NAD+-Consuming Enzymes in the Regulation of Lung Immune Responses

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Abstract: Nicotinamide adenine dinucleotide (NAD+) plays a role as a coenzyme in numerous oxidation-reduction reactions and mediates not only energy metabolism and mitochondrial functions, but also calcium homeostasis, aging, and cell death. Moreover, extensive evidence attests to the great importance of the non-redox functions of NAD+ *via* NAD+-consuming enzymes. Indeed, ADP-ribose transferases, cADP-ribose synthases and sirtuins emerge as NAD+ dependent enzymes with potent regulatory function in many physiological and pathophysiological processes. They have been shown to be involved in several neurological and cardiovascular disorders as well as in cancer and inflammation. In this review we will summarize the current knowledge about function of NAD+-consuming enzymes in the regulation of the immune system with particular emphasis on lung inflammatory disorders.

INTRODUCTION

The function of the mammalian immune system is to provide protection against exogenous pathogen antigens, as well as dysfunctional or aberrant endogenous antigens such as cancer cells. Immune cells can have highly lethal effects on their targets, which allows rapid and efficient clearance of undesired antigens, cells and microorganisms. However, if left uncontrolled, excessive immune response can cause damage of normal cells and tissues. This often constitutes the basis of chronic inflammatory conditions like rheumatoid arthritis, inflammatory bowel disease, etc.

The respiratory tract is constantly exposed to a broad range of pathogenic and non-pathogenic antigens, and immune cells play a crucial role in maintenance of airway homeostasis. Uncontrolled inflammation contributes to airway dysfunction and tissue remodelling, characteristic of acute lung injury and chronic lung diseases including asthma, chronic obstructive pulmonary disease (COPD) and lung fibrosis. These diseases represent a major health problem worldwide. For that reason, there is a growing need for the development of efficient therapies to treat lung inflammation.

Nicotinamide adenine dinucleotide (NAD+) has been known for years to play a role as a coenzyme in numerous oxidation-reduction reactions. The chemistry of this molecule allows it to serve both as an electron acceptor (in its oxidized form, NAD+) and as an electron donor (in its reduced form, NADH) in reactions catalyzed by enzymes of the mitochondrial electron transport chain, leading to the generation of adenosine triphosphate (ATP). Despite its role in energy metabolism, intracellular NAD+ is involved in various biological processes, including calcium homeostasis, antioxidation/generation of oxidative stress, gene expression, aging, and cell death [1]. Moreover when released into the extracellular compartment *via* lytic or non-lytic mechanisms [2], NAD+ can also act as a mediator or modulator of multiple immune and inflammatory responses [3]. However, the origin of endogenous extracellular NAD+ is still a matter of debate. Recently it has been shown that a significant level of NAD+ is present in exudates during the early phase of the inflammatory response and that this concentration is sufficient to provoke NAD+-induced T cell death (NICD) *in vitro* [4]. Injection of NAD+ into wild type mice induces massive depletion of T cells [4, 5] confirming the inhibitory function of NAD+ in T cell proliferation and cytotoxic activity *in vitro* [5]. It has been demonstrated by Seman *et al.* [6] that NAD+ selectively induces apoptosis of mouse T cells (both CD4+ and CD8+) but not B cells. Furthermore, the sensitivity to NICD is dependent on the developmental stage of lymphocytes [7]. Primed T cells escape the deleterious effect of NAD+, as fraction of activated/memory cells (CD44high, CD62Llow) is strongly enlarged after NAD+ treatment [4]. It has been shown as well that sensitivity to NICD is high in CD4+CD25+ regulatory T cell. This suggests that NAD+ treatment during the early phase of the immune response could contribute toward improved response through the elimination of naïve and immunosuppressive regulatory T cells [8].

Recent evidence demonstrated the involvement of NAD+ in the regulation of lung immune responses *via* NAD+-consuming enzymes. Indeed, NAD+ can serve as a substrate for covalent protein modification catalyzed by three classes of enzymes: (i) adenosine diphosphate (ADP)-ribose transferases (ART) or poly(ADP-ribose) polymerases (PARP), (ii) ADP-ribose cyclases: CD38 and CD157, and (iii) sirtuins (type III protein lysine deacetylases). In this review we will address the question of what the role is of NAD+ depending enzymes in the maintenance of lung homeostasis and whether modulation of their activity could potentially serve as a target in anti-inflammatory treatment.

(ADP-RIBOSE) TRANSFERASES

Over 45 years ago Chambon *et al.* [9] demonstrated that addition of NAD+ to hen liver nuclear extracts stimulated

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synthesis of poly-ADP-ribose. This was followed by the discovery of mono-ADP-ribosylation in studies of bacterial toxins [10]. Mono-ADP-ribosylation is a phylogenetically ancient and covalent posttranscriptional modification of proteins in which the mono-ADP-ribosyltransferases (mARTs) transfer the ADP-ribose moiety of NAD+ onto a specific amino acid residue in target proteins while nicotinamide is released [11]. Poly-ADP-ribose is a homopolymer of ADP-ribose units linked by glycosidic bonds and synthesized by members of the poly(ADP-ribose)-polymerase (PARP) family [12]. Similarly to mono-ADP-ribose synthesis, poly-ADP-ribose synthesis requires NAD+ as a precursor. The simultaneous presence of ADP-transferase and hydrolase activity of most of the mARTs and PARPs, and the existence of ADP-ribosylhydrolases suggest that ADP-ribosylation of proteins acts as a reversible regulatory mechanism [13, 14]. ADP-ribosylation reactions have recently been shown to play important roles in many physiological and pathophysiological processes, including inter- and intracellular signalling, transcription, DNA repair, cell cycle regulation, mitosis as well as necrosis and apoptosis [15-17]. In the next sections we will summarize the current knowledge about the role of ADP-ribosylating enzymes in the modulation of immune responses with particular emphasis on lung inflammatory disorders.

Mono-(ADP-Ribose)-Transferases

In mammalian cells mono-ADP-ribosylation is catalyzed by arginine-specific mARTs, which share less than 10% sequence identity, but are structurally similar in their catalytic sites. The mammalian mART family includes four human subtypes (ART1, 3, 4, 5) and six murine subtypes (ART1, 2.1, 2.2, 3, 4, 5) [18]. All mARTs are linked to the cell surface *via* a glycosylphosphatidylinositol-anchor, except for ART5, which is a secreted protein [19]. The ART1 and ART2 proteins are the best characterized members of mART family at the genetic, biochemical and cell physiological level [15, 20]. As no enzyme activity has yet been demonstrated for ART3 and ART4 [18] and as ART5 functions are not yet known, this review will be focusing on ART1 and ART2 and their potential role in immunity.

ART1

ART1 has been shown to be expressed in skeletal muscle [21], heart [22] and epithelial cells [23] as well as activated granulocytes [24]. However its function and protein targets are still not well elucidated. Allport *et al.* [25] demonstrated an important role of ART1-dependent ADP-ribosylation in inflammation through modulation of granulocyte functions. In human neutrophils, they showed a close correlation between ART1 activity and actin polymerization as well as chemotaxis. It has been also shown that ART1, which is constitutively expressed on the surface of cells lining the airway lumen [23], modifies defensin human neutrophil peptide-1 (HNP-1), an antimicrobial peptide released by neutrophils. Indeed, ADP-ribosylation by ART1 and not other mART decreased antimicrobial and cytotoxic activities of HNP-1 but not the ability to stimulate T cell chemotaxis and interleukin-8 (IL-8) release from lung epithelial cells [26]. Moreover ADP-ribosylated HNP-1 was identified in bronchoalveolar lavage fluid (BALF) from patients with asthma, idiopathic pulmonary fibrosis, or a history of smoking but not in normal volunteers [27]. ART1 has been shown to possess pro-inflammatory functions as its inhibitors, meta-iodobenzyl-guanidine and novobiocine, suppress the lipopolysaccharide (LPS)-induced production of IL-6 and IL-8 in human lung epithelial cells [28]. Furthermore, ART1 was upregulated in lung epithelial cells after stimulation with lipotheicoic acid and flagellin but not with LPS, peptidoglycan or poly(I:C) [29], revealing a potentially important role of mono-ADP-ribosylation in innate immune responses in the airways.

ART2

The murine ART2 gene encodes two homologous isoforms, ART2.1 and ART2.2, whereas human beings do not express ART2 due to a functional inactivation of the ART2 gene by premature stop codons [30]. Mouse ART2 homologs are coexpressed on the surface of most mature peripheral T lymphocytes and show similar target specificities [31]. Yet ART2.1 is active only in the presence of reducing agent such as dithiothreitol (DTT) or cystein while ART2.2 appears to be constitutively active [32]. The ART2 protein has been shown to play a potentially important role in the control of immune responses by regulating T cell function [33], however its role in the lung immunity is not yet known.

In the presence of micromolar concentrations of extracellular NAD+, ART2 ADP-ribosylates several membrane proteins including lymphocyte function-associated antigen 1 (LFA-1) [34], CD8, CD44, CD45 and CD11a [35], which inhibits cell contact and T cell trafficking [35]. ART2-mediated modification of surface proteins would thus have an inhibitory effect on the expansion of T cell responses. As previously mentioned, NAD+ was shown to induce T cell apoptosis [5]. This was demonstrated to be an ART2-dependent mechanism [33, 36]. Indeed two crucial findings confirmed ART2 as a necessary mediator of NICD. First, cells derived from ART2-deficient mice were resistant to NICD [37]. Second, pre-incubation of cells with ART2-specific antibodies effectively protected cells from NICD as well [6, 31, 36]. Seman *et al.* [6] have identified cytolytic P2X7 purinergic receptor (P2X7) as a key effector of NICD. Indeed, P2X7 in mouse T cells can be directly activated by ART2.2-mediated ADP-ribosylation, which induces calcium flux, pore formation, phosphatidylserine exposure, shedding of CD62L, cell shrinkage, and propidium iodide uptake [6]. Recently it has been shown that T cell activation induces down modulation of P2X7 expression and shedding of ART2 by TACE metalloproteinase leading to resistance to NICD [4, 7, 38]. Alternatively, CD4+Foxp3+ regulatory cells were demonstrated to be highly sensitive to NICD through the ART2-P2X7 pathway [8, 39]. This suggests that elimination of part of the naïve and regulatory T cells *via* ART2-dependent ADP-ribosylation would allow the expansion of Ag-primed T cell populations and thus increase T cell responses. However the exact role of ART2 in the development of the immune responses needs to be elucidated.

ART2 was shown as well to play an important role in autoimmune responses, since a deficiency of ART2 was observed in a model of autoimmune diabetes [39]. In the BB rat model of human type 1 diabetes, transfusion of ART2+ T cells protects the animals against autoreactive cells targeted to pancreatic beta cells [40]. In another study, a deficiency in a novel immunoregulatory population of ART2+ intestinal

intraepithelial lymphocytes has been associated with the development of autoimmune diabetes [41]. On the other hand, mice lacking ART2 exhibit increased survival in a model of natural killer T cell-mediated autoimmune hepatitis [37, 42]. Hence other studies are necessary to clarify the role of ART2 protein in autoimmune diseases.

Although ART2 expression on T cells is extensively studied, its expression on in other cell types is ill-defined. It was shown that neither spleen macrophages nor peripheral macrophages displayed ADP-ribosyltransferase activity [35]. Recently, this has been reported as well for bone marrow derived macrophages (BMDMs), which were shown to lack constitutive expression of any of the six murine mARTs. However, stimulation of BMDMs with LPS, interferon (IFN)-β or IFN-γ induced high expression of ART2.1, but not ART2.2. The catalytic function of the induced cell surface ART2.1 was strictly dependent on the presence of extracellular thiol-reducing cofactors, suggesting that *in vivo* activity of ART2.1-expressing macrophages may be potentiated in hypoxic conditions often present at inflammatory sites [43]. As macrophages and T cells are potent regulators of lung immunity, these recent findings support a potential role of ART2 protein in the development and maintenance of immune responses in the lungs. Yet, no study has been performed to test whether ART2 deficient mice are less susceptible to inflammatory stimuli. Thus, it would be interesting to investigate whether abrogation of ART2 can attenuate immune responses in models of chronic lung inflammatory diseases.

Poly(ADP-Ribose)-Polymerases

PARP-encoding genes now constitute a superfamily of at least 18 members that share homology with the catalytic domain of the founding member, PARP-1. The characterization of all family members is incomplete so there is still concern that some PARP-like homologues may not be catalytically active and thus are not actually PARPs [12]. PARP-1 protein is the best studied member of PARP superfamily and its activity is responsible for approximately 90% of the total poly(ADP-ribose) formation in a living cell. This was confirmed in PARP-1 deficient mice, which show a residual 10% polymer production after challenge [44]. The role of PARP-1 was broadly investigated and it was shown to play an important role in DNA repair, maintenance of genomic integrity, replication and transcriptional regulation [45]. As such, the key acceptors of poly-ADP-ribose are histones, DNA repair proteins and transcription factors. On the other hand, high PARP-1 activity induces cell death either by apoptosis or by necrosis *via* depletion of intracellular NAD+ [46]. Therefore PARP-1 was shown to be involved in several neurological [47-49] and cardiovascular disorders [50] as well as in cancer [51] and inflammation [17, 52, 53]. Focusing on the connection between PARP-1 dependent cell death and PARP-1 regulated inflammation, selected publications related to the lung pathologies will be discussed below.

PARP-1 in Lung Disorders

PARP-1 was shown to play an important role in several lung disorders including fibrotic lung diseases, ischemia/reperfusion injury, sepsis and endotoxic shock. For PARP-1 function in fibrotic lung diseases, the reader is referred to the review article of Virlág [54], while the other lung diseases will be discussed herein.

Ischemia/reperfusion (I/R) occurs when blood flow is temporarily blocked and then restored. Oxidative stress and inflammation occurring during reperfusion may cause severe tissue injury in various organs including the lungs, heart and brain. A protective activity of PARP-1 inhibition was shown in I/R-induced brain and heart injury [55]. In the lungs, the PARP inhibitors INO-1001 and 3-aminobenzamide (3-AB) decreased vascular permeability and alveolar leukocyte accumulation caused by I/R injury [56, 57]. This was correlated with decreased nuclear translocation of nuclear factor-κB (NF-κB) and activator protein 1 (AP-1) and secretion of cytokine-induced neutrophil chemoattractants (CINC) and monocyte chemoattractant protein 1 (MCP-1) into the alveolus (Fig. **1A**) [58]. The beneficial effects of INO-1001 were also shown in a dog model of cardioplegic arrest and extracorporeal circulation [59] and a rat model of obliterative bronchiolitis occurring after lung transplantation [60]. Allografted airways treated with INO-1001 demonstrated attenuated NF-κB nuclear translocation and reduced transcription of tumour necrosis factor (TNF)-α, indicating pro-inflammatory effects of PARP-1 activation [60]. Recently, Su *et al.* [61] have demonstrated elevated levels of poly-ADP-ribose in the lungs following I/R. Moreover, nicotinamide reduced the expression of inducible nitric oxydase synthase (iNOS) and pro-inflammatory cytokines, as well as the levels of nitric oxide (NO) and free radicals, while restoring ATP levels. These effects were suggested to be due to PARP-1 inhibition [61].

Oxidative stress induced by hyperoxia was shown to induce PARP activity in oxygen-exposed mouse lungs [62]. Moreover, in this model, PARP-1 deficient mice exhibited significantly higher lung cell hyperplasia and proliferation than wild type (WT) animals. This was accompanied by an increased inflammatory response in PARP-/- mice characterized by increased neutrophil infiltration and IL-6 levels in BALF. However, in contrast to WT mice, the lesions caused by hyperoxia were reversible and did not result in fibrosis [63].

Recently, over-activation of PARP-1 has been shown to play an important role in transcriptional pathogenesis of ventilator-induced lung injury (VILI), and PARP-1 inhibition has potentially beneficial effects on the prevention and treatment of VILI [64]. Indeed PJ-34, a PARP-1 inhibitor, decreased the levels of IL-6 and active plasminogen activator inhibitor 1 (PAI-1) in the lungs, attenuated leukocyte lung transmigration, and reduced pulmonary oedema and apoptosis (Fig. **1B**) [65]. PARP-1 inhibitors were also demonstrated to reduce the lung injury caused by bleomycin, a DNA damaging agent [66].

The influence of PARPs activity during sepsis has been tested extensively [53, 67, 68]. In 2002, Liaudet *et al.* [69] demonstrated that the absence of functional PARP-1 reduced LPS-induced cytokine expression (TNF-α, MIP-1α and IL-6), NO production, and lipid peroxidation (Fig. **1C**). Alveolar neutrophil accumulation, hyperreactivity and lung damage were also attenuated [69]. PARP-1 gene silencing or inhibition by niacinamide, but not by PJ-34, also diminished IL-1β and MIP-2 expression [69, 70]. The beneficial effect of pharmacological PARP-1 inhibition was also shown in an

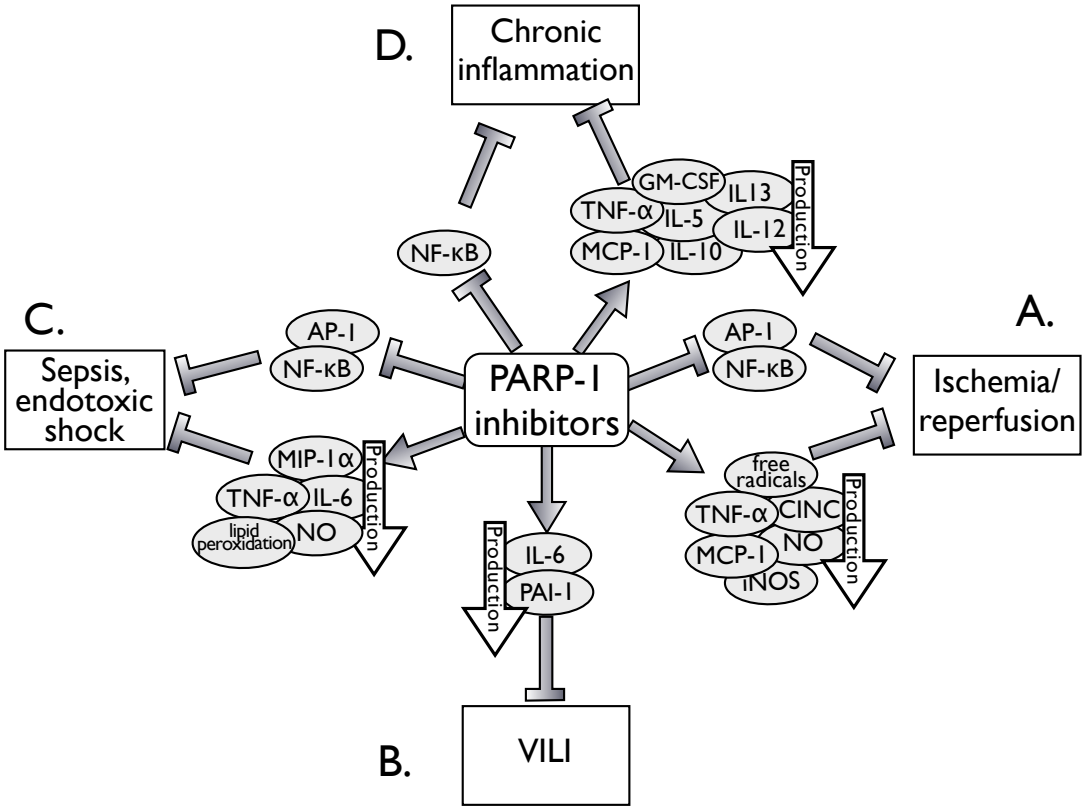

Fig. (1). Beneficial effect of PARP-1 inhibition in lung disorders. (**A**) Use of PARP-1 inhibitors reduced lung injury caused by ischemia/reperfusion through inhibition of transcription factors NF-κB and AP-1, and a reduction of CINC, MCP-1, TNF-α, iNOS, NO and free radicals levels. (**B**) PARP-1 inhibition potentially has beneficial effects on the prevention and treatment of VILI as pharmacological inhibitors of PARP-1 decreased IL-6 and active PAI-1 levels. (**C**) PARP-1 inhibitors reduced AP-1 and NF-κB activity as well as MIP-1α, TNF-α and IL-6 levels and prevented against sepsis/endotoxic shock. (**D**) In several models of chronic lung inflammation, pharmacological inhibition or genetic ablation of PARP-1 reduced inflammation through decreased NF-κB activity and pro-inflammatory mediator production (IL-5, IL-10, IL-12, IL-13, GM-CSF, TNF-α and MCP-1). AP-1, activator protein 1; CINC, cytokine-induced neutrophil chemoattractant; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN, interferon; iNOS, inducible nitric oxydase synthase; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein 1α; NF-κB, nuclear factor-κB; NO, nitric oxide; PAI-1, plasminogen activator inhibitor 1; PARP, poly(ADP-ribose)-polymerase; TNF-α, tumour necrosis factor α; VILI, ventilator-induced lung injury.

ovine model of acute lung injury caused by smoke inhalation and *Pseudomonas aeroginosa* instillation [71] as well as in LPS-induced lung injury in rabbits [72]. The pretreatment of rabbits with 3-AB attenuated LPS-induced AP-1 activation but enhanced NF-κB activation [72]. On the other hand, several groups already demonstrated that PARP-1 inhibitors, like 4-hydroxyquinazoline (4-HQN) or 3-AB, decreased activation of NF-κB and AP-1 in murine model of LPS-induced endotoxic shock (Fig. **1C**) [58, 60, 73]. Taken together, although PARPs inhibition has been proved to be effective against endotoxin induced organ failure, additional studies are necessary to elucidate the molecular mechanism of this process.

PARP-1 in Chronic Inflammatory Diseases

Important roles of PARP-1 were proposed in chronic lung inflammatory diseases as well. Indeed the percentage of poly-ADP-ribose polymer-positive lymphocytes appeared to be higher in COPD patients than in healthy age-matched controls, while the blood level of NAD+ was significantly reduced in COPD patients [74]. Moreover, it was demonstrated that theophylline, a widely used compound in the treatment of COPD, inhibits PARP-1 in a dose dependent manner [75], suggesting PARP-1 as a potential target in COPD treatment. Recently, systemic PARP-1 activation was found in alveolar macrophages in response to prolonged exposure to the fuel compound JP-8 [76]. This suggests that an extensive induction of PARP-1 may coordinate persistent expression of pro-inflammatory mediators and stimulate chronic inflammatory responses. Consistent with these findings, several studies demonstrated the beneficial role of PARP-1 inhibitors in chronic allergic inflammation – asthma. The first study directly addressing the role of PARP activation in asthma was published by Boulares *et al.* [77]. They showed increased activity of PARP-1 in a murine model of asthma evoked by intranasal application of ovalbumin (OVA) in OVA-sensitized mice. Use of 3-AB prevented OVA-elicited airway inflammation. Moreover, PARP-1-/- mice were resistant to OVA-induced inflammation. Another group using the same animal model showed that asthma prevention by PARP-1 inhibition is strongly dependent on the severity of lung inflammation [78]. Indeed, severe inflammation was not affected by a PARP-1 inhibitor (PJ-34), and expression of MIP-2 or production of eosinophil

recruiting IL-5 or IL-13 was not impaired. However, inflammatory cytokine (TNF-α and IL-12), and chemokine (MIP-1) production was strongly suppressed [78]. Also in a guinea pig model of OVA–induced asthma, the PARP-1 inhibitors 3-AB and 5-aminoisoquinolinone (5-AIQ) decreased TNF-α amounts in BALF and improved all the respiratory parameters assayed [79]. In contrast to the results of Virág *et al.* [78], Oumuna *et al.* [80] demonstrated that PARP-1 is involved in allergen-induced lung inflammation by preventing eosinophilic infiltration into the airways. They have shown that production of IL-5, IL-10, IL-13 and granulocyte macrophage-colony stimulating factor (GM-CSF) was completely abrogated in lungs of OVA-challenged PARP-1-/- mice, while production of IL-4 was only moderately affected (Fig. **1D**). These effects were mimicked by a single injection of the PARP inhibitor TIQ-A prior to OVA challenge in WT mice [80]. A single injection of TIQ-A one or 6h post-allergen challenge also conferred similar reduction in OVA challenge-induced eosinophilia [81]. Recently, Naura *et al.* [82] have demonstrated a reciprocal relationship between iNOS and PARP-1 as a potential regulatory mechanism of allergen-induced eosinophilia. iNOS expression was shown to be required for PARP-1 activation upon OVA challenge and PARP-1 was required for iNOS expression. However the expression of iNOS seems to be dispensable for eosinophilia as iNOS inhibition is ineffective in preventing allergen-induced inflammation in humans [82, 83]. Therefore, further studies are necessary to elucidate the exact function of poly-ADP-ribosylation in the regulation of allergic inflammation.

ADP-RIBOSE CYCLASES

The ADP-ribose cyclase family in mammals consists of 2 membrane ecto-enzymes, known as CD38 and CD157, which are highly conserved and have been found in organisms ranging from sea slugs to mammals [84]. Expressed in distinct patterns in most of tissues, CD38 and CD157 cleave NAD+ and NADP+, generating cyclic ADP-ribose (cADPR), nicotinic acid adenine dinucleotide phosphate (NAADP+) and ADP-ribose, which play important roles in calcium signalling [85]. Members of the ADP-ribose cyclase family control complex processes, including cell activation and proliferation, muscle contraction, hormone secretion, and immune responses [86]. Moreover they serve as receptors with enzyme-dependent or –independent signalling properties [84, 87].

CD157 protein was demonstrated to play a role in B cell development and antibody production *in vivo* and to regulate critical functions of human neutrophils, promoting polarization, chemotaxis and diapedesis. However its function in immunity still needs to be elucidated.

Contrarily to CD157, CD38 function has been broadly studied and several reviews are available concerning its role in immunity [84, 86, 87]. CD38 is used as a convenient marker of immune cell development and indicator of progression of several diseases like human leukemias and myelomas. It is directly involved in the pathogenesis and outcome of human immunodeficiency virus infection and chronic lymphocytic leukaemia, and controls insulin release and the development of diabetes [86]. The loss of CD38 function is associated with impaired immune responses, metabolic disturbances, and behavioural modifications in mice [86]. Moreover Deshpande *et al.* [88] have shown decreased lung responsiveness to different doses of metacholine in CD38-/- mice, as determined by changes in lung resistance and dynamic compliance. Airway hyperreactivity (AHR) was also shown to be diminished in CD38-/- mice in several mouse models of allergic inflammation [89-91]. CD38-/- mice develop significant airway and parenchymal inflammation after nasal IL-13 challenges and both WT and CD38-/- mice develop AHR. However, the magnitude of AHR observed in WT mice was significantly greater than that of CD38-/- mice [91]. This was confirmed in an *Aspergillus fumigatus*-induced model of allergic asthma, where again allergen-associated AHR was decreased in CD38-/- mice, despite preserved airway inflammation [90]. CD38-/- mice sensitized and airway challenged with OVA exhibited strongly reduced AHR in comparison with WT mice. However, this was accompanied by a decrease in typical hallmarks of pulmonary inflammation, including eosinophilia and lymphocytic lung infiltrates, as well as IL-4, IL-5 and IL-13 levels [89]. Antigen-specific immunoglobulin (Ig) E and IgG1 antibody titers were substantially reduced. Transferring CD38-/- bone marrow into WT mice showed reduced AHR levels, while reconstitution of lethally irradiated CD38-/- mice with WT bone marrow did not restore WT levels of AHR [89]. These studies demonstrate that CD38 acts as a modulator of lung inflammation and furthermore, the CD38

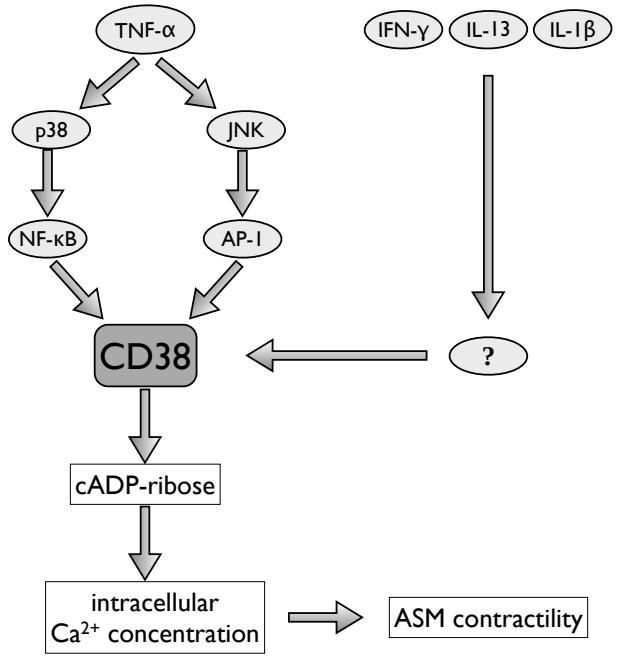

Fig. (2). Regulation of CD38 expression in ASM cells. TNF-α ligation to its cell surface receptor results in p38 and JNK MAPK activation. p38 and JNK activate transcription factors NF-κB and AP-1 which in turn increase the expression of CD38. IFN-γ, IL-1β and IL-13 increase CD38 expression as well, through yet unknown molecular pathways. Increased activity of CD38 results in cADP-ribose production, increased intracellular calcium concentration and subsequently increased ASM contractility. AP-1, activator protein 1; ASM, airway smooth muscle cells; IFN, interferon; IL, interleukin; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; TNF-α, tumour necrosis factor α.

function in AHR development was attributed to changes in calcium homeostasis in airway smooth muscle cells (ASM) induced by inflammatory and Th2 cytokines [92]. *In vitro* studies showed that CD38 expression and its enzymatic activities are increased by TNF-α, IFN-γ, IL-1β and IL-13 (Fig. **2**) [93-95]. TNF-α-induced CD38 expression is transcriptionally regulated and involves NF-κB-dependent and -independent mechanisms [96]. Recently it has been shown that CD38 expression in ASM cells can be regulated by NF-κB and AP-1 activation involving both p38 and JNK MAP kinases [97]. The increase in CD38 expression is associated with augmented intracellular calcium concentration, which are largely attributable to cADP-ribose and subsequent increased ASM contractility (Fig. **2**) [94]. These studies together with murine models of allergic inflammation implicate CD38 in normal airway function and AHR.

SIRTUINS

Sirtuins are evolutionary conserved proteins, discovered in *Sacharomyces cerevisiae* by Klar *et al.* [98] during studies on the silencing at the mating-type loci. Later, a set of four genes was discovered in yeast (SIR1-4) [99]. Due to the global deacetylation of yeast histones observed when SIR2 was overexpressed, sirtuins have been classified into the class III of histone deacetylases (HDAC) [100], as they are strictly NAD+-dependent in contrast to class I, II and IV (HDAC) that are Zn+- dependent enzymes. Indeed, sirtuins cleave NAD+ to produce nicotinamide and O-acetyl-ADP-ribose [101]. Moreover Frye [102] has demonstrated that SIR2 proteins from bacteria, yeast and mammals were able to transfer an ADP-ribose group from NAD+ to protein carriers revealing an ADP-ribosyltransferase activity of sirtuins. In mammals seven homologues of SIR2 have been identified (SIRT1-7) [102, 103]. They are ubiquitously expressed in human tissues, although higher mRNA expression levels are detected in the brain and testis for most of the sirtuins [102, 104]. The most studied mammalian sirtuin, SIRT1 has been shown to regulate histones (H1, H3 and H4) [104] as well as non-histone substrates including p53 [105], forkhead transcription factors (FOXOs) proteins [106], p300 [107], NF-κB [108] and peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) [109], implicating sirtuins in apoptosis, cell survival, transcription, caloric restriction and aging. SIRT1 was also suggested to play a role in inflammation *via* the regulation of pro-inflammatory transcription factors. Indeed, it was demonstrated that SIRT1 regulates NF-κB-dependent transcription in response to TNF-α [108] through deacetylation of the RelA/p65 subunit of NF-κB, thereby inhibiting NF-κB activity [108, 110]. This was supported by the studies of Pfluger *et al.* [111] where it was shown that mice over-expressing SIRT1 are protected from hepatic inflammation and display marked LPS hypersensitivity due to lower NF-κB activation. In addition, SIRT1 has been shown to repress transcriptional activity of AP-1, another pro-inflammatory transcription factor [112]. However, its role *in vivo* is not yet elucidated. Recently, the role of sirtuins in lung inflammatory diseases has been assessed. Indeed, reduced levels of SIRT1 and increased activation of NF-κB were shown in lungs of smokers and patients with COPD. This may result in an abnormal NF-κB-mediated chronic inflammatory effect [113]. The importance of SIRT1 in lung inflammation was confirmed in SIRT1-deficient mice which present increased neutrophil infiltration in the lungs [114]. These observations therefore suggest that SIRT1 inhibition leads to exaggerated lung inflammation.

Environmental stress, such as cigarette smoke exposure, has been shown to decrease SIRT1 levels both in macrophages *in vitro* and in rat lungs *in vivo* [115]. This was associated with increased IL-8 and TNF-α release. Moreover, a pharmacological activator of SIRT1, resveratrol, attenuated cigarette smoke extract-induced pro-inflammatory cytokine release in human monocyte/macrophage cells (MonoMac6), supporting an anti-inflammatory potential of SIRT1 [115]. The recent studies of Rajendrasozhan *et al.* [113] have demonstrated that decreased SIRT1 levels in cigarette smoke extract-treated MonoMac6 cells was associated with increased acetylation of RelA/p65 (Fig. **3**). Loss of SIRT1 resulted in increased acetylation of RelA/p65 and IL-8 release, whereas overexpression of SIRT1 decreased IL-8 release in response to cigarette smoke extracts [113]. Altogether further studies are necessary to investigate the mechanism of sirtuins inhibition *in vivo* and the potential use of SIRT1 modulators in the treatment of inflammation.

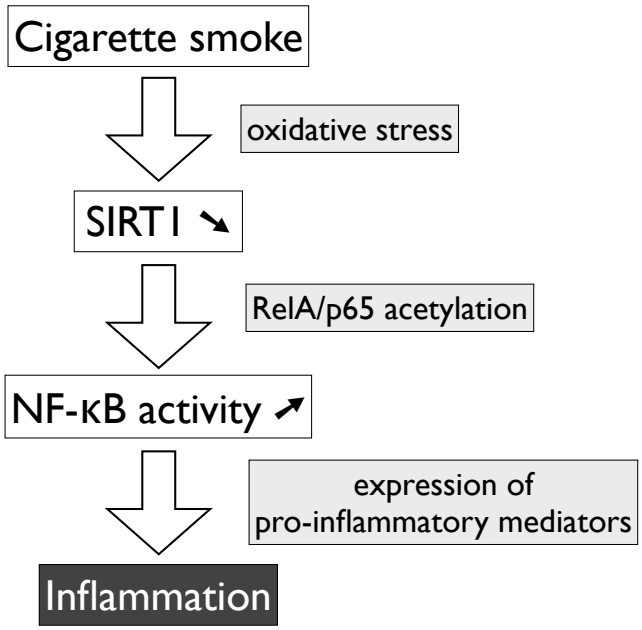

Fig. (3). Cigarette smoke inhibits SIRT1 leading to increased inflammation. Cigarette smoke-mediated oxidative stress reduces the expression of SIRT1, resulting in increased acetylation of the RelA/p65 subunit of NF-κB. Enhanced activity of NF-κB leads to increased transcription of pro-inflammatory mediators and subsequently to inflammation. NF-κB, nuclear factor-κB; SIRT1, sirtuin 1.

CONCLUSIONS

Extensive evidence presented in this review attests to the great importance of the non-redox functions of NAD+ in inflammation and inflammatory disorders. Although the role of NAD+ in inflammatory lung disease is not completely elucidated, recent findings suggest that NAD+-consuming enzymes are crucial regulators of immune responses. As summarized herein, various studies showed that NAD+-dependent enzymes are active in a majority of immune and non-immune cells involved in the maintenance of lung ho-

meostasis. These enzymes include ADP-ribosyltransferases in neutrophils, lymphocytes and epithelial cells, ADP-cyclases in ASM, neutrophils and B cells, and sirtuins in macrophages (Fig. **4**). Moreover, several observations suggest that there is an important interplay between these three groups of enzymes. Indeed, ARTs, ADP-cyclases and sirtuins consume the same substrate, NAD+, consequently limiting its availability to each other. For instance, SIRT1 and ART2 activity has been shown to be downregulated by CD38 [116, 117] and PARPs inhibits SIRT1 activity by declining the NAD+ level and rising nicotinamide levels [118]. As the NAD+ consuming enzymes often function in the same processes but in opposite ways, the balance between their activities may be essential for the regulation of the amplitude and duration of the inflammatory responses. Consequently, it would be of great interest to understand how the cross talk between these NAD+- consuming enzymes occurs at a subcellular level and how NAD+ homeostasis is regulated, under normal conditions and upon inflammatory challenges. This knowledge will likely be crucial for the development of NAD+-dependent enzyme modulators and their potential therapeutic use. Indeed, there is a growing body of experimental evidence demonstrating a protective activity of PARP-1 inhibitors in several animal models of lung inflammatory disorders. Moreover, pharmacological activator of sirtuins, resveratrol, diminished inflammation through decreased proinflammatory cytokine release. Thus, specific inhibition or activation of NAD+-consumers during lung inflammation may be a novel and powerful tool in therapeutic intervention of chronic inflammatory lung diseases in the future.

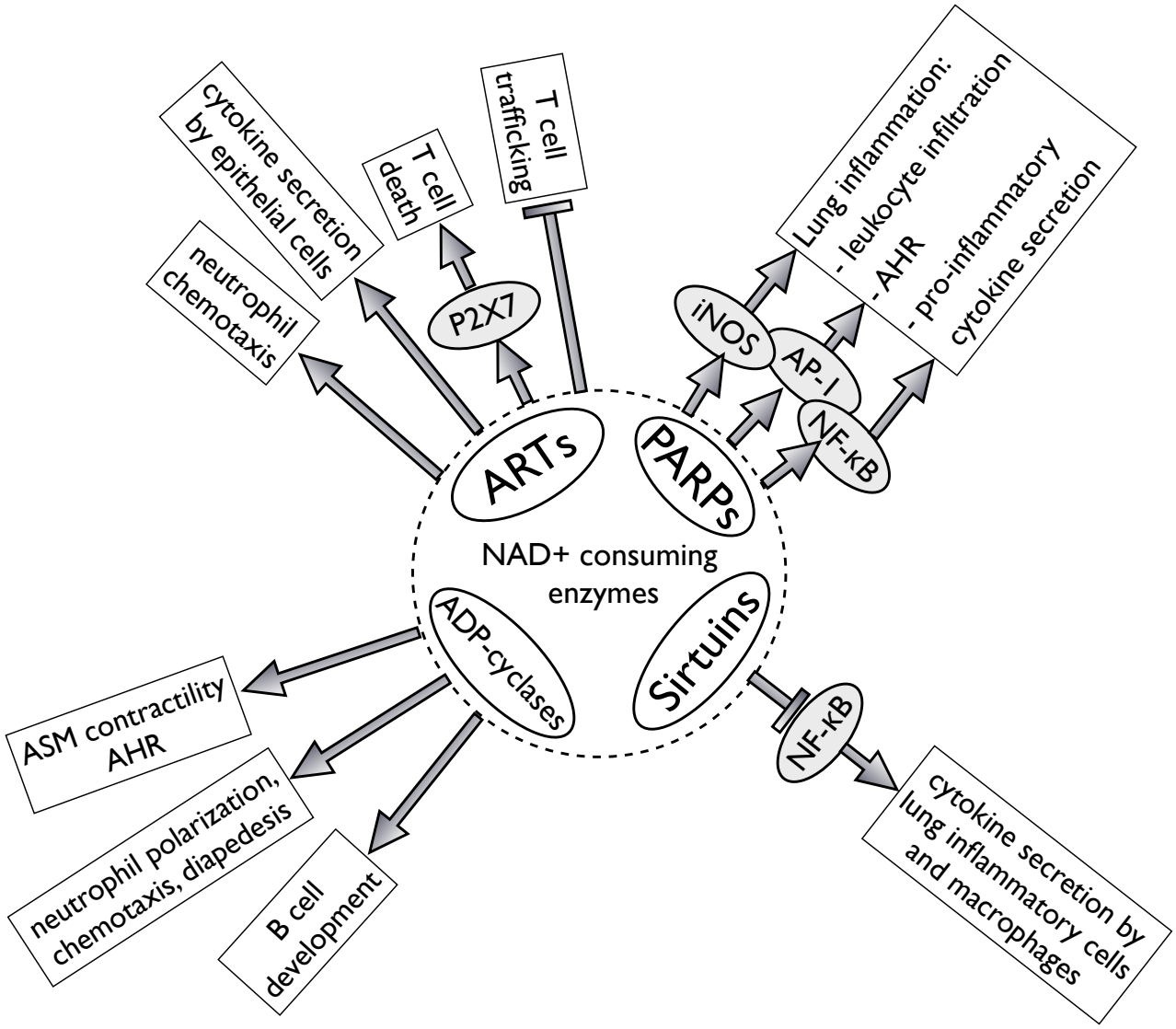

Fig. (4). NAD+-consuming enzymes in the regulation of lung immune responses. ART proteins play important roles in immunity by regulating immune cell functions such as T cell trafficking and apoptosis (*via* P2X7 receptor), neutrophil chemotaxis, as well as cytokine secretion by epithelial cells. PARPs regulate lung inflammation, as increased activity of PARPs was correlated with activation of NF-κB, AP-1 and iNOS leading to pro-inflammatory cytokine release, leukocyte infiltration and hyperresponsiveness. ADP-cyclases play a role in B cell development, neutrophil polarization, chemotaxis and diapedesis. In the lungs, ADP-cyclases regulate smooth muscle contractility. Sirtuins were shown to possess anti-inflammatory properties through inhibition of NF-κB and subsequently reduced cytokine secretion by lung inflammatory cells and macrophages *in vitro*. AP-1, activator protein 1; iNOS, inducible nitric oxydase synthase; NF-κB, nuclear factor-κB; P2X7, cytolytic P2X7 purinergic receptor.

REFERENCES

[1] Ying W. NAD+/NADH and NADP+/NADPH in cellular functions and cell death: Regulation and biological consequences. Antioxid Redox Signal 2008; 10: 179-206.

[2] Bruzzone S, Guida L, Zocchi E, Franco L, De Flora A. Connexin 43 hemichannels mediate Ca2+-regulated transmembrane NAD+ fluxes in intact cells. FASEB J 2000; 15(1): 10-2.

[3] Haag F, Adriouch S, Braß A, *et al*. Extracellular NAD and ATP: Partners in immune cell modulation. Purinergic Signal 2007; 3: 71-81.

[4] Adriouch S, Hubert S, Pechberty S, Koch-Nolte F, Haag F, Seman M. NAD+ released during inflammation participates in T cell homeostasis by inducing ART2-mediated death of naive T cells *in vivo*. J Immunol 2007; 179: 186-94.

[5] Liu Z-X, Azhipa O, Okamoto S, Govindarajan S, Dennert G. Extracellular nicotinamide adenine dinucleotide induces T cell apoptosis *in vivo* and *in vitro*. J Immunol 2001; 167: 4942-7.

[6] Seman M, Adriouch S, Scheuplein F, *et al.* NAD-Induced T cell death: ADP-ribosylation of cell surface proteins by ART2 activates the cytolytic P2X7 purinoceptor. Immunity 2003; 19: 571-82.

[7] Haag F, Freese D, Scheublein F, *et al.* T cells of different developmental stages differ in sensitivity to apoptosis induced by extracellular NAD. Dev Immunol 2002; 9: 197-202.

[8] Aswad F, Kawamura H, Dennert G. High sensitivity of CD4+CD25+ regulatory T cells to extracellular metabolites nicotinamide adenine dinucleotide and ATP: a role for P2X7 receptors. J Immunol 2005; 175: 3075-83.

[9] Chambon P, Weill J, Mandel P. Nicotinamide mononucleotide activation of new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. Biochem Biophys Res Commun 1963; 11: 39-43.

[10] Gill D, Pappenheimer AJ, Brown R, Kurnick J. Studies on the mode of action of diphtheria toxin. VII. Toxin-stimulated hydrolysis of nicotinamide adenine dinucleotide in mammalian cell extracts. J Exp Med 1969; 129: 1-21.

[11] Okazaki IJ, Moss J. Characterization of glycosylphosphatidylinositiol-anchored, secreted, and intracellular vertebrate mono-ADP-ribosyltransferases. Annu Rev Nutr 1999; 19: 485-509.

[12] Amé J-C, Spenlehauer C, de Murcia G. The PARP superfamily. Bioessays 2004; 26: 882-93.

[13] Koch-Nolte F, Kernstock S, Mueller-Dieckmann C, Weiss M, Haag F. Mammalian ADP-ribosyltransferases and ADP-ribosylhydrolases. Front Biosci 2008; 13: 6716-29.

[14] Okazaki I, Moss J. Mono-ADP-ribosylation: a reversible posttranslational modification of proteins. Adv Pharmacol 1996; 35: 247-80.

[15] Koch-Nolte F, Adriouch S, Bannas P, *et al.* ADP-ribosylation of membrane proteins: Unveiling the secrets of a crucial regulatory mechanism in mammalian cells. Ann Med 2006; 38: 188-99.

[16] Hassa PO, Haenni SS, Elser M, Hottiger MO. Nuclear ADP-ribosylation reactions in mammalian cells: Where are we today and where are we going? Microbiol Mol Biol Rev 2006; 70: 789-829.

[17] Beneke S. Poly(ADP-ribose) polymerase activity in different pathologies - The link to inflammation and infarction. Exp Gerontol 2008; 43: 605-14.

[18] Glowacki G, Braren R, Firner K, *et al*. The family of toxin-related ecto-ADP-ribosyltransferases in humans and the mouse. Protein Sci 2002; 11: 1657-70.

[19] Glowacki G, Braren R, Cetkovic-Cvrlje M, Leiter EH, Haag F, Koch-Nolte F. Structure, chromosomal localization, and expression of the gene for mouse ecto-mono (ADP-ribosyl)transferase ART5. Gene 2001; 275: 267-77.

[20] Seman M, Adriouch S, Haag F, Koch-Nolte F. Ecto-ADP-ribosyltransferases (ARTs): emerging actors in cell communication and signaling. Curr Med Chem 2004; 11: 857-72.

[21] Zolkiewska A, Moss J. Integrin alpha 7 as substrate for a glycosylphosphatidylinositol- anchored ADP-ribosyltransferase on the surface of skeletal muscle cells. J Biol Chem 1993; 268: 25273-6.

[22] Braren R, Glowacki G, Nissen M, Haag F, Koch-Nolte F. Molecular characterization and expression of the gene for mouse NAD+:arginine ecto-mono(ADP-ribosyl)transferase, Art1. Biochem J 1998; 336: 561-8.

[23] Balducci E, Horiba K, Usuki J, Park M, Ferrans VJ, Moss J. Selective expression of RT6 superfamily in human bronchial epithelial cells. Am J Respir Cell Mol Biol 1999; 21: 337-46.

[24] Donnelly LE, Rendell NB, Murray S, *et al.* Arginine-specific mono(ADP-ribosyl)transferase activity on the surface of human polymorphonuclear neutrophil leucocytes. Biochem J 1996; 315: 635-41.

[25] Allport J, Donnelly L, Hayes B, *et al.* Reduction by inhibitors of mono(ADP-ribosyl)transferase of chemotaxis in human neutrophil leucocytes by inhibition of the assembly of filamentous actin. Br J Pharmacol 1996; 118: 1111-8.

[26] Paone G, Wada A, Stevens LA, *et al*. ADP ribosylation of human neutrophil peptide-1 regulates its biological properties. Proc Natl Acad Sci USA 2002; 99: 8231-5.

[27] Paone G, Stevens LA, Levine RL, *et al.* ADP-ribosyltransferase-specific modification of human neutrophil peptide-1. J Biol Chem 2006; 281: 17054-60.

[28] Del Vecchio M, Balducci E. Mono ADP-ribosylation inhibitors prevent inflammatory cytokine release in alveolar epithelial cells. Mol Cell Biochem 2008; 310: 77-83.

[29] Balducci E, Micossi LG, Soldaini E, Rappuoli R. Expression and selective up-regulation of toxin-related mono ADP-ribosyltransferases by pathogen-associated molecular patterns in alveolar epithelial cells. FEBS Lett 2007; 581: 4199-204.

[30] Haag F, Koch-Nolte F, Kühl M, Lorenzen S, Thiele H-G. Premature stop codons inactivate the RT6 genes of the human and chimpanzee species. J Mol Biol 1994; 243: 537-46.

[31] Koch-Nolte F, Duffy T, Nissen M, *et al.* A new monoclonal antibody detects a developmentally regulated mouse ecto-ADP-ribosyltransferase on T cells: Subset distribution, inbred strain variation, and modulation upon T cell activation. J Immunol 1999; 163: 6014-22.

[32] Hara N, Badruzzaman M, Sugae T, Shimoyama M, Tsuchiya M. Mouse Rt6.1 is a thiol-dependent arginine-specific ADP-ribosyltransferase. Eur J Biochem 1999; 259: 289-94.

[33] Adriouch S, Ohlrogge W, Haag F, Koch-Nolte F, Seman M. Rapid induction of naive T cell apoptosis by ecto-nicotinamide adenine dinucleotide: requirement for mono(ADP-ribosyl)transferase 2 and a downstream effector. J Immunol 2001; 167: 196-203.

[34] Nemoto E, Yu Y, Dennert G. Cell surface ADP-ribosyltransferase regulates lymphocyte function- associated molecule-1 (LFA-1) function in T cells. J Immunol 1996; 157: 3341-9.

[35] Okamoto S, Azhipa O, Yu Y, Russo E, Dennert G. Expression of ADP-ribosyltransferase on normal T lymphocytes and effects of nicotinamide adenine dinucleotide on their function. J Immunol 1998; 160: 4190-8.

[36] Scheuplein F, Adriouch S, Glowacki G, Haag F, Seman M, Koch-Nolte F. Triggering of T-cell apoptosis by toxin-related ecto-ADP-ribosyltransferase ART2. Ann N Y Acad Sci 2003; 1010: 296-9.

[37] Ohlrogge W, Haag F, Lohler J, *et al.* Generation and characterization of ecto-ADP-ribosyltransferase ART2.1/ART2.2-deficient mice. Mol Cell Biol 2002; 22: 7535-42.

[38] Kahl S, Nissen M, Girisch R, *et al.* Metalloprotease-mediated shedding of enzymatically active mouse ecto-ADP-ribosyltransferase ART2.2 upon T cell activation. J Immunol 2000; 165: 4463-9.

[39] Bortel R, Waite D, Whalen B, *et al.* Levels of Art2+ cells but not soluble Art2 protein correlate with expression of autoimmune diabetes in the BB rat. Autoimmunity 2001; 33: 199-211.

[40] Rigby M, Bortell R, Greiner D, *et al.* The rat T-cell surface protein RT6 is associated with src family tyrosine kinases and generates an activation signal. Diabetes 1996; 45: 1419-26.

[41] Todd DJ, Forsberg EM, Greiner DL, Mordes JP, Rossini AA, Bortell R. Deficiencies in gut NK cell number and function precede diabetes onset in BB rats. J Immunol 2004; 172: 5356-62.

[42] Kawamura H, Aswad F, Minagawa M, Govindarajan S, Dennert G. P2X7 receptors regulate NKT cells in autoimmune hepatitis. J Immunol 2006; 176: 2152-60.

[43] Hong SBA, Seman M, Haag F, Koch-Nolte F, Dubyak GR. Lipopolysaccharide, IFN-gamma, and IFN-beta induce expression of the thiol-sensitive ART2.1 Ecto-ADP-ribosyltransferase in murine macrophages. J Immunol 2007; 179: 6215-27.

[44] Shieh WM, Ame J-C, Wilson MV, *et al.* Poly (ADP-ribose) polymerase null mouse cells synthesize ADP-ribose polymers. J Biol Chem 1998; 273: 30069-72.

[45] Kraus WL. Transcriptional control by PARP-1: chromatin modulation, enhancer-binding, coregulation, and insulation. Curr Opin Cell Biol 2008; 20: 294-302.

[46] van Wijk SJL, Hageman GJ. Poly(ADP-ribose) polymerase-1 mediated caspase-independent cell death after ischemia/reperfusion. Free Radic Biol Med 2005; 39: 81-90.

[47] Moroni F. Poly(ADP-ribose)polymerase 1 (PARP-1) and postischemic brain damage. Curr Opin Pharmacol 2008; 8: 96-103.
[48] Kauppinen TM. Multiple roles for poly(ADP-ribose)polymerase-1 in neurological disease. Neurochem Int 2007; 50: 954-8.
[49] Pacher P, Szabó C. Role of the peroxynitrite-poly(ADP-ribose) polymerase pathway in human disease. Am J Pathol 2008; 173: 2-13.
[50] Pacher P, Szabó C. Role of poly(ADP-ribose) polymerase 1 (PARP-1) in cardiovascular diseases: The therapeutic potential of PARP inhibitors. Cardiovasc Drug Rev 2007; 25: 235-60.
[51] Miwa M, Masutani M. PolyADP-ribosylation and cancer. Cancer Sci 2007; 98: 1528-35.
[52] Hassa PO, Hottiger MO. The functional role of poly(ADP-ribose)polymerase 1 as novel coactivator of NF-κB in inflammatory disorders. Cell Mol Life Sci 2002; 59: 1534-53.
[53] Cuzzocrea S. Shock, inflammation and PARP. Pharmacol Res 2005; 52: 72-82.
[54] Virág L. Poly(ADP-ribosyl)ation in asthma and other lung diseases. Pharmacol Res 2005; 52: 83-92.
[55] Virág L, Szabó C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol Rev 2002; 54: 375-429.
[56] Woolley SM, Farivar AS, Naidu BV, *et al.* Role of Poly (ADP) ribose synthetase in lung ischemia--reperfusion injury. J Heart Lung Transplant 2004; 23: 1290-6.
[57] Koksel O, Yildirim C, Cinel L, *et al.* Inhibition of poly(ADP-ribose) polymerase attenuates lung tissue damage after hind limb ischemia-reperfusion in rats. Pharmacol Res 2005; 51: 453-62.
[58] Farivar AS, Woolley SM, Fraga CH, *et al.* Intratracheal poly (ADP) ribose synthetase inhibition ameliorates lung ischemia reperfusion injury. Ann Thorac Surg 2004; 77: 1938-43.
[59] Szabó G, Soós P, Mandera S, *et al.* INO-1001 a novel poly(ADP-ribose) polymerase (PARP) inhibitor improves cardiac and pulmonary function after crystalloid cardioplegia and extracorporal circulation. Shock 2004; 21: 426-32.
[60] Farivar AS, Woolley SM, Naidu BV, *et al.* Poly (ADP) ribose synthetase inhibition reduces obliterative airway disease in rat tracheal allografts. J Heart Lung Transplant 2004; 23: 993-1002.
[61] Su CF, Liu DD, Kao SJ, Chen HI. Nicotinamide abrogates acute lung injury caused by ischaemia/reperfusion. Eur Respir J 2007; 30: 199-204.
[62] Pagano A, Pitteloud C, Reverdin C, Metrailler-Ruchonnet I, Donati Y, Barazzone Argiroffo C. Poly(ADP-ribose)polymerase activation mediates lung epithelial cell death *in vitro* but is not essential in hyperoxia-induced lung injury. Am J Respir Cell Mol Biol 2005; 33: 555-64.
[63] Pagano A, Metrailler-Ruchonnet I, Aurrand-Lions M, Lucattelli M, Donati Y, Argiroffo CB. Poly(ADP-ribose) polymerase-1 (PARP-1) controls lung cell proliferation and repair after hyperoxia-induced lung damage. Am J Physiol Lung Cell Mol Physiol 2007; 293: 619-29.
[64] Kim J, Suk M, Yoon D, *et al.* Inflammatory and transcriptional roles of poly (ADP-ribose) polymerase in ventilator-induced lung injury. Crit Care 2008; 12: R108.
[65] Vaschetto R, Kuiper JW, Chiang SR, *et al.* Inhibition of poly(adenosine diphosphate-ribose) polymerase attenuates ventilator-induced lung injury. Anesthesiology 2008; 108: 261-8.
[66] Genovese T, Mazzon E, Di Paola R, *et al.* Inhibitors of poly(ADP-ribose) polymerase modulate signal transduction pathways and the development of bleomycin-induced lung injury. J Pharmacol Exp Ther 2005; 313: 529-38.
[67] Horváth EM, Benko R, Gero D, Kiss L, Szabó C. Treatment with insulin inhibits poly(ADP-ribose)polymerase activation in a rat model of endotoxemia. Life Sci 2008; 82: 205-9.
[68] Tasatargil A, Aksoy NH, Dalaklioglu S, Sadan G. Poly (ADP-ribose) polymerase as a potential target for the treatment of acute renal injury caused by lipopolysaccharide. Ren Fail 2008; 30: 115-20.
[69] Liaudet L, Pacher P, Mabley JG, *et al.* Activation of poly(ADP-Ribose) polymerase-1 is a central mechanism of lipopolysaccharide-induced acute lung inflammation. Am J Respir Crit Care Med 2002; 165: 372-7.
[70] Kao S, Liu D, Su C, Chen H. Niacinamide abrogates the organ dysfunction and acute lung injury caused by endotoxin. J Cardiovasc Pharmacol 2007; 50: 333-42.
[71] Murakami K, Enkhbaatar P, Shimoda K, *et al.* Inhibition of poly (ADP-ribose) polymerase attenuates acute lung injury in an ovine model of sepsis. Shock 2004; 21: 126-33.
[72] Kiefmann R, Heckel K, Doerger M, *et al.* Role of PARP on iNOS pathway during endotoxin-induced acute lung injury. Intensive Care Med 2004; 30: 1421-31.
[73] Veres B, Radnai B, Gallyas F Jr, *et al.* Regulation of kinase cascades and transcription factors by a poly(ADP-ribose) polymerase-1 inhibitor, 4-hydroxyquinazoline, in lipopolysaccharide-induced inflammation in mice. J Pharmacol Exp Ther 2004; 310: 247-55.
[74] Hageman GJ, Larik I, Pennings H-J, Haenen GR, Wouters EF, Bast A. Systemic poly(ADP-ribose) polymerase-1 activation, chronic inflammation, and oxidative stress in COPD patients. Free Radic Biol Med 2003; 35: 140-8.
[75] Moonen HJ, Geraets L, Vaarhorst A, Bast A, Wouters EF, Hageman GJ. Theophylline prevents NAD+ depletion *via* PARP-1 inhibition in human pulmonary epithelial cells. Biochem Biophys Res Commun 2005; 338: 1805-10.
[76] Espinoza LA, Smulson ME, Chen Z. Prolonged poly(ADP-ribose) polymerase-1 activity regulates JP-8-induced sustained cytokine expression in alveolar macrophages. Free Radic Biol Med 2007; 42: 1430-40.
[77] Boulares AH, Zoltoski AJ, Sherif ZA, Jolly P, Massaro D, Smulson ME. Gene knockout or pharmacological inhibition of poly(ADP-Ribose) polymerase-1 prevents lung inflammation in a murine model of asthma. Am J Respir Cell Mol Biol 2003; 28: 322-9.
[78] Virág L, Bai P, Bak I, *et al.* Effects of poly(ADP-ribose) polymerase inhibition on inflammatory cell migration in a murine model of asthma. Med Sci Monitor 2004; 10: 77-83.
[79] Suzuki Y, Masini E, Mazzocca C, *et al.* Inhibition of poly(ADP-Ribose) polymerase prevents allergen-induced asthma-like reaction in sensitized guinea pigs. J Pharmacol Exp Ther 2004; 311: 1241-8.
[80] Oumouna M, Datta R, Oumouna-Benachour K, *et al.* Poly(ADP-ribose) polymerase-1 inhibition prevents eosinophil recruitment by modulating Th2 cytokines in a murine model of allergic airway inflammation: A potential specific effect on IL-5. J Immunol 2006; 177: 6489-96.
[81] Naura AS, Hans CP, Zerfaoui M, *et al.* Post-allergen challenge inhibition of poly(ADP-ribose) polymerase harbors therapeutic potential for treatment of allergic airway inflammation. Clin Exp Allergy 2008; 38: 839-46.
[82] Naura AS, Datta R, Hans CP, *et al.* Reciprocal regulation of iNOS and PARP-1 during allergen-induced eosinophilia. Eur Respir J 2009; 33(2): 252-62.
[83] Singh D, Richards D, Knowles RG, *et al.* Selective inducible nitric oxide synthase inhibition has no effect on allergen challenge in asthma. Am J Respir Crit Care Med 2007; 176: 988-93.
[84] Malavasi F, Deaglio S, Ferrero E, *et al.* CD38 and CD157 as receptors of the immune system: a bridge between innate and adaptive immunity. Mol Med 2006; 12: 334-41.
[85] Fliegert R, Gasser A, Guse AH. Regulation of calcium signalling by adenine-based second messengers. Biochem Soc Trans 2007; 35: 109-14.
[86] Malavasi F, Deaglio S, Funaro A, *et al.* Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol Rev 2008; 88: 841-86.
[87] Lund F. Signaling properties of CD38 in the mouse immune system: enzyme-dependent and -independent roles in immunity. Mol Med 2006; 12: 328-33.
[88] Deshpande DA, White TA, Guedes AGP, *et al.* Altered Airway Responsiveness in CD38-Deficient Mice. Am J Respir Cell Mol Biol 2005; 32: 149-56.
[89] Gally F, Hartney JM, Janssen WJ, Perraud A-L. CD38 plays a dual role in allergen-induced airway hyperresponsiveness. Am J Respir Cell Mol Biol 2008; (In press).
[90] Jain D, Keslacy S, Tliba O, *et al.* Essential role of IFN[beta] and CD38 in TNF[alpha]-induced airway smooth muscle hyperresponsiveness. Immunobiology 2008; 213: 499-509.
[91] Guedes AGP, Paulin J, Rivero-Nava L, Kita H, Lund FE, Kannan MS. CD38-deficient mice have reduced airway hyperresponsiveness following IL-13 challenge. Am J Physiol Lung Cell Mol Physiol 2006; 291: 1286-93.
[92] Deshpande DA, White TA, Dogan S, Walseth TF, Panettieri RA, Kannan MS. CD38/cyclic ADP-ribose signaling: role in the regulation of calcium homeostasis in airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 2005; 288: 773-88.

[93] Deshpande DA, Dogan S, Walseth TF, *et al.* Modulation of calcium signaling by interleukin-13 in human airway smooth muscle: role of CD38/cyclic adenosine diphosphate ribose pathway. Am J Respir Cell Mol Biol 2004; 31: 36-42.

[94] Deshpande DA, Walseth TF, Panettieri RA, Kannan MS. CD38-cyclic ADP-ribose-mediated Ca2+ signaling contributes to airway smooth muscle hyperresponsiveness. FASEB J 2003; 17: 452-4.

[95] Sieck GC, White TA, Thompson MA, Pabelick CM, Wylam ME, Prakash YS. Regulation of store-operated Ca2+ entry by CD38 in human airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 2008; 294: 378-85.

[96] Kang B-N, Tirumurugaan KG, Deshpande DA, *et al.* Transcriptional regulation of CD38 expression by tumor necrosis factor-{alpha} in human airway smooth muscle cells: role of NF-{kappa}B and sensitivity to glucocorticoids. FASEB J 2006; 20: 1000-2.

[97] Tirumurugaan KG, Jude JA, Kang BN, Panettieri RA, Walseth TF, Kannan MS. TNF-{alpha} induced CD38 expression in human airway smooth muscle cells: Role of MAP kinases and transcription factors NF-{kappa}B and AP-1. Am J Physiol Lung Cell Mol Physiol 2007; 292: 1385-95.

[98] Klar AJS, Fogel S, Macleod K. MAR1- a regulator of the HMa and HMalpha loci in saccharomyces cerevisiae. Genetics 1979; 93: 37-50.

[99] Rine J, Herskowitz I. Four genes responsible for a position effect on expression from HML and HMR in Saccharomyces cerevisiae. Genetics 1987; 116: 9-22.

[100] Braunstein M, Rose AB, Holmes SG, Allis CD, Broach JR. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. Genes Dev 1993; 7: 592-604.

[101] Denu JM. The Sir2 family of protein deacetylases. Curr Opin Chem Biol 2005; 9: 431-40.

[102] Frye RA. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem Biophys Res Commun 1999; 260: 273-9.

[103] Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun 2000; 273: 793-8.

[104] Michishita E, Park JY, Burneskis JM, Barrett JC, Horikawa I. Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Mol Biol Cell 2005; 16: 4623-35.

[105] Vaziri H, Dessain SK, Eaton EN, *et al.* hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 2001; 107: 149-59.

[106] Motta MC, Divecha N, Lemieux M, *et al.* Mammalian SIRT1 represses forkhead transcription factors. Cell 2004; 116: 551-63.

[107] Bouras T, Fu M, Sauve AA, *et al.* SIRT1 deacetylation and repression of p300 involves lysine residues 1020/1024 within the cell cycle regulatory domain 1. J Biol Chem 2005; 280(11): 10264-76.

[108] Yeung F, Hoberg J, Ramsey C, *et al.* Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J 2004; 23: 2369-80.

[109] Rodgers JT, Lerin C, Gerhart-Hines Z, Puigserver P. Metabolic adaptations through the PGC-1[alpha] and SIRT1 pathways. FEBS Lett 2008; 582: 46-53.

[110] Salminen A, Kauppinen A, Suuronen T, Kaarniranta K. SIRT1 longevity factor suppresses NF-kappaB -driven immune responses: regulation of aging *via* NF-kappaB acetylation? Bioessays 2008; 30: 939-42.

[111] Pfluger PT, Herranz D, Velasco-Miguel S, Serrano M, Tschöp MH. Sirt1 protects against high-fat diet-induced metabolic damage. Proc Natl Acad Sci USA 2008; 105: 9793-8.

[112] Gao Z, Ye J. Inhibition of transcriptional activity of c-JUN by SIRT1. Biochem Biophys Res Commun 2008; 376: 793-6.

[113] Rajendrasozhan S, Yang S-R, Kinnula VL, Rahman I. SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008; 177: 861-70.

[114] McBurney MW, Yang X, Jardine K, *et al.* The mammalian SIR2{alpha} protein has a role in embryogenesis and gametogenesis. Mol Cell Biol 2003; 23: 38-54.

[115] Yang S-R, Wright J, Bauter M, Seweryniak K, Kode A, Rahman I. Sirtuin regulates cigarette smoke-induced proinflammatory mediator release *via* RelA/p65 NF-{kappa}B in macrophages *in vitro* and in rat lungs *in vivo*: implications for chronic inflammation and aging. Am J Physiol Lung Cell Mol Physiol 2007; 292: 567-76.

[116] Aksoy P, Escande C, White TA, *et al.* Regulation of SIRT 1 mediated NAD dependent deacetylation: A novel role for the multifunctional enzyme CD38. Biochem Biophys Res Commun 2006; 349: 353-9.

[117] Krebs C, Adriouch S, Braasch F, *et al.* CD38 controls ADP-ribosyltransferase-2-catalyzed ADP-ribosylation of T cell surface proteins. J Immunol 2005; 174: 3298-305.

[118] Zhang J. Are poly(ADP-ribosyl)ation by PARP-1 and deacetylation by Sir2 linked? Bioessays 2003; 25: 808-14.

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