The Role of Nicotinamide Adenine Dinucleotide in the Pathogenesis of Rheumatoid Arthritis: Potential Implications for Treatment

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Abstract

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory, autoimmune disease characterised by small joint swelling, deformity, and dysfunction. Its exact aetiology is unclear. Current treatment approaches do not control harmful autoimmune attacks or prevent irreversible damage without considerable side effects. Nicotinamide adenine dinucleotide (NAD^+), an important hydrogen carrier in mitochondrial respiration and oxidative phosphorylation, is the major determinant of redox state in the cell. NAD^+ metabolites act as degradation substrates for a wide range of enzymes, such as sirtuins, poly-ADP-ribose polymerases, ADP-ribosyltransferases, and CD38. The roles of NAD^+ have expanded beyond its role as a coenzyme, linking cellular metabolism to inflammation signalling and immune response. The aim of this review is to illustrate the role of NAD^+-related enzymes in the pathogenesis of RA and highlight the potential therapeutic role of NAD^+ in RA.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterised by synovial inflammation, synovial hyperplasia, pannus formation with subsequent joint swelling, space narrowing, and destruction of articular cartilage and bone. The exact causes of RA are still unclear. However, it is well recognised that a combination of factors, including abnormal autoimmune response, genetic susceptibility, and some environmental or biologic triggers, such as viral infection or hormonal changes, are involved in the development of RA.[1] Despite the use of biological disease-modifying antirheumatic drugs, such as anti-TNF-α inhibitors, and targeted synthetic disease-modifying antirheumatic drugs, such as JAK inhibitors,[2] there are still a significant number of RA patients who have poorly controlled disease. Therefore, the development of new therapies is urgently needed.

T cell immune responses to self-antigens are known to play an important role in the development and progression of RA. T cells

can differentiate toward the T helper (Th) 1 or Th17 lineages, imposing a hyper-inflammatory phenotype.[3-5] The activation of T cells, which produce proinflammatory cytokines, such as TNF-α, IL-1, IL-6, IL-8, and IL-17, is involved in the pathogenesis of RA.[6,7] NFκB, which regulates the activity of many genes that code for cytokines, contributes to rheumatoid synovial inflammation. Therefore, the NFκB pathway is one of the most important signalling pathways involved in the development of synovitis.[8]

Recently, scientists found that abnormal energy metabolism is associated with the development of RA.[9] During preclinical RA, when autoreactive T cells expand and immunological tolerance is broken, the main sites of disease are the secondary lymphoid tissues. Naïve CD4+ T cells from patients with RA have a defect in glycolytic flux due to the upregulation of glucose-6-phosphate dehydrogenase.[3] This therefore leads to high levels of NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) and thus depleted levels of intracellular reactive oxygen species, which facilitates T cell hyperproliferation and development of proinflammatory effector functions. In clinical RA, immune cells coexist with stromal cells in the acidic milieu of the inflamed joint. This microenvironment is rich in metabolic intermediates that are released into the extracellular space to shape cell–cell communication and the functional activity of tissue-resident cells.[10] However, it is still unclear how energy metabolites influence the pathogenic behaviour of T cells and regulate signalling pathways in RA.

BIOLOGICAL EFFECT OF NICOTINAMIDE ADENINE DINUCLEOTIDE IN ENERGY METABOLISM AND IMMUNE RESPONSE

NAD^+, an important hydrogen carrier in mitochondrial respiration and oxidative phosphorylation, is the major determinant of redox state within the cell.[11] NADPH is the phosphorylated form of NAD^+. Broadly speaking, proton carriers are required for energy metabolism. NAD^+ can be synthesised from five major precursors and intermediates: nicotinamide (Nam), nicotinamide mononucleotide (NMN), nicotinamide riboside (NR), nicotinic acid (NA), and tryptophan.[12] NAD^+ is mainly synthesised by two pathways (the *de novo* synthesis and salvage synthesis pathways). Nicotinamide phosphoribosyltransferase (Nampt) is a rate-limiting enzyme that plays an important role in the synthesis of NAD^+ via the salvage pathway.[13]

However, the roles of NAD^+ have been discovered to extend beyond its role as a coenzyme. NAD^+ and its metabolites also act as degradation substrates for a wide range of enzymes, such as the Class III NAD^+-dependent deacetylases (sirtuins), poly-ADP-ribose polymerases (PARP), ADP-ribosyltransferases (ART), and the cyclic ADP-ribose synthases (CD38 ectoenzymes).[13,14] Through its activities, NAD^+ links cellular metabolism to changes in inflammation signalling and immune response. It has been reported that NAD^+ is able to promote an impressive allograft survival through a robust systemic IL-10 production, suggesting IL-10 may be a key molecule involved in NAD^+-mediated immune regulation.[15] Administration of NAD^+ protects against experimental autoimmune encephalomyelitis (EAE) and reverses disease progression by regulating CD4+ T cell differentiation and apoptosis;[16] this suggests a potential role in the pathogenesis of NAD^+ and indicates potential therapeutic effects in RA by regulating the immune response. Here, the authors explore how NAD^+-related substrates contribute to the progress of RA and summarise the biological effects of NAD^+ in the treatment of RA.

Levels of Nam and tryptohan, the precursors of NAD^+, are decreased in patients with RA.[17-19] Nam has been shown to be a potent inhibitor of glucose-6-phosphate dehydrogenase, which may have benefits for conditions like RA.[20] However, tryptophan has been shown to be a poor NAD^+ precursor *in vivo*.[21] NR has been found to reduce obesity-related inflammation, which may apply to other inflammatory diseases, such as RA.[22] Nampt has been shown to play a major role in inflammatory arthritis because expression of Nampt is increased in both the sera and in the arthritic paw in a collagen-induced arthritis (CIA) mouse model. Furthermore, a specific competitive inhibitor of Nampt was shown to effectively reduce arthritis severity and progression of arthritis with comparable

activity to the TNF inhibitor etanercept. Moreover, Nampt inhibition has been shown to reduce intracellular NAD^+ concentration in inflammatory cells and circulating TNF-α level during endotoxaemia in mice.[23,24] However, no papers have been published on the targeting of Nampt in human patients with RA.

SIRTUINS INVOLVED IN SYNOVITIS AND T CELL DIFFERENTIATION IN RHEUMATOID ARTHRITIS

The mammalian sirtuins family of proteins has seven members, named Sirt1–7. Sirtuins have NAD^+-dependent deacetylase activity and belong to the Type III histone deacetylase. They are involved in the regulation of various biological processes, such as cell survival, apoptosis, proliferation, lipid metabolism, senescence, and systemic inflammation, as well as in bone and cartilage remodelling.[25,26] Three sirtuins are located in the mitochondria (Sirt3, 4, and 5), while Sirt1, 6, and 7 are predominantly located in the nucleus and Sirt2 is found in the cytoplasm.[27] Sirt1 is the most important member of the sirtuins family. Sirt1 is activated during times of energy deficit and reduced carbohydrate energy sources, such as during exercise or when hungry. Herranz et al.[28] showed that Sirt1 overexpression helps to reduce metabolic and age-related complications in mice, promoting healthy ageing.[29]

Besides exerting an anti-ageing effect, Sirt1 is also involved in the development of synovitis. Sirt1 is upregulated in synovial tissues, human synovial fibroblasts, and chondrocytes in patients with RA.[30] Sirt1 has been shown to decrease apoptosis in synovial cells and to promote the production of proinflammatory cytokines. TNF-α-induced Sirt1 overexpression contributes to chronic inflammation by promoting inflammatory cytokine production and inhibiting apoptosis in RA synovial cells. Knockdown of Sirt1 results in a reduction in proinflammatory IL-6 and IL-8 and proliferation of RA fibroblast-like synoviocytes (FLS).[30,31] However, Sirt1 exhibits anti-inflammatory properties in RA by enhancing macrophage polarisation to an anti-inflammatory phenotype or inhibiting NFκB signalling.[32,33]

Sirt1 can mediate the differentiation of inflammatory T cell subsets in an NAD^+-dependent manner.[34] Sirt1 is highly expressed in the thymus, suggesting the involvement of Sirt1 in T cell development. Furthermore, T cell-specific Sirt1 deletion and treatment with pharmacological Sirt1 inhibitors has been shown to suppress Th17 differentiation and exert a protective effect in a mouse model of multiple sclerosis.[35] The loss of Sirt1 has been shown to compromise the survival of regulatory T (Treg) cells, resulting in antigen-induced T cell proliferation and inflammation in two mouse models.[36] A deficiency of Sirt1 in mouse or human T cells has been shown to enhance IL-9 production, suggesting that Sirt1 negatively regulates Th9 cell differentiation.[37] Myeloid deletion of Sirt1 impairs Th1 and Th17 cell differentiation and dendritic cell maturation in CIA.[38] In contrast, Gardner et al.[39] found that Sirt1 activators contribute to the suppression of T cell proliferation. Oral Sirt1 activator treatment has been shown to suppress antigen-specific T cell responses and the production of proinflammatory cytokines, including IL-6, IL-17A, and IFN-γ, in experimental autoimmune uveoretinitis mice.[39] Overall, the role of Sirt1 in controlling synovitis and the differentiation of effector T cells in RA is still controversial.

Sirt6, another NAD^+-dependent protein lysine deacetylase, is known to interfere with the NFκB signalling pathway and thereby has an anti-inflammatory function.[40] An adenovirus containing Sirt6 complementary DNA delivering Sirt6 to human RA FLS *in vitro* was shown to suppress TNF-α-induced NFκB target gene expression; additionally, this adenovirus containing Sirt6 complementary DNA was also used to deliver Sirt6 to mice with collagen-induced arthritis, which resulted in reduced arthritis severity.[40] In contrast, Sirt6 has been shown to enhance the proinflammatory and matrix-destructive potential of RA-FLS through TNF-α.[41] Sirt6 has also been shown to increase the intracellular levels of ADP-ribose, an activator of the Ca^{2+} channel. Sirt6 can also enhance the production of IL-8 and TNF-α via a Ca^{2+}-dependent mechanism, showing that cell metabolism can connect with inflammatory responses through a Sirt6-mediated pathway.[42]

POLY-ADP-RIBOSE POLYMERASE-1 IS INVOLVED IN INFLAMMATION AND T CELL DIFFERENTIATION IN RHEUMATOID ARTHRITIS

PARP-1 catalyses 80% of cellular poly(ADP-ribosyl) action, while the other PARP family members, PARP-2, PARP-3, PARP-4, and tankyrases (PARP-5 a and b) account for the remaining 20%. PARP-1 has been shown to increase the risk of RA and promote the development of arthritis inflammation.[1,43] PARP-1 deficiency or inhibition suppresses the activation of JAK, activator protein-1, and NFκB signalling.[1,44,45] PARP-1 inhibition has been shown to decrease TNF-α-induced RA-FLS proliferation and significantly reduces expression of cytokines and chemokines in FLS from patients with RA.[44] Furthermore, PARP-1 inhibitor treatment has been found to significantly attenuate the severity of experimental arthritis by downregulating inflammation, Th1, and Th17 cells, and upregulating Treg cells.[45,46] The deletion of PARP-1 in mice (*PARP-1−/−*) has been shown to lead to T cells generating more thymic Treg cells and converting more naïve T cells into induced Treg cells both *in vitro* and *in vivo*.[47] Additionally, the inhibition of PARP-1 enzyme activity was found to result in an increased expression of *FOXP3* and *TGF-β* receptor I genes in human CD4+ T cells.[47]

ADP-RIBOSYLTRANSFERASES ARE A CRUCIAL REGULATOR OF T CELL FUNCTION

ADP-ribosylation is a post-translational modification regulating protein function in which amino acid-specific ART transfer ADP-ribose from NAD^+ to specific target proteins. Five paralogs (ART1–5) have been cloned, but only four of them are expressed in humans due to a defective *ART2* gene, and six in mice as the result of *ART2* gene duplication.[48] NAD can activate the purinergic receptor P2X7 via ART1 and cause cell depletion in murine models; however, NAD^+ does not induce a cell death in human CD4+CD39+ Treg cells. Further experimentation showed that the expression of P2X7 is lower in human CD4+ CD39+ Treg cells than in CD4+CD39-Treg cells, while the expression of ART1 is relatively higher.[49] This implies that ART1–P2X7 signalling participates in the resistance against cell death of Treg cells induced by NAD^+.[49]

In patients with RA, Treg cells are functionally defective or unstable and are converted to Th17 cells in the presence of inflammatory cytokines, such as IL-6.[50] ART1 may be related to the stability of Treg cells in patients with RA.[49,50]

ART2 could activate the cytolytic purinergic receptor in turn to affect T cell differentiation in mice. For instance, the conversion of Treg cells into Th17 cells is promoted in the presence of IL-6, primarily through the NAD^+-ART2.2-P2X7 pathway. Activation of P2X7 in T cells by ATP or by NAD^+-dependent ADP-ribosylation initiates a cascade of events, including the influx of calcium, the shedding of the L-selectin homing receptor, the externalisation of phosphatidylserine on the outer leaflet of the cell membrane, DNA fragmentation, and, ultimately, cell death.[51,52] This mechanism is called NAD^+-induced cell death.[53] It has been shown that ART2.2-overexpressing mice with normal T lymphocytes are sensitive to NAD^+ and prone to death. It has also been shown that the fewer B cells that express ART2.2, the lower the amount of cell death. Bannas et al.[54] showed that ART2.2 transgenic T cells, but not B cells, are sensitive to NAD^+-induced cell death. NAD^+ can also induce apoptosis of naïve CD4+ $CD62L^{high}$ T cells. Conversely, activated $CD44^{high}$ $CD69^{high}$ T cells are resistant to NAD^+-induced cell death.[55] A study has shown that CD4+ CD25+ FoxP3+ Treg cells express the ART2.2 enzyme and high levels of P2X7, and that these Treg cells can be depleted by intravenous injection of NAD^+.[54] This can be used to promote an antitumour immune response.[54] This mechanism may provide a means by which NAD^+ released during immune diseases controls T cell functions. This suggests that the NAD^+-ART2.2-P2X7 signalling pathway is an important part of T cell death in mice. However, ART2 is not expressed in human T cells.

CD38 IS INVOLVED IN T REGULATORY CELL HOMEOSTASIS AND THE PRODUCTION OF THE PROINFLAMMATORY CYTOKINES IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLAST CELLS

CD38 and CD157 are two prominent enzymes that catalyse the synthesis and hydrolysis of cyclic ADP-ribose, a Ca^{2+} messenger molecule responsible for regulating a wide range of cellular functions.[56] The catalytic efficiency of CD38 is significantly higher when compared with CD157. CD38 is an important NAD^+ consumer; loss of CD38 function in mice led to a 30-fold increase in NAD^+ levels in different tissues.[56] CD38 is expressed by lymphocytes, endothelial cells, and several other cells.[57] CD38 is the main enzyme involved in the degradation of the NAD precursor NMN *in vivo*, indicating that CD38 has a key role in the modulation of NAD-replacement therapy for aging and metabolic diseases. Cells from CD38-deficient mice do not metabolise NAD^+ efficiently.[58]

Most importantly, CD38 can be used as a marker for mice Treg cells with a high suppressive activity. CD38 is expressed mainly in a subset of Foxp3+ CD25+ CD4+ T cells. $CD38^{high}$ Treg cells have a superior suppressive activity compared to $CD38^{low}$ Treg cells.[59] Lower Treg cell numbers are found in CD38-deficient mice, indicating the role of CD38 in Treg cells homeostasis. Chang et al.[60] found that CD38 expression is increased in the synovial membranes of patients with RA. IL-1α and IL-β levels are significantly decreased after treatment with siRNAs targeting the *CD38* or *E2F2* genes.[60] Mice deficient in CD38 develop attenuated collagen-induced arthritis.[61] In addition, CD38 is a surface marker for regulatory B cells in human disease. However, the number and the frequency of regulatory B cells do not change in patients with RA compared to healthy controls.[62]

POTENTIAL VALUE OF NICOTINAMIDE ADENINE DINUCLEOTIDE AND RELEVANT CONSUMING ENZYMES IN THE TREATMENT OF RHEUMATOID ARTHRITIS

One study showed that NAD^+ promotes the conversion of effector Th1 cells (CD4+ IFNγ+) into Type 1 regulatory T cells (CD4+ IL-10+ IFNγ+, Tr1) and blocks chronic inflammation independently of the cytokine milieu.[16] Furthermore, after NAD^+ administration, mast cells exclusively promote CD4+ T cell differentiation *in vivo* and *in vitro*, both in the absence of antigen and independently of major antigen-presenting cells. Moreover, mast cell-mediated CD4+ T cell differentiation is independent of major histocompatibility complex II and T cell receptor signalling.[63] It has been reported that NAD^+ promotes Treg cell conversion into Th17 cells *in vitro* and *in vivo*.[15] NAD^+ has been shown to promote allograft survival by promoting a robust systemic IL-10 production, which suggested that IL-10 is a key molecule involved in immune tolerance and immune regulation.[15] However, Elkhal et al.[15] did not consider that NAD^+ is associated with apoptosis of Treg cells, which may affect the conversion of Treg into Th17. The other factor is that Treg cells are unstable in the anti-graft-dependent inflammatory state and are easily converted into Th17.[17] Therefore, whether NAD^+ promotes the differentiation of Treg cells towards Th17 cells and whether they directly affect Th17 cells and facilitate their differentiation remains to be further studied.

Several drugs are in development or are already available in clinics that could be useful to suppress inflammation in autoimmune diseases. Nam, a precursor of NAD^+, is able to inhibit activation and modulate the activity of B lymphocytes, suggesting a potential role of this agent in regulating antibody-mediated autoimmune disorders like RA.[64] There are abundant sources of Nam, NR, and NA in natural food and milk, suggesting they are generally safe.[65,66] Indeed, new studies have demonstrated the therapeutic potential of supplementing NAD^+ intermediates, such as NR and NMN, providing a proof of concept for the development of an effective intervention.[12] NR is widely used as an NAD^+ precursor vitamin.

Single doses of 100, 300, and 1,000 mg of NR produced dose-dependent, safe increases in the blood NAD^+ metabolome in the first clinical trial of NR pharmacokinetics in humans.[67] Also, re-establishing cellular NAD^+ levels with NAD^+ or Nam has been shown to exert a protective effect against axonal degeneration in EAE.[16,68] Restoring NAD^+ levels with NR or PARP inhibitors has been shown to have a therapeutic effect on nonalcoholic steatohepatitis.[69,70] Jonas et al.[71] found that Nam treatment improved the global symptoms of patients with osteoarthritis, joint flexibility, and reduced inflammation when compared to placebo in patients with osteoarthritis.

NAD^+ plays a crucial role in inflammatory response and autoimmune diseases through Sirts, PARP, ART, or CD38. Although most studies suggest that Sirt1 plays a proinflammatory role in the development of RA, the role of Sirt1 in synovitis and T cell differentiation remains unclear and is controversial.[30,32,33,35-39] Intracellular NAD^+ levels regulate tumour necrosis factor protein synthesis in a Sirt6-dependent manner.[72] Sirt6 overexpression suppresses the expression of NFκB target gene in RA FLS and significantly decreases arthritis severity. Intra-articular injections of an adenovirus containing Sirt6 complementary DNA was shown to decrease arthritis severity in mice.[40] This demonstrates that the NAD^+-Sirt6-NFκB pathway may be an important target for the treatment of RA.

Additionally, PARP-1 inhibiton can reduce the production of proinflammatory cytokines through physically interacting with NFκB. A study showed that PARP-1 inhibition either with specific inhibitors or by siRNA transfection significantly reduced TNF-α-induced proliferation, cytokine, and chemokine expression in RA-FLS via suppressing NFκB signalling. This suggests that PARP-1 inhibitors could have therapeutic benefits in RA.[44,45,47] In fact, many clinical trials using PARP-1 inhibiton have been carried out for the treatment of different tumours. For instance, PARP-1 inhibition has been shown to suppress tumour progression in breast cancer patients by limiting the rate of cell proliferation and activation of NFκB, which results in the suppression of inflammation and the expression of genes related to tumour progression.[73] Common side effects, such as nausea and vomiting, should be monitored.[74]

Studies have shown that ART2 is specifically expressed on T cells in mice. NAD^+ can regulate murine CD4+ T cell differentiation through the NAD^+-ART2.2-P2X7 signalling pathway.[51-53] Due to inactivated ART2 pseudogenes in the human genome, ART2 is deficient in humans. Recently, scientists reported a higher expression level of ART1 in human CD4+ CD39+ Treg cells. ART1 participates in the resistance against cell death of Treg cells induced by NAD^+.[49] It is known that Treg cells are functionally defective or unstable in patients with RA and are converted to Th17 cells in the presence of proinflammatory cytokines, such as IL-6.[50] ART1 may be related to the stability of Treg cells in patients with RA.

CD38 is highly expressed in synovial membranes and plasma cells from RA patients.[75] The IL-1α and IL-β levels are significantly decreased after treatment with siRNA targeting CD38.[59] Mice deficient in CD38 develop an attenuated collagen-induced arthritis.[59,61] Inhibitors or therapeutic antibodies targeting CD38 should be tested for their ability to raise the concentration of NAD^+.[76,77] It is not easy to benefit from therapy through simply targeting CD38 from bench to bedside because targeting CD38 may impair the function of Treg cells and regulatory B cells.

CONCLUSION

Many studies have revealed the importance of NAD^+ biosynthesis in energy metabolism and in the immune response process. It is clear that levels of NAD^+ precursors, Nam and tryptophan, are decreased in patients with RA. Nam, NR, NMN, and NA are promising candidates to replenish NAD^+ and reduce inflammation in patients with RA and experimental arthritis model. IL-10 may be a key molecule involved in immune tolerance and immune regulation after treatment with NAD^+. Administration of NAD^+ protects against autoimmune reaction and reverses disease progression by regulating CD4+ T cell differentiation and apoptosis. The authors argue that the focus of study should be moved from other diseases, like EAE, nonalcoholic steatohepatitis, or OA, to RA and the therapeutic effect of NAD^+ and its precursors should be explored.

Different NAD+-consuming enzymes, such as Sirt1, Sirt6, PARP-1, ART-1, and CD38, are involved in T cell differentiation and homeostasis and synovial inflammation in RA pathogenesis. NAD+ has diverse biological functions through these consuming enzymes. Therefore, NAD+ and Sirt1/Sirt6, NAD+ and PARP-1, NAD+ and ART-1, and NAD+ and CD38 signalling pathways are more affected after NAD+ supplementation. These enzymes can be targeted to efficiently improve arthritis through inhibition of synovitis or regulation of T effector cells differentiation. Published data show that Sirt6 overexpression and PARP-1 inhibitor both have therapeutic benefits in RA animal models. In the near future, human clinical studies are needed to further confirm the therapeutic effect of NAD+ biosynthesis, especially regarding NAD+ precursors and their related consuming coenzymes in RA.

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