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Uric acid, hyperuricemia and vascular diseases

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1. ABSTRACT

Uric acid is the product of purine metabolism. It is known that hyperuricemia, defined as high levels of blood uric acid, is the major etiological factor of gout. A number of epidemiological reports have increasingly linked hyperuricemia with cardiovascular and neurological diseases. Studies highlighting the pathogenic mechanisms of uric acid point to an inflammatory response as the primary mechanism for inducing gout and possibly contributing to uric acid's vascular effects. Monosodium urate (MSU) crystals induce an inflammatory reaction, which are recognized by toll-like receptors (TLRs). These TLRs then activate NALP3 inflammasome. MSU also triggers neutrophil activation and further produces immune mediators, which lead to a proinflammatory response. In addition, soluble uric acid can also mediate the generation of free radicals and function as a pro-oxidant. This review summarizes the epidemiological studies of hyperuricemia and cardiovascular disease, takes a brief look at hyperuricemia and its role in neurological diseases, and highlights the studies of the advanced pathological mechanisms of uric acid and inflammation.

2. INTRODUCTION

Uric acid is the end product of nucleic acid metabolism. High levels of blood uric acid have long been associated with gout. Gouty arthritis (gout) is a medical condition characterized by red, tender, hot, and swollen joints caused by recurrent attacks of acute inflammatory arthritis. The prevalence of gout in the United States has increased from 2.9 cases per 1,000 persons in 1990 to 5.2 cases per 1,000 persons in 1999 (1), due to increasing age of the population. Men have a higher risk of developing gout than women due to higher baseline levels of blood uric acid. Pathologically, gout is caused by an increase of blood uric acid levels, which leads to crystal deposits in joints, tendons, and other tissues and uric acid renal stones (2).

Recently, gout has been linked to cardiovascular disease. Epidemiological data supports the strong association between cardiovascular disease and gout (3-5). Furthermore, multiple studies have also associated hyperuricemia with the precursors of cardiovascular diseases, including hypertension, metabolic syndrome, and coronary artery disease, as well as with closely related

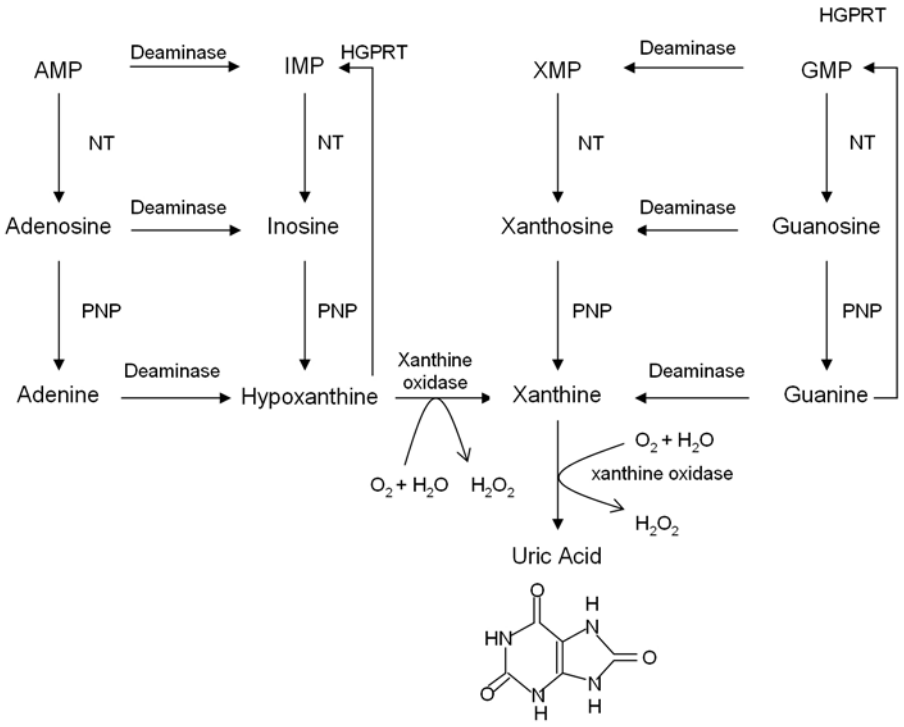

Figure 1. Biosynthesis of uric acid, Abbreviations: AMP: adenosine monophosphate; IMP: inosine monophosphate; XMP: xanthosine monophosphate; GMP: guanosine monophosphate; NT: nucleotidase; PNP: purine nucleoside phosphorylase; HGPRT: hypoxanthine-guanine phosphoribosyltransferase

vascular diseases such as cerebrovascular disease, vascular dementia, preeclampsia, and kidney disease (6-12). In 2004, a prospective cohort study showed that hyperuricemia may be an independent risk factor for cardiovascular disease in middle aged men (13). However, other multivariate analyses have not supported this claim (14, 15). Although a consensus on the association of hyperuricemia with cardiovascular disease has not been reached, these debates have motivated investigators to further determine the mechanisms underlining uric acid and its associated diseases. Recent studies on gout have advanced the understanding of pathological mechanisms of uric acid crystal-induced inflammation (16-18). These mechanisms may also play a role in uric acid's link to vascular disease. In this article, we review the relationship of hyperuricemia and vascular disease by highlighting uric acid's role in inflammation and gout.

3. BIOCHEMISTRY AND METABOLISM OF URIC ACID

Uric acid is a heterocyclic organic compound with the formula $C_5H_4N_4O_3$ (7,9-dihydro-1H-purine-2,6,8(3H)-trione) and a molecular weight of 168 Daltons. Uric acid is the final metabolic product of purine metabolism in humans, and is excreted in urine. Many enzymes are involved in the conversion of the two purine nucleic acids, adenine and guanine, to uric acid. Initially, adenosine monophosphate (AMP) is converted to inosine by two different mechanisms; either first removing an amino group by deaminase to form inosine monophosphate (IMP) followed by dephosphorylation with nucleotidase to form inosine, or by first removing a phosphate group by nucleotidase to form adenosine followed by deamination to form inosine. Guanine monophosphate (GMP) is converted to guanosine by nucleotidase. The nucleosides, inosine and guanosine, are further converted to purine base, hypoxanthine and guanine, respectively, by purine nucleoside phosphorylase (PNP). Hypoxanthine is then oxidized to form xanthine by xanthine oxidase, and guanine is deaminated to form xanthine by guanine deaminase. Xanthine is again oxidized by xanthine oxidase to form the final product, uric acid (Figure 1). At physiologic pH, uric acid is a weak acid with a pKa of 5.8. Uric acid exists majorly as urate, the salt of uric acid. As urate concentration increases in blood, uric acid crystal formation increases. The normal reference interval of uric acid in human blood is 1.5 to 6.0 mg/dL in women and 2.5 to 7.0 mg/dL in men. The solubility of uric acid in water is low, and in humans, the average concentration of uric acid in blood is close to the solubility limit (6.8 mg/dL). When the level of uric acid is higher than 6.8 mg/dL, crystals of uric acid form as monosodium urate (MSU). Studies have found that MSU triggers the inflammation seen in gout (14-16), and may also contribute to the pathogenesis of vascular diseases (see the section 10 for the details).

Humans cannot oxidize uric acid to the more soluble compound allantoin due to the lack of uricase enzyme. Normally, most daily uric acid disposal occurs via the kidneys. In most other mammals, the enzyme uricase (urate oxidase) further oxidizes uric acid to allantoin.

Uricase gene likely underwent a functional mutation during the early stages of hominoid evolution (19). As a result, humans and several other primates have no functional uricase, which consequently leads to higher blood uric acid levels when compared to rodents (19). Hyperuricemia has detrimental effects for multiple organ systems. Uricase gene deficient mice have a 10 fold increase in the serum uric acid level, and are found to have urate nephropathy with infiltration of plasma cells, lymphocytes, and macrophages (20). More than half of the mutant mice die before 4 weeks of age (20). On the other hand, hyperuricemia may have some beneficial effects. Although it has been debated whether the loss of urate oxidase was simply an evolutionary accident (21), Watanabe *et al* has suggested that hyperuricemia maintains blood pressure during low salt intake environments, which may have provided a survival advantage during the course of primate evolution (22). Alternatively, many investigators hypothesize that hyperuricemia developed due to the antioxidant properties of uric acid (19, 21, 23-25). For example, in the nervous system, hyperuricemia has been linked to more favorable outcomes in stroke and other neurological diseases (26) (see section 9 for details).

4. CAUSES OF HYPERURICEMIA

Hyperuricemia is defined as blood uric acid levels above the normal reference interval. Generally, hyperuricemia in adults is defined as a blood uric acid concentration greater than 7.0 mg/dL in men and 6.0 mg/dL in women. In normal humans, uric acid is excreted in urine. However, uric acid excretion may be impaired by kidney disease, leading to hyperuricemia. Hyperuricemia also occurs in babies born with fewer nephrons. These babies process less uric acid compared to healthy controls, and/or have excessive uric acid transferred from their mothers (27). In diseases such as leukemia or lymphoma, chemotherapeutic treatments cause a marked increase in the excretion of uric acid resulting from the nucleic acid metabolism and can obstruct renal tubules, causing acute renal failure ("tumor lysis syndrome") (28).

In addition to problems with uric acid excretion due to kidney dysfunction, hyperuricemia can also result from the increased generation of uric acid. Diets heavy in purine or fructose, or exposure to lead can also contribute to high uric acid levels. Fructose is a unique sugar molecule in that it rapidly depletes ATP and increases the amounts of uric acid (29). A study conducted by Johnson's group (28, 49) using rodents showed a drastic increase in uric acid after the consumption of fructose.

In certain humans, a deficiency of enzymes resulting from genetic mutations may also cause increased blood uric acid levels. For example, hypoxanthine-guanine phosphoribosyl transferase (HGPRT) catalyzes the formation of IMP and GMP for recycling purine bases (Figure 1) with 5-phoshoribosyl-alpha-pyrophosphate (PRPP) as a co-substrate. Lesch-Nyhan syndrome, a rare inherited X-linked disorder caused by the deficiency of HGPRT, leads to the accumulation of purine and PRPP, which are used in the salvage pathway of hypoxanthine and guanine. The HGPRT defect results in the accumulation of hypoxanthine and guanine, which further leads to high uric acid levels. The excess PRPP also increases the rate of *de novo* synthesis of purine, and consequently promotes the production of its end degradation product, uric acid. Lesch-Nyhan syndrome is the result of the buildup of high levels of uric acid in the body beginning in infancy, which leads to severe gout, kidney dysfunction, mental retardation, neurological dysfunction, and self-mutilating behaviors.

Glucose-6-phosphatase (G6Pase) is an enzyme that catalyzes the release of glucose. A deficiency of G6Pase causes von Gierke's disease, one of the glycogen storage diseases, and increases uric acid levels. Lack of functioning G6Pase increases glucose-6-phosphate levels, which stimulates the pentose phosphate pathway, and consequently increases PRPP, purine biosynthesis, and uric acid. However, the issue of whether these diseases have any vascular complications remains unknown.

5. URIC ACID AND CORONARY HEART DISEASE

Coronary heart disease (CHD) is a disease characterized by reduced blood supply to the heart muscle, usually due to atherosclerotic lesions and vessel narrowing in the coronary arteries. Hyperuricemia has been shown to increase the risk of CHD related-events independently of other CHD risk factors, and is linked to higher mortality rates of CHD in women. A recent meta-analysis of a prospective cohort study showed that there is a 12% increase in mortality with each extra 1 mg/dL of uric acid in a person with CHD (30). The presence of hyperuricemia increases the risk of CHD by approximately 70% in women, but not in men. Another meta-analysis published in 2005 investigated the association between hyperuricemia and CHD, and found an increased risk ratio of 1.12 in men and 1.22 in women with hyperuricemia (31). The mechanisms underlying the higher risk of CHD in women with hyperuricemia remain poorly defined (see section 10.2 for the details). However, a combined analysis of all studies demonstrated an odds ratio of 1.13, which dropped to 1.02 after adjusting for other established risk factors and possible cofounders. Serum uric acid concentrations were not significant in independently predicting coronary heart disease (31).

6. URIC ACID AND HYPERTENSION

Recent studies have consistently concluded that hyperuricemia may be an independent risk factor for hypertension (32). Although it seems possible that high uric acid levels are a mere consequence of disease, high uric acid levels always precede the development of hypertension. Hypertension has become increasingly prevalent worldwide, and uric acid levels are rising in correlation (31, 70).

High plasma uric acid levels are positively associated with increased incidences of hypertension in adults (33, 34). More specifically, plasma uric acid levels significantly predict diastolic hypertension, but not systolic

hypertension (35, 36). However, this association decreases as patients age (37) and is not found in elderly patients (36, 38-40). The weakening relationship between uric acid and hypertension with age has a few possible explanations. Uric acid damages small renal vessels, which leads to irreversible salt-sensitive hypertension. This hypertension persists regardless of uric acid levels (22). When hypertension develops in the elderly, other pathophysiological mechanisms such as decreased arterial compliance may play a larger role in hypertension than hyperuricemia (41).

An increase in fructose intake over the past 200 years also corresponds with the increasing prevalence of hypertension and hyperuricemia. As discussed in the previous section, fructose rapidly depletes ATP and increases levels of blood uric acid (29). The modern human diet contains excessive fructose, in the form of high-fructose corn syrup, table sugar, and artificial sweeteners. This may be responsible for the trends of increasing uric acid and hypertension. In a study of rats by Nakagawa (42), animals who ingested fructose without uric acid lowering drugs (allopurinol) suffered from metabolic syndrome, elevated insulin, higher triglycerides, hypertension, and higher body weights. Those who received uric acid lowering drugs (allopurinol or benzbromarone) averted hyperinsulinemia, systolic hypertension, hypertriglyceridemia, and weight gain.

Further evidence of the association of hyperuricemia and hypertension can be found in babies with low birth weight (27). Low birth weight babies appear to have an increased risk of hypertension later in life, and is associated with high levels of uric acid (43). It has been hypothesized that the increased risk of hypertension is a result of low nephron counts common in babies with low birth weights (44). One study by Keller *et al* (45) tested ten Caucasians with essential hypertension (age range 35 to 59 years) who had died in traffic accidents. The ten patients all had fewer nephrons than a control group with age-matched people who had died similarly. Their data supports the hypothesis that there is a reduced number of nephrons in patients with primary hypertension.

Hyperuricemia has also been established as an independent predictor of microalbuminuria (46) and renal dysfunction (25, 47, 48). In healthy normotensive individuals, increased uric acid levels correlate with decreased kidney function (49). Both interstitial and vascular inflammation may also occur. Thus, high levels of uric acid can induce a vasoreactive hypertension, which can further develop into kidney-dependent hypertension.

7. URIC ACID AND METABOLIC SYNDROME

Metabolic syndrome is a disorder characterized by abdominal obesity, dyslipidemia, hypertension, insulin resistance, and a prothrombotic and proinflammatory state, based on the definition given by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) (50). The diagnosis of metabolic syndrome is made when three or more of the risk determinations including waist circumference (greater than 102 cm for men, and greater than 88 cm for women), triglycerides (greater than or equal to 150 mg/dL), high-density lipoprotein (HDL) cholesterol (less than 40 mg/dL for men, and less than 50 mg/dL for women), blood pressure (greater than or equal to 130/85 mmHg), and fasting glucose level (greater than or equal to 110 mg/dL) are met. The prevalence of metabolic syndrome in the United States has drastically increased over the past few decades, with a prevalence of approximately 27% (51) in the year 2000. Persons with metabolic syndrome have an increased risk of developing cardiovascular disease (52). Hyperuricemia has been associated with the development of metabolic syndrome in industrialized nations around the world (53-55). A mirroring trend can be seen in the rise of plasma uric acid levels. In the general population, plasma uric acid levels have risen from averages of 3.5 mg/dL in the 1920s to 6.5 mg/dL in the 1970s (56, 57). One possible culprit to the recent rise of uric acid is the increased consumption of fructose in industrialized nations (58, 59). Fructose intake has been directly linked to hyperuricemia (60, 61), which may result in metabolic syndrome (62, 63) and hypertension (64, 65). The mechanisms in which uric acid may promote the development of metabolic syndrome may involve uric acid inhibition of nitric oxide synthase (66, 67), One study by Cook *et al* showed that knockout mice lacking nitric oxide synthase had features of metabolic syndrome including hypertension, hypercholesterolemia, hypertriglyceridemia, and increased insulin resistance (68).

8. URIC ACID AND STROKE

Hyperuricemia has been implicated as playing a part in the development of cardiovascular disease, including stroke (30). Stroke accounts for roughly 1 out of every 18 deaths and is the third leading cause of death in the United States. According to a recent report, approximately 795,000 Americans experience a stroke every year, which approximates to 1 stroke every 40 seconds (69). In 2009, Kim *et al* published the only large systemic review and meta-analysis known to date investigating the relationship of stroke and hyperuricemia (70). A total of sixteen prospective cohort studies from over 230,000 patients were included. Patients with hyperuricemia were found to be at a significantly higher risk for both stroke incidence (relative risk 1.41) and mortality (relative risk 1.26) than controls with normal levels of uric acid. After adjusting for known risk factors of stroke, the significance between hyperuricemia and stroke remain. In the same year, Holme *et al* examined uric acid as a potential risk factor for acute myocardial infarctions, congestive heart failure, and stroke in the Apolipoprotein Mortality RISK study (AMORIS). Increasing uric acid levels were found to be associated with increased risk of both hemorrhagic and ischemic strokes (71).

9. URIC ACID AND NEURODEGENERATIVE DISORDERS

As the aging population expands, the potential association between serum uric acid levels and a variety of

neurodegenerative disorders has been of particular interest. While high levels of uric acid have been linked with gout, hypertension, cardiovascular disease, and stroke (30, 32, 70, 72), reduced serum levels of uric acid have been associated with Parkinson's disease, Huntington's disease, and multiple sclerosis (73-77). A study by Annanmaki *et al* in 2007 showed significantly lower plasma uric acid levels in patients with Parkinson's disease when compared with matched controls. A later study by Andreadou *et al* confirmed Annanmaki's conclusions that Parkinson's disease patients have significantly lower mean serum uric acid levels when compared with controls (73). It was also found that in men, there was a significant inverse correlation between uric acid levels and disease duration, with lower levels associated with longer duration of Parkinson's.

It has been argued that if lower levels of serum uric acid are associated with Parkinson's disease, then higher levels, particularly the hyperuricemic levels seen in gout may have a protective effect. Alonso *et al* performed a prospective case-controlled study to determine the association between gout and the risk of developing Parkinson's disease (78). Individuals with a history of gout had a significantly lower risk of developing Parkinson's than ones without a history of gout. However, this finding was only significant for men, but not for women. De Vera *et al* later published a large population-based cohort study in Canada, which also studied the relationship between gout and Parkinson's disease on patients whose age was greater than 65 (76). Using the British Columbia Linked Health Database and PharmaCare data, the incidence rates of Parkinson's disease in 11,258 gout patients were compared with 56,199 matched controls. Over an 8-year median follow up, there was a 30% reduction in the risk of developing Parkinson's disease in both male and female patients with a history of gout, independent of age, sex, prior comorbid conditions, and nonsteroidal anti-inflammatory drugs (NSAIDs) and diuretic use. Recognition of uric acid as a possible biomarker and its function as a scavenger in Parkinson's disease raises the possibility that urate may bear the therapeutic potential for the disease (79).

A recent 2010 study explored the relationship between uric acid levels and Huntington's disease (HD). By performing a secondary analysis of the Huntington's disease CARE-HD trial, Auinger *et al* found an association between higher serum uric acid levels and slower HD progression (75). In addition, there was a trend of decreased worsening of motor function with increasing uric acid levels, hinting that uric acid may aid as a therapeutic target for the slowing of the motor component of HD progression. In contrast, cognitive, behavioral, and neuropsychological functions were not found to be related to uric acid levels. One of the major cellular defenses against the superoxide anion (O_2^{*-}) and formation of peroxynitrite is the superoxide dismutases (SODs). These include cytosolic Cu/ZnSOD (Cu/ZnSOD or SOD1), manganese SOD (MnSOD or SOD2), and extracellular Cu/ZnSOD (ecSOD or SOD3) (80). The protection roles of uric acid may result from its inhibition of peroxidase activity of both cytosolic CuSOD and ecSOD (80). It is thought the antioxidative effects of uric acid may be neuroprotective, particularly in Parkinson's disease, where oxidative stress has a leading role in the degeneration of dopaminergic neurons in the substantia nigra (77, 81).

10. PATHOGENIC MECHANISMS OF URIC ACID AND INFLAMMATION

The significance of high plasma uric acid levels as a risk factor for vascular disease has been discussed in other sections of this review. The issue is still under debate whether uric acid levels can have direct effects on vascular cells or serve as only a functional marker of xanthine oxidase activity. In this section, we review the pro-inflammatory effects of uric acid, which supports the direct cause-effect relationship of hyperuricemia and vascular pathology.

10.1. Effects of soluble uric acid on vascular cells

As a byproduct of hydrogen peroxide generated from hypoxanthine and xanthine, uric acid has long been considered an anti-oxidant reagent (21, 82, 83). As an anti-oxidant, uric acid decreases the violability of superoxide, and therefore may have a protective role in vascular inflammation and dysfunction. However, uric acid also mediates the production of aminocarbonyl radicals, which have pro-oxidant effects on several molecules (84-86) including low-density lipoprotein (LDL) cholesterol. Patterson *et al* showed that uric acid functioned as an antioxidant in the presence of native LDL taken from human plasma, but in response to mildly oxidized LDL, when the oxidation had occurred, uric acid became a pro-oxidant (86, 87). In certain local conditions, uric acid also directly reacts with other small chemicals including nitric oxide. Normal nitric oxide generation is essential for vascular relaxation whereas uric acid reduces nitric oxide bioavailability by converting nitric oxide into other molecules such as glutathione (88) or by decreasing nitric oxide production (67, 89).

Studies have shown that soluble uric acid induces vascular smooth muscle cell (VSMC) proliferation (90). Further studies demonstrated that the induced proliferation of VSMC is mediated by soluble uric acid via the mitogen-activated protein kinase (MAPKs) pathway. In addition, uric acid also has pro-inflammatory effects on vascular cells. In VSMC, uric acid induces chemokine monocyte chemoattractant protein-1 (MCP-1) generation by activating transcription factor nuclear factor κ-B (NF-kappaB), MAPKs, and cyclooxygenase-2 (COX-2) (91). Uric acid also increases the up-regulation of C-reactive protein in both VSMCs and endothelial cells (92), which adds to the pro-atherogenic properties of soluble uric acid. Studies with rat models have found that hyperuricemia-induced hypertension and renal injury are due to stimulation of the renin-angiotensin system, lower endothelial nitric oxide levels, and inhibition of neuronal nitric oxide synthase in the kidney (66). Uric acid also stimulates vascular smooth muscle cell proliferation. This directly causes the development of renal microvascular disease and afferent arteriolopathy, possibly increasing

blood pressure. Hyperuricemia leads to impaired endothelium-mediated vasodilation even in the absence of existing cardiovascular disease (93). Studies in rat and cell culture models have shown that this endothelial dysfunction is due to the inhibition of endothelial generation of nitric oxide by uric acid (67). Subsequent studies have shown that hyperuricemia-induced renovascular damage, resulting in hypertension and renal injury, is caused by endothelial dysfunction (94). Similarly, endothelial dysfunction may underly the causes of other aspects of hyperuricemia-related cardiovascular diseases. Decreases in uric acid levels in patients with hyperuricemia results in distinct improvements in endothelial dysfunction (95), vasodilator capacity and blood flow (96). Soluble uric acid has complex pro- and con- effects on vascular pathology depending on different cellular environments. A better understanding of how the microenvironment affects this trend is needed and may lead to the identification of future therapeutic targets in hyperuricemic patients. Several pharmacological agents are available for the treatment of hyperuricemia in gout, including allopurinol and febuxostat, whereas pepgloticase, a recombinant, pegylated formulation of a mammalian urate oxidase, is under review by the U.S. Food and Drug Administration. It will be very interesting to determine whether these drugs can lower the risk of cardiovascular diseases in addition to lower urate levels (87).

10.2. Uric acid crystals and inflammation response

Though the role of soluble uric acid in vascular disease remains controversial, it is established that uric acid crystals strongly induce inflammation and vascular dysfunction in humans. As discussed in section 3, when uric acid levels are over 6.8 mg/dL, crystals form as MSU. Thus, MSU generations are mainly caused by hyperuricemia. However, other factors also involved in uric acid crystallization include pH, temperature, ionic strength, and the binding of urate to plasma macromolecules (97). The role of MSU in triggering inflammation was first proposed by rheumatologists when a strong causal relationship of crystal deposition in joint tissues and the inflammatory response during the pathogenesis of gout was observed (98). MSU has garnered more attention since 2003 when it was first recognized as an endogenous danger signal released from dying cells (99). In humans, the innate immune system can detect specific danger signals in addition to non-self molecules in order to establish an efficient immune response. This hypothesis is known as the “danger model” (100). In instances of gout and hyperuricemia, crystallized uric acid is produced by dying cells and signals “danger” to immune system.

Though the strong immunogenic effect of MSU has been recognized, the mechanism of how MSU is sensed by immune cells remains unknown. Several different cell membrane receptors have been reported to be involved in MSU recognition. Research data from Naccache’s group demonstrated that blocking antibodies, directed against CD16 and CD11b, selectively inhibits the activation of neutrophils by MSU (101), indicating a possible role of antigen receptors in MSU-induced inflammation. In addition, more research has been focused on the relationship of pathogen-associated molecular patterns (PAMPs) recognition receptors (PRRs) and MSU detection. Four major families of PRRs have been identified as important components of innate immunity, participating in host defense sensory systems against the invasion of infectious agents and danger signals. Toll-like receptors (TLRs) recognize a variety of conserved microbial PAMPs as well as many other molecules. Furthermore, TLRs work in synergy with three cytosolic sensing receptor families including NLRs [NOD (nucleotide binding and oligomerization domain)-like receptors], RLRs [RIG-I (retinoic acid-inducible gene 1)-like receptors], and CLRs (C-type lectin receptors). In one study using a subcutaneous air pouch gout model, it was reported that TLR2, TLR4, and myeloid differentiation factor 88 (MyD88)-deficient bone marrow-derived macrophages (BMDMs) were insufficient in sensing MSU crystals and MSU-induced generation of pro-inflammatory cytokines. These observations were accompanied by a reduction in neutrophil infiltration at the site of injection (102), suggesting that MSU-induced inflammation is TLRs-dependent. The progress in the field demonstrated that another PRR in the NLRs family, NACHT, LRR and PYD domains-containing protein 3 (NALP3), may also act as a sensor in the presence of MSU (103). MSU-induced activation of NALP3, a type of NLR, leads to an assembly of a protein complex termed inflammasome. The NALP3 inflammasome, composed of NALP3, apoptosis-associated speck-like protein containing a caspase recruitment domain (ACS), caspase-1, and cardinal, is functional for activation of proinflammatory caspase-1. This protein complex processes caspase-1 precursor into its p20-p10 heterodimer mature form, which further cleaves the precursor molecules of the well-known proinflammatory cytokines, pro-interleukin-1beta (IL-1beta) and pro-IL-18, to their active secreted products. IL-1beta and IL-18 are believed to play a critical role in MSU-mediated inflammation. A pilot study of IL-1 inhibitor in treating patients with acute gouty arthritis indicates its rapid beneficial effects and validates the essential role of IL-1 in gouty inflammation (104). In addition, there is an expanding spectrum of acute and chronic inflammatory diseases thought to be “autoinflammatory diseases”. These autoinflammatory diseases, as a new concept, are distinct from the traditional definition of autoimmune diseases, the latter of which are associated with dysfunctional T cells and can be treated with new T cell suppressive biological drugs, including anti-tumor necrosis factor-alpha (TNF-alpha), inhibitory receptor cytotoxic T lymphocyte antigen 4-Ig (CTLA-Ig) fusion preparation, anti-IL-12/23, anti-CD20, anti-IL-17, and anti-IL-6 receptor (105). In contrast, autoinflammatory diseases are uniquely attributed to dysfunctional caspase1 activity, inflammatory cell death (pyroptosis) (106-108), and the secretion of IL-1beta. Indeed, blocking IL-1beta results in a rapid and sustained reduction in the severity of most autoinflammatory diseases. Flares of gout, type 2 diabetes, heart failure, and smoldering multiple myeloma are examples of seemingly unrelated diseases, which are uniquely responsive to IL-1beta neutralization (105).

Whether uric acid needs to upregulate the expression of NLR/inflammasome components in order to

activate inflammasome/caspase-1 and IL-1beta/IL-18 maturation remains unknown. To address this question, Tschopp's team found that granulocytes, monocytes (very weakly), dendritic cells, and B and T cells all express NALP1 and NALP3. The highest levels of NALP1 are found in T cells and Langerhans cells. Furthermore, NALP1 is present in glandular epithelial structures such as the stomach, gut, lungs, and surprisingly, in neurons and testis. In contrast to NALP1, NALP3 shows a more restricted tissue distribution with its expression mainly focused in non-keratinizing epithelia in the oropharynx, esophagus, and ectocervix. NALP3 expression is also found in the urothelial layer in the bladder. A difference in the subcellular distribution between NALP1 and NALP3 is observed because NALP1 is localized mainly in the nucleus, whereas NALP3 is predominantly cytoplasmic. The functional significance in the different subcellular locations of NALP1 and NALP3 remains unknown. To further enhance our understanding of the expression status of TLRs, NLRs, inflammasome components, and proinflammatory caspases, we took different approaches and examined the mRNA transcript levels of these genes. We have made several important findings: (1) Among 11 tissues examined, vascular and heart tissue express fewer types of TLRs and NLRs than immune system tissues such as blood, lymph nodes, thymus, and trachea; (2) based on the expression data of three characterized inflammasomes [NALP1, NALP3, and ICE protease-activating factor (IPAF) inflammasomes], the examined tissues can be classified into three tiers: the first tier tissues including brain, placenta, blood, and thymus express inflammasome(s) in a constitutive status; the second tier tissues have inflammasome(s) in nearly-ready expression status (with the requirement of upregulation of one component); the third tier tissues, like heart and bone marrow, require upregulation of at least two components in order to assemble functional inflammasomes. Based on the expression readiness of inflammasomes in tissues, we propose a new working model of a three-tier responsive expression of inflammasomes in tissues and suggest a new concept of third tier tissues' inflammation privilege, which provides insight on the differences of tissues in initiating acute inflammations in response to the stimulations of uric acid crystals (Figure 2) and other risk factors. This model suggests that (a) the first-tier tissues with constitutively expressed inflammasomes initiate inflammation quicker than the second-tier and third-tier tissues; and (b) the second tier tissues (requiring one component upregulation) including vascular tissue and the third tier tissues including heart (requiring more than one component upregulation) are in an inducible expression state of inflammasomes. The inducible expressions of inflammasomes are presumably mediated through various signal pathways and the interplay between the signal pathways take longer time and overcome a higher threshold than the first tier tissue in initiating inflammation. Traditional concepts of immune privilege suggests a protective mechanism from autoimmune destruction based on lack of expression of antigen-presenting self-major compatibility complex (MHC) molecules in tissues (109). The lack of self-MHC expression in immune privileged tissues including testis, results in the failure of self-antigen presentation to stimulate the host immune system. Thus, it protects immune privileged tissues from autoimmune destruction. Similarly, we propose a new concept of tissues' inflammation privilege that emphasizes a protective mechanism against tissue destruction mediated by inflammasome/IL-1beta-based innate immune responses. In this new concept of tissues' inflammation privilege, vascular tissue and heart disproportionally express fewer types of TLRs and NLRs and may only inducibly express inflammasomes. The lower expression can be explained by the "inflammation privilege" of the tissue against uncontrolled inflammation destruction that is mediated by inflammasome-based innate immune responses (110). Our new concept and model may also explain the potential differences between cardiovascular tissues and other tissues in initiating acute inflammation in response to uric acid crystals. The first-tier tissues may have a higher probability of developing acute inflammation than the second-tier and third-tier tissues. Future work is required to elucidate the specific roles of different tiers of tissue inflammasomes in uric acid crystals-induced vascular inflammation and the regulatory signal pathways (111).

A few hypothetical mechanisms have been proposed on how a solid structure such as MSU can directly activate, post-translationally, intracellular receptors like NALP3 (Figure 2). One model suggests that NALP3 inflammasome is able to sense ionic perturbations induced by MSU crystals (112), especially potassium efflux. Indeed, low intracellular potassium has been reported as a requirement for MSU-induced NALP3 activation (113). The issue remains undetermined whether MSU can activate specific ion channels or induce a non-selective increase in ion permeability due to membrane damage. A second model proposes that the generation of reactive oxygen species (ROS) induced by MSU is essential for NALP3 inflammasome activation. Accompanied with inflammasome activation, ROS production is observed in MSU treated macrophages (114, 115). ROS blockade via knockdown of NAPDH subunit expressions or the use of antioxidants inhibits MSU-induced inflammasome activation (114, 115). These findings indicate that ROS may transmit danger signals and activate NALP3 inflammasome either directly or indirectly. A third model claims that considering the size of crystalline, MSU is too large to be efficiently phagocytosed, and thus induces destabilization of lysosomes, which leads to the release of cathepsin B, a proinflammatory protease. This process may be sensed by NALP3 which triggers inflammasome activation. Supporting evidence for this model shows that inhibition of cathepsin B and phagocytosis impairs NALP3 inflammasome activation induced by MSU as well as other NALP3 agonists (116, 117). Though evidence can be found to support each of these three models, much is still unknown regarding the specific details delineating

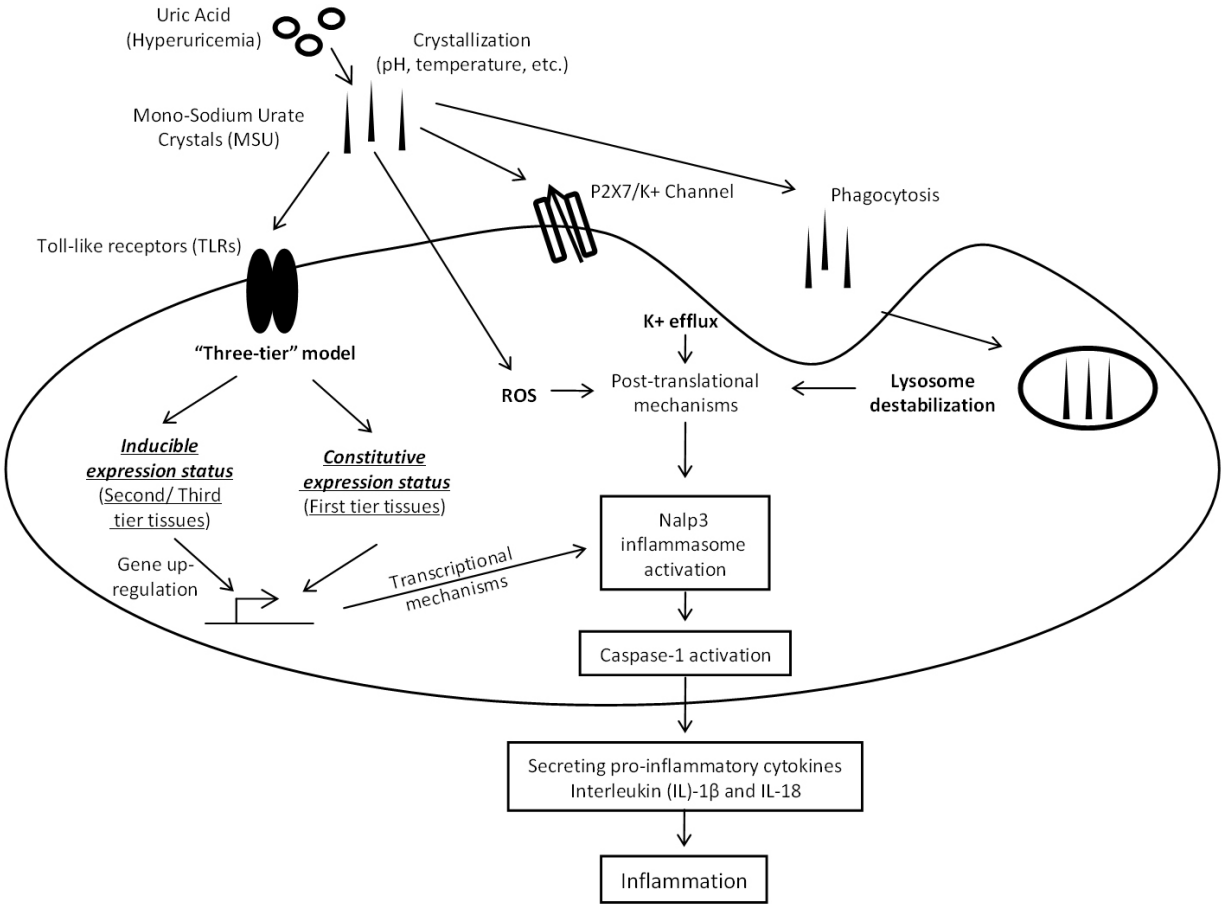

Figure 2. Mechanisms and pathways in MSU-mediated inflammation

each step. It is possible that all the models may work in parallel or in tandem subsequently for inflammasome activation.

To clearly demonstrate the role of ASC/inflammasome in vascular inflammation and injury, Yajima *et al* showed that ASC was markedly expressed at the site of vascular injury. Neointimal formation was significantly attenuated in ASC gene deficient (-/-) mice after injury. IL-1beta and IL-18 were expressed in the neointimal lesion in wild-type mice but showed decreased expression in the lesion of ASC-/- mice. To further define the roles of ASC in bone marrow cells and residential vascular cells for vascular inflammation, the investigators replaced mouse bone marrow with ASC-/- bone marrow cells and found that neointimal formation was significantly decreased (118). In addition, NALP3 inflammasome, that can be activated by MSU crystals (112) and cholesterol crystals (119, 120), plays an important role in promoting vascular inflammation and atherosclerosis. Furthermore, uric acid has also been found to enhance platelet adhesiveness (121) and stimulate vascular smooth muscle cell growth (90). Taken together, uric acid and MSU crystals promote vascular inflammation via several different mechanisms.

11. CONCLUSION

Hyperuricemia has long been associated with gout, and more recently, may be associated with coronary heart disease, hypertension, stroke, metabolic syndrome, and other disorders. Emerging studies of the pathological mechanism of gout have provided insight into the underlying mechanisms of MSU crystal-induced inflammation intimately involved in the pathology of gout. MSU crystal is recognized by TLRs, which trigger the synthesis of active proinflammatory cytokine IL-1beta through NALP3 inflammasome activation. Neutrophil activation also triggers the production of immune mediators associated with a proinflammatory function. In addition to the MSU crystal triggering an inflammatory reaction, soluble uric acid can also mediate the generation of radicals and function as a pro-oxidant. These mechanisms by MSU or soluble uric acid found in gout may also contribute to the development of vascular diseases seen in patients with hyperuricemia (Figure 3). However, current models of the pathophysiological mechanisms of uric acid are not yet fully sufficient to explain the relationship between hyperuricemia and vascular

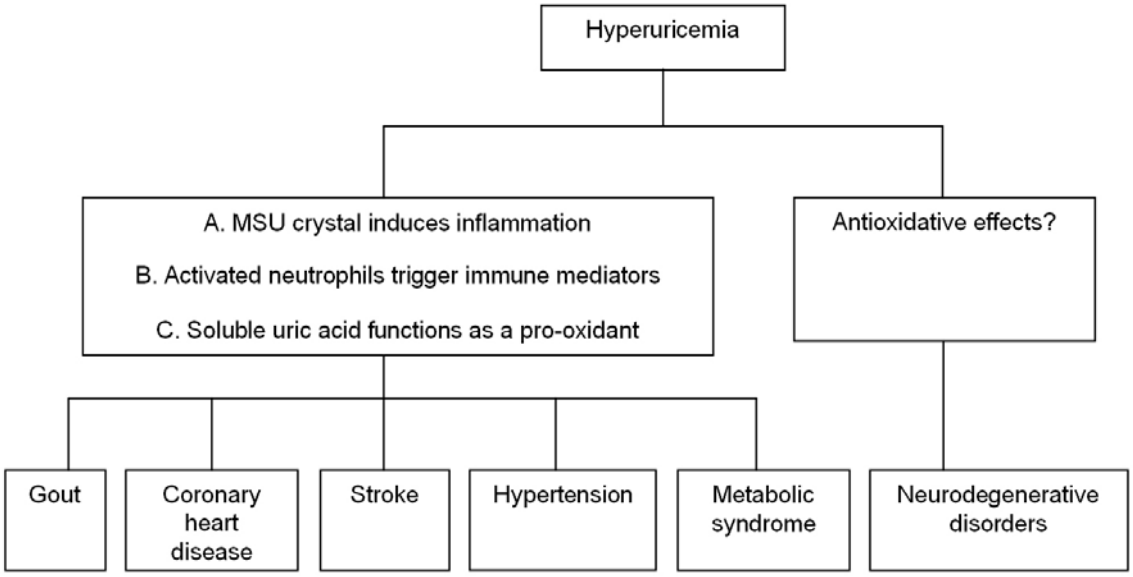

Figure 3. Hyperuricemia and diseases

disease. Further research is needed to better understand the biological roles of uric acid, and identify new therapeutic targets in the prevention and treatment of hyperuricemic-related diseases.

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Abbreviations: PAMPs: pathogen-associated molecular patterns; PRR: PAMPs recognition receptors; TLRs: toll-like receptors; NLRs: [NOD (nucleotide binding and oligomerization domain)-like receptors]; RLRs: [RIG-I (retinoic acid-inducible gene 1)-like receptors]; CLRs: C-type lectin receptors; IPAF: ICE protease-activating factor. NALP: NACHT, LRR and PYD domains-containing protein (NACHT: domain present in NAIP, class II transactivator, heterokaryotic incompatibility factor-E, telomerase-associated protein 1; LRR: leucine-rich repeat; PYD: pyrin domain)

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