓ Immunoglobulin gene conversion ↓ IgG2a ↑ IgA ↑ IgG2b	Cell number in lymphatic organs: Normal/small increase in T-cell number, CD4, CD8 cells; Normal B-cell number; Increased $Foxp3^+$ Treg cell number
PARP-2	Genetic deletion or silencing provides protection in: Cerebral ischaemia Colitis Doxorubicin-induced vascular smooth muscle damage Impaired astrocyte activation	↓ IL-1β production ↓ TNF-α production ↓ iNOS expression	Altered thymopoiesis: Reduced thymus size Increased Noxa expression and apoptosis in DP thymocytes Reduced TCR expression and skewed TCR-α chain repertoire
PARP-14	Reduced airway inflammation	↓ Th2 cell responses and IgE production ↓ IgA production in antigen-specific response ↓ IL-4-induced B-cell survival	Normal overall cell numbers in thymus, spleen, and lymph nodes Fewer marginal zone B cells More follicular B cells

AP-1, activator protein 1; DP, double positive ($CD4^+$ $CD8^+$); Foxp3, forkhead box P3; ICAM, intercellular cell adhesion molecule; Ig, immunoglobulin; IL, interleukin; iNOS, inducible nitric oxide synthase; KO, knock out; LPS, lipopolysaccharide; PARP, poly(ADP-ribose)-polymerase; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; *Noxa*, Latin for damage; TCR, antigen-specific T cell receptor; TNF, tumour necrosis factor; ↓, inhibition; ↑, upregulation.

when stimulated with transforming growth factor-β and IL-6.[54] Consistently, the beneficial effects of PARP inhibitors observed in experimental autoimmune encephalomyelitis (EAE) models are not associated with a reduction in Th17 cells.[32]

PARP-1 was also described as being involved in CD8 T-cell homeostasis. In particular oxidative stress can induce apoptosis in CD8 T (and natural killer) cells through PARP-1 activation,[63] this mechanism being exploited by tumour cells to evade the immune response.[64]

Roles of PARP in B-cell development

Some critical events in B-cell life require DNA resolution and rejoining, pointing to possible roles of PARP enzymes in B-cell development.[65] Although B-cell numbers are normal in peripheral lymphatic organs from PARP-1 knockout mice [66] (and our unpublished observations), several studies showed that PARP-1 is involved in B-cell differentiation and functions. Mutations in the DNA-PK gene causes defective V(D)J recombination with arrest of B- and T-lymphocyte development, resulting in the severe combined immune deficiency. In T cells the deficient V(D)J recombination can be partially rescued by knocking out PARP-1, indicating that PARP-1 exerts an anti-recombinogenic function during T-cell maturation.[67] Indeed, V(D)J recombination induces a DNA damage response in severe combined immune deficiency cells and PARylation supports coding end resolution, as shown in a B-cell precursor-derived cell line.[68]

Upon antigen stimulation, mature B cells undergo affinity maturation through the generation of immunoglobulin variants by somatic hypermutation or by gene conversion, followed by selection of the B-cell clones producing high-affinity antibody variants. Somatic hypermutation and gene conversion require the introduction of DNA lesions by the activation-induced cytidine deaminase (AID), followed by fixation via a mutagenic DNA repair mechanism.[69,70] PARP-1 is highly expressed in germinal centres and promotes (unexpectedly) mutagenic rather than conservative repair mechanisms through its BRCT domain.[71] Indeed, PARP-1 deficiency results in reduced gene conversion and in increased fidelity repair of AID-induced lesions in immunoglobulin chains.[71]

PARP-1 is also involved in the other AID-mediated process, that is class switch recombination.[69] Indeed, depending on the stimuli provided to B cells, PARP-1 inhibition or gene knock down increases the frequency of immunoglobulin class recombination to IgA, IgG1 or IgG2b whereas it decreases switching to IgG2a, facilitating alternative end-joining.[66,72,73] At variance, a selective impairment in IgA production associated with reduced marginal zone and increased follicular B-cell numbers was observed in PARP-14 knockout mice.[74] Interestingly, during class switch recombination, PARP-2 acts as a suppressor that prevents illegitimate recombination between the immunoglobulin heavy chain locus and other genes (in particular c-myc).[73]

PARP-1 binds in a sequence-specific way to the first intron of Bcl-6, where it acts as a transcriptional repressor, and its enzymatic inhibition or silencing by small interfering RNA induces Bcl-6 mRNA expression.[75] As down-regulation of Bcl-6 expression occurs during differentiation to plasma cells, PARP-1, by inhibiting Bcl-6 transcription, could sustain this process.

While PARPs take part in many critical events in B-cell maturation, there is only one study showing that ART7.1 is expressed in chicken B cells within the bursa of Fabricius where it is involved in B-cell signalling.[76]

Poly-ADP-ribosylation in immune-mediated diseases: a new therapeutic target?

The involvement of PARP enzymes in several immune-mediated diseases has been demonstrated in pre-clinical models using pharmacological inhibitors or gene-specific knockout mice [18,27] (Table 2). As revealed more recently, many of the inhibitors commonly used in PARP research bind to and dampen the enzymatic activity of more than one family member.[77] Although the beneficial effects of PARP pharmacological inhibition cannot be ascribed to the inhibition of a unique PARP, some of the effects were confirmed with knockout mice, lacking only one functional PARP family member (Table 1).

The first evidence showing that PARP-1 is involved in immune-mediated diseases was obtained in a model of rheumatoid arthritis. PARP-1 knockout mice display a less severe rheumatoid arthritis with reduced destruction of bone and cartilage associated with a lower IL-1β and monocyte chemoattractant protein 1 expression in arthritic joints compared with wild-type mice.[78] When given to mice affected by collagen-induced arthritis, AIQ, an enzymatic inhibitor that targets PARP-1, -2 and -3,[77] down-regulates the production of various inflammatory cytokines and chemokines, decreases the antigen-specific Th1-cell expansion, and induces the production of IL-10.[79] In humans, specific PARP-1 promoter haplotypes seem to be associated with susceptibility to RA [80] while a single nucleotide polymorphism in exon 17 is not.[81]

Several studies investigated the effects of PARP inhibition in EAE. Pharmacological inhibitors dampened NF-κB and AP-1 activation, inflammatory gene transcription, adhesion molecule expression and cell migration resulting in a reduced demyelination and in a better clinical score.[82–84] In secondary progressive multiple sclerosis/EAE, PARP inhibition impaired the expression of inflammatory factors and prevented new relapses, by interfering with the activation of microglia, macrophages and astrocytes induced by 15a-hydroxicholestene.[32] In contrast with these findings Selvaraj *et al.* [85] reported that, upon immunization with myelin oligodendrocyte glycoprotein, PARP-1 knockout mice had an earlier onset and developed a more severe EAE compared with wild-type mice. In EAE spinal cords from wild-type mice, PARP-1 was down-regulated and PARP-3 (but not PARP-2) was drastically up-regulated in both PARP-1 knockout and wild-type mice.[85] The PARP-1 inhibitors that induced the beneficial effects described above [32,82–84] are not exclusive to PARP-1 and target other members of the family.[77] Further studies are therefore needed to clarify whether different PARPs could play distinct roles in central nervous system immune-mediated diseases and how the use of pharmacological inhibitors could be exploited for therapeutic purposes.

Several studies showed that PARP-1 deficiency and PARP inhibition confer resistance to inflammatory bowel disease in rodent models of dinitrobenzene/trinitrobenzene sulphonic acid (DNBS/TNBS)-induced colitis by dampening AP-1 and NF-κB activation, inflammatory cytokine production and apoptosis, with consequent reduced colon damage.[26,86,87] Noteworthy, also PARG inhibitors, which hamper PAR degradation and induce PARP-1 inhibition by stabilizing its PARylated status, exert therapeutic effects in DNBS-induced inflammatory bowel disease.[88] Therapeutic effects were also obtained in IL-10-deficient mice (which develop spontaneous colitis) using PARP inhibitors [89] or targeting PARP-2 with antisense oligonucleotides.[90]

During infection of gastric epithelial cells, *H. pylori* activates intracellular PARP-1, a process associated with

Table 2. Synopsis on poly(ADP-ribose)-polymerase inhibitors use in immune-mediated disease models

Disease model	Inhibitor(s) used	Findings	Notes
Rheumatoid arthritis	AIQ	Reduced expression of inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-1β and IL-12), and chemokines (RANTES and MIP-2) in the joints of arthritic mice Increased IL-10 production Decreased collagen-specific Th1-cell expansion[79]	PARP-1KO mice display reduced RA when immunized with collagen or treated with peroxynitrite[78]
Experimental autoimmune encephalomyelitis	AIQ[32] PJ34[82] PHE[83] PJ34 and PHE[84]	Reduced NF-κB and AP-1 activation, inflammatory gene transcription (TNF-α, IFN-γ, iNOS), adhesion molecule expression, cell migration Reduced activation of macrophages, microglia, dendritic cells and astrocytes Reduced demyelination, better clinical score[32, 82–84]	AIQ targets PARP-1, -2, -3[77]; PJ34 targets PARP-1 and PARP2 and less efficiently PARP-3[77]; PHE binds to PARP-1, -2 and -3 and Tankyrases-1 and -2[77] In contrast to the results obtained with the inhibitors, PARP-1KO mice display more severe EAE compared with wild type mice[85]
Colitis	GPI6150[27] Nicotinamide[86] DiQ (1,5-dihydroxyisoquinoline)[86] 3-aminobenzamide[89]	In DNBS/TNBS model: reduced AP-1 and NF-κB activation, inflammatory cytokine production, adhesion molecules, COX2, PGE_2, apoptosis, with consequent reduced colon damage[27,86] In IL-10-deficient mice: reduced TNF-α, IFN-γ, iNOS, normalized colonic permeability[89]	DiQ targets PARP-1, -2, -3[77] PARP-1KO mice displayed reduced colon damage, apoptosis, JNK and AP-1 activation, and increased Bcl-2 expression[87]
Allergic airway inflammation	TIQ-A[94] PJ34[59]	Reduced production of IL-5, IL-10, IL-13, and GM-CSF Reduced eosinophil recruitment[94]	TIQ-A targets PARP-1, -3, and other family members including tankyrase-1[77] PJ34 targets PARP-1, -2 and, to a lesser extent other members, including tankyrase-1, -2 and PARP-14[77] PARP-1KO[94] and PARP-14KO[59] mice show reduced Th2 cytokine production and allergic airway inflammation

AIQ, 5-aminoisoquinolinone; COX2, cyclooxygenase-2; DNBS/TNBS, dinitrobenzene/trinitrobenzene sulphonic acid; DiQ, 1,5-dihydroxyisoquinoline; EAE, experimental autoimmune encephalomyelitis; GM-CSF, Granulocyte-macrophage colony-stimulating factor; GPI6150, 1,11b-dihydro-[2H]benzopyrano [4,3,2-de]isoquinolin-3-one; JNK, c-Jun N-terminal kinases; MIP-2, macrophage inflammatory protein-2 (CXCL2); PGE2, prostaglandin E2; PJ34, *N*-(6-Oxo-5,6-dihydrophenanthridin-2-yl)-(*N*,*N*-dimethylamino)acetamide; PHE, 6(5H)-phenanthridinone; RA, rheumatoid arthritis; Rantes, regulated on activation, normal T cell expressed and secreted (CCL5); TIQ-A, 4H-Thieno[2,3-c]isoquinolin-5-one; see also footnote in Table 1.

the development of peptic ulcer disease and gastric cancer in human chronic infection.[91] In mouse models, PARP inhibitors reduce gastric inflammation, prevent the T-cell-driven immunopathology and the formation of gastric precancerous lesions, and revert pre-existing lesions.[92]

The role of PARylation was also investigated in allergic airway inflammation. Following allergen challenge PARP-1 protein expression and activity are greatly increased in murine lungs.[93] Even if with some specific differences, both PARP-1 knockout and PARP-14 knockout mice show reduced IL-5 and IL-13 production (also IL-4 in PARP-14 knockout), eosinophilic recruitment, allergic airway inflammation, and IgE levels compared with wild-type animals.[59,94] Similar results were obtained using TIQ-A and PJ34 inhibitors,[59,94] both of which target PARP-1 and to a lesser extent other family members.[77] As discussed above, both PARP-1 and PARP-14 play a role in Th2 cell differentiation through different mechanisms, having an impact in allergic airway inflammation.[57–59]

Altogether the findings discussed above show that PARP pharmacological inhibition exerts protective effects

in a variety of immune-mediated disease models affecting both innate and adaptive components of the immune system.

Conclusions and perspectives

Several PARP-1 inhibitors are undergoing clinical trials (at different stages) for cancer therapy with promising results.[17] Their use is supported by the role PARP-1 plays in DNA damage detection and in base excision repair. As BRCA1 and BRCA2 proteins are critical for homologous recombination repair, the use of PARP inhibitors in BRCA-defective cancer cells is believed to result in unsustainable DNA damage and consequent tumour cell death. Pre-clinical data gave promising results also for the treatment of haematopoietic malignancies associated with ATM mutations.[95] However, some setbacks[96] should provoke reconsideration of our understanding of the mechanisms underlying the effects of PARP inhibitors, which might involve pathways other than DNA repair. Indeed, recent evidence revealed that PARP-1 inhibition limits tumour growth and development by affecting tumour-related gene expression and by inhibiting inflammatory signals and angiogenesis.[97–100] This should not be surprising as inflammatory mediators are known to play critical roles in both tumour induction and tumour growth.

As shown in the pre-clinical models discussed above, almost all acute and chronic inflammatory conditions, having different aetiopathogenesis and occurring in different specific organs or being systemic, can be attenuated by PARP inhibition, pointing to a central role of these enzymes in immune-mediated diseases and making them a suitable therapeutic target. Yet few studies have been conducted in humans to explore this possibility. Considering the role of reactive oxygen species in the activation of PARP-1, its inhibition (or the inhibition of PARG) to limit tissue damage during reperfusion in acute events, such as myocardial infarction or brain stroke, might represent one of the prime indications to develop. Infarct and brain stroke treatment could require a few days of therapy limiting the risk of potential side effects. However, despite many promising pre-clinical studies,[23,24] the beneficial effects of PARP inhibition in heart and cerebral ischaemia have been studied in only a small number of trials. In a phase I trial, aimed at the evaluation of safety, and pharmacokinetics/dynamics, a PARP inhibitor (INO-1001) reduced the levels of IL-6 and C-reactive protein in patients with myocardial infarction.[101] Pre-clinical studies also suggest a possible use of PARP-1 inhibitors in chronic inflammatory diseases, such as multiple sclerosis[102] and allergy/asthma,[103] but no clinical studies are available. However, because of the long treatments needed for these diseases, caution should be used in considering therapeutic effects versus side effects.

As summarized in this review, the available findings point to a relevant pro-response/danger/activating role of PARylation in response to external cues. Many of the pro-inflammatory effects of PARPs are mediated by PARP-1 and PARP-2, which display partially overlapping functions, PARylate each other, and can form heterodimers. Both PARP-1 and PARP-2 activate inflammatory gene expression in several cell types but they also appear to play distinct roles in lymphocyte development. The available PARP inhibitors dampen the enzymatic activity of both PARP-1 and PARP-2. Also mono-ADP-ribosylation, as catalysed by ARTs in response to cell damage occurring during inflammation, acts as an amplifying danger signal. Remarkably, some PARP inhibitors can also inhibit other NAD-using enzymes including ARTs and sirtuins.[18] Further research on poorly studied PARPs is needed to assess whether they also play an immunological role, and how different PARPs (for instance PARP-3 and PARP-1) interact and at which levels they regulate immune functions. In this context, as the specific inhibition of single PARPs is still an unmet issue, studies performed using pharmacological inhibitors should include confirmation experiments with PARP-deficient cells or small interfering RNA. Nevertheless, pluri-PARP inhibition might be useful in the treatment of certain diseases, whereas single PARP inhibition could be suitable in many others, especially to reduce side effects. The recently solved crystal structures of some PARPs in complex with inhibitors and a large study on the interactions between PARPs and a wide array of inhibitors open new possibilities in the development of inhibitors specific for a determined family member.[77,104,105] In this context, the development of inhibitors targeting sites other than the catalytic domain and interfering with the interaction between a specific PARP and other molecules should also be considered, as they could be useful for therapeutic purposes in both inflammation and cancer.

Therapeutic targeting of PARPs in human immune-mediated diseases is a potentially fruitful field deserving further and specific studies.

Disclosures

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