Review

Crystal ball gazing: new therapeutic targets for hyperuricaemia and gout

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Recent studies in diverse disciplines have led to significant advances in the understanding of the basic biology of hyperuricaemia and gout, with important implications for future treatment. These findings include genetic variation within SLC2A9 as a key regulator of urate homeostasis, and identification of urate–anion exchanger urate transporter 1 (URAT1) and other renal uric acid transporters. Recognition of urate as an endogenous danger signal and activator of the adaptive immune response suggests an important role for urate crystals in non-microbial immune surveillance. The central role of NALP3 inflammasome activation and IL-1β signalling in the initiation of the acute gout attack raises the possibility of new therapeutic targets. Disordered osteoclastogenesis in patients with chronic gout highlights potential therapies for prevention of joint damage. This review summarizes these findings and the potential relevance for future management of gout.

KEY WORDS: Gout, Hyperuricaemia, Genetics, Inflammation, Treatment.

Introduction

Gout is a common inflammatory disease of metabolic origin. This disorder is characterized by intermittent attacks of severe joint inflammation, and in the presence of persistent hyperuricaemia, development of tophaceous disease and chronic gouty arthropathy. The central role of hyperuricaemia and MSU crystal deposition in the pathogenesis of gouty inflammation has been recognized for decades (reviewed in [1]), and new urate-lowering drugs such as febuxostat and PEG-uricase should lead to major improvements in long-term management of gout [2, 3]. However, many questions remain about the basic mechanisms of this disease. These questions include: why certain individuals are predisposed to hyperuricaemia; why certain hyperuricaemic individuals are predisposed to gout; how uric acid is handled by the renal tubule; what molecular pathways are involved in initiation of the acute gout attack; and what factors mediate joint damage in chronic gout. Recent laboratory research has shed light on a number of the issues. This review summarizes this research and focuses on the implications for future treatment of hyperuricaemia and gout.

Genetic polymorphisms of SLC2A9 as a key regulator of urate homeostasis

The solute transporter 2A9 was first identified as a member of the SLC2A gene family of hexose transporters [4], with its major physiological role assumed to be in the transport of glucose and fructose. More recently, genome-wide association scanning and follow-up studies have demonstrated a role in Caucasian populations for genetic variation within SLC2A9 in the control of serum urate levels [5–9] and susceptibility to gout [5, 6, 8, 10]. These novel findings have revealed SLC2A9 to be a transporter of uric acid that can be inhibited by a uricosuric agent [8]. The inheritance of one predisposing variant of SLC2A9 increases the risk for an individual to develop gout by 30–70% [odds ratio (OR) = 1.3–1.7] [5, 6, 8, 10]; the inter-study variability is likely to be due to differing criteria for ascertainment of gout.

While the precise molecular mechanism by which SLC2A9 increases the risk of hyperuricaemia and gout is not yet understood, it is likely that this variant increases expression of the shorter isoform 2 of SLC2A9 [6], encoding a protein with a shorter N-terminal region (GLUT9AN, resulting from transcriptional editing of exons 1a and 2, using an alternative translational initiation codon in exon 1b) [4]. Expression of this variant is detectable only in kidney and placenta in humans. SLC2A9v2 localizes exclusively to the apical membrane of the renal proximal tubule [4] (Fig. 1). Thus, given that it has been shown to transport uric acid [8], SLC2A9v2 appears to function, as does the urate–anion exchanger urate transporter 1 (URAT1) [11], in the reabsorption of uric acid [12]. Reabsorbed uric acid exits the kidney tubular cell into the serum via the full-length variant (SLC2A9v1) situated in the basolateral membrane [13]. Demonstration that genetic variation within SLC2A9 influences serum urate emphasizes SLC2A9 as a checkpoint in control of serum urate levels. SLC2A9 can be regarded as a potential therapeutic target and warrants concerted pharmaceutical research in order to develop superior uricosuric agents.

Ingestion of fructose-sweetened, but not artificially sweetened, soft drinks is associated with increased risk of hyperuricaemia and gout (OR = 1.8 for hyperuricaemia at ≥ 4 servings/day and OR = 1.9 for gout at ≥ 2 servings/day) [14, 15]. Fructose is the only sugar known to increase serum urate levels [16]. The observation that SLC2A9, genetically associated with hyperuricaemia and gout, transports both fructose and uric acid (with maximal transport of fructose occurring in the absence of uric acid), suggests a possible gene/environment interaction in development of hyperuricaemia and gout. Inclusion of data on fructose ingestion as a covariate in genetic studies of SLC2A9 in hyperuricaemia and gout may be illuminating.

Other candidate genes

The genome-wide association scans clearly demonstrated SLC2A9 to have the major single effect on serum urate levels in Caucasian populations [6–9]. No other loci reached the genome-wide level of significance. However, SLC2A9 explains <5% of variance in serum urate levels, indicating that a number of other factors controlling serum urate levels—environmental, epigenetic and genetic—remain to be discovered. It is reasonable to expect that meta-analysis of the genome-wide scan data will reduce the background ‘noise’ in the association data and enable identification of other genes that control serum urate levels, which can be
regarded as validated therapeutic targets. Common genetic variations in \textit{URAT1}, the β3 adrenergic receptor and methylene tetrahydrofolate reductase genes have also been implicated in regulation of serum urate levels in more than one population [17–25] (Table 1). The β3 adrenergic receptor data are particularly intriguing, suggesting a genetic link between serum urate levels and insulin resistance, a frequent comorbid feature of gout [26, 27]. Study of these genes in other populations, including Caucasian, is warranted.

Most studies to date have identified genetic associations with serum urate and hyperuricaemia. At present it is not known as to what factors determine the development of gout in individuals with hyperuricaemia, noting that the majority of those with hyperuricaemia do not develop gout [28]. Answering this question should enable novel therapeutic opportunities of this disease. Genome-wide association scanning in gout may provide critical insights into this issue. This will require significant international effort in recruiting thousands of cases. Accurate phenotyping will be essential to reduce clinical heterogeneity, ideally with gout proven by the gold standard method of microscopic MSU crystal diagnosis.

**The renal uric acid transportasome**

We envisage that pharmacogenomics will be an important part of decision-making in future clinical care, perhaps in optimizing treatment using existing therapies. One prominent example is the decision to use the xanthine oxidase inhibitor allopurinol vs uricosuric agents. At present, the latter tend to be used when allopurinol is not tolerated or has proven ineffective, often the case in clinical practice [2, 29]. The major regulator of serum urate is renal excretion, with insufficiency in this process a feature of gout [30–32]. The use of uricosuric agents as initial therapy in gout might be justified in patients who could be demonstrated, using a simple test, to be insufficient in renal uric acid excretion. Genetic testing at individual uric acid transport genes (such as \textit{SLC2A4}) would lack the specificity required for clinical decision-making. However, a uric acid ‘transportasome’ genetic test (in combination with standard urine testing for uric acid excretion) may give the necessary specificity and sensitivity. The renal uric acid excretion capability of an individual is a net effect of the uric acid secretion and reabsorption activities of the various renal uric acid transporters [12]. This renal exchange is mediated by specialized molecules expressed in renal proximal tubule cells (Fig. 1; reviewed in [33]). Identified molecules include SLC2A9 (see above), URAT1, organic anion transporters 1, 3, and 4 (OAT1, OAT3, OAT4), multi-drug resistance protein 4 (MRP4) and sodium-coupled monocarboxyl transporters SMCT1,2 (SLC5A8, SLC5A12). In addition to \textit{SLC2A4}, genetic variation in \textit{URAT1} has been demonstrated to influence serum urate levels in Caucasian and Japanese cohorts [17, 18] (Table 1). The influence of genetic variation in other uric acid transportasome molecules has not been adequately tested. The fact that association at other transportasome genes was not reported in the genome-wide association scans for genetic variants controlling serum urate levels does not rule out a role for variation in such genes in control of serum urate. Furthermore, current genome-wide genotyping SNP microarrays do not have adequate coverage of some genes,
including \textit{URAT1} [6]. It is not unreasonable to hypothesize that the combined influence of genetic variation in the uric acid transportasome (excluding \textit{SLC2A9}) could exceed the influence of \textit{SLC2A9} on serum urate levels. What is required is exhaustive genetic association experiments of the influence of genetic variation in transportasome genes on serum urate levels and gout, with the ultimate goal being development of a transportasome genetic risk algorithm to inform decision-making on the optimal therapy for a newly diagnosed patient.

**MSU crystals as an endogenous danger signal**

The immune system identifies infections through cellular activation by microbial adjuvants (reviewed in [34]); immune responses to non-microbial stimuli (such as tumours or transplanted cells) also require adjuvants to generate an immune response, and dying cells are thought to be important activators of this response through release of endogenous adjuvants (or ‘danger signals’) [35]. Until recently, the identity of these danger signals has not been known. In 2003, Shi et al. [36] reported that most of the endogenous activity involved in priming CD8 T-cell responses to dying cells is due to MSU crystals. This study demonstrated that dying cells have super-saturating concentrations of urate (likely due to liberation of purines through degradation of DNA and RNA), and that in \textit{vivo} elimination of urate using allopurinol and uricase inhibits the adjuvant activity, and reduces priming of cytotoxic T lymphocytes (CTLs) by about 90%. This effect was found to occur through stimulation of dendritic cells to increase expression of costimulatory molecules, such as CD86 and CD80. Thus, formation of MSU crystals plays a key role in immune surveillance and generation of adaptive immunity to non-microbial stimuli.

Further work has shown that in mouse tumour models, MSU crystals induce an IL-5 Th2 immune response, suggesting that MSU crystals can also enhance humoral immunity [37]. These observations may be of relevance to the generation of therapeutic immune responses. Adjuvants are frequently used in human vaccines to activate the immune system to foreign antigen. Alum is the most frequently used adjuvant in human vaccines, and predominantly induces humoral immunity, possibly through activation of Th2 cells. A recent study has shown that high concentrations of urate are present after injection of alum into the peritoneal cavities of mice [38]. Intra-peritoneal injection of alum was associated with an intense inflammatory response that was entirely abrogated by treatment with uricase, and was not observed in MyD88-deficient mice (see below). These findings suggest that alum acts as an adjuvant by inducing formation of MSU crystals, which in turn promotes differentiation and activation of inflammatory dendritic cells.

The potential clinical relevance of these findings has been explored further in animal models of non-microbial immunity. In a mouse tumour model, elevated urate levels are found in tumours undergoing immune rejection [39]. Subcutaneous injection of MSU crystals close to the site of the tumour enhances the tumour immune response, and both allopurinol and uricase delay tumour immune rejection [39]. In a tumour protection model, addition of MSU crystals to dying tumour cells suppresses subsequent tumour growth in a dose-dependent manner [37]. In a mouse model of transplant immunity, elimination of urate using a combination of allopurinol and uricase reduces generation of CTLs to an antigen in transplanted syngeneic cells by ~80% [40]. Urate depletion also inhibits proliferation of auto-reactive T cells in a transgenic mouse diabetes model. This effect is related to reduced activation of endogenous antigen-presenting cells [40].

Together, these data provide strong and consistent evidence for the role of MSU crystals in animal models of non-microbial immune surveillance. These data suggest a potential biological advantage to hyperuricaemia and formation of MSU crystals in immune surveillance and generation of adaptive immunity. However, it should be noted that serum urate levels are low in mice and most other non-primate mammals due to persistence of uricase. Studies of human immune responses are needed to clarify whether similar mechanisms of immune activation are relevant in human disease.

If these effects are confirmed in human subjects, there are several implications for treatment of human disease. First, these observations suggest that gout therapies that suppress the serum urate to very low levels for prolonged periods may be associated with increased risk of immunosuppression, and reduced immune activation in response to non-microbial danger signals and vaccines. At present, there is no documented evidence to support this hypothesis, but long-term safety monitoring of newly developed potent urate-lowering therapies will be needed to clarify the optimal serum urate levels in treatment of gout. Second, these data suggest potential new therapeutic indications for potent urate-lowering therapies, particularly in the fields of transplantation and autoimmunity.

**The central role of IL-1β in the pathogenesis of acute gouty inflammation**

It has been known for many years that MSU crystals stimulate monocytes and macrophages to produce IL-1β [41]. However, the central importance of IL-1β in the initiation and amplification phases of the acute gout attack has been recently demonstrated. The NALP3 inflammasome (cryopyrin) is a complex of intracellular proteins that is activated on exposure to microbial elements, such as bacterial RNA and toxins [42, 43], and is required for adequate responses to adjuvants such as alum [44]. Activation

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**Table 1. Summary of replicated genetic associations with gout and contributing phenotypes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
<th>Effect size (OR)</th>
<th>Population</th>
<th>Molecular mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC2A9/GLUT9</td>
<td>Hyperuricaemia</td>
<td>1.7–1.9</td>
<td>Caucasian</td>
<td>Refer to text herein</td>
<td>[5–9]</td>
</tr>
<tr>
<td></td>
<td>Gout</td>
<td>1.3–1.7</td>
<td>Caucasian</td>
<td></td>
<td>[8, 10]</td>
</tr>
<tr>
<td>SLC2A9/URAT1</td>
<td>Reduced renal uric acid excretion</td>
<td>1.4</td>
<td>Caucasian</td>
<td>A possible influence on isoform levels</td>
<td>[17]</td>
</tr>
<tr>
<td>Serum urate</td>
<td>Not applicable</td>
<td></td>
<td>Japanese</td>
<td></td>
<td>[18]</td>
</tr>
<tr>
<td>Serum urate</td>
<td>Not applicable</td>
<td></td>
<td>Chinese</td>
<td>The 64Arg variant is associated with higher serum urate levels in three of the four studies [not the Masuo et al. (22)] study. This may induce insulin resistance as a result of lower lipolysis and increased adipose tissue.</td>
<td>[19]</td>
</tr>
<tr>
<td>Hyperuricaemia</td>
<td>1.6</td>
<td>Korean</td>
<td></td>
<td>The 677T allele is associated with hyperuricaemia in the three studies. It is possible that this variant allows greater availability of 5,10-methyltetrahydrofolate for de novo synthesis of purines.</td>
<td>[20]</td>
</tr>
<tr>
<td>Hyperuricaemia</td>
<td>2.4</td>
<td>Italian</td>
<td></td>
<td></td>
<td>[21]</td>
</tr>
<tr>
<td>Hyperuricaemia</td>
<td>1.1</td>
<td>Japanese</td>
<td></td>
<td></td>
<td>[22]</td>
</tr>
<tr>
<td>Hyperuricaemia</td>
<td>1.7</td>
<td>Korean</td>
<td></td>
<td></td>
<td>[23]</td>
</tr>
<tr>
<td>Methylenetetrahydrofolate reductase (MTHFR)</td>
<td>Hyperuricaemia</td>
<td>1.5</td>
<td>Iranian</td>
<td></td>
<td>[24]</td>
</tr>
<tr>
<td>Hyperuricaemia</td>
<td>Not given</td>
<td>Japanese</td>
<td></td>
<td></td>
<td>[25]</td>
</tr>
</tbody>
</table>

*Restricted to common variants (polymorphisms) of genes. For single copy of associated variant.
of this protein complex leads to release of caspase-1, which is required for cleavage of pro-IL-1β to the active form of IL-1β. A recent report has demonstrated that the NALP3 inflammasome is essential for acute gouty inflammation [45]. MSU crystals activate caspase-1 and lead to release of IL-1β in human monocytes. These effects are reduced in macrophages from mice lacking components of the NALP3 inflammasome. Neutrophil influx following injection of crystals into the peritoneal cavity is impaired in mice lacking components of the NALP3 inflammasome, and also in mice deficient in the IL-1 receptor. Interestingly, colchicine, a drug that is frequently used to prevent and treat acute gout attacks, blocks IL-1β maturation by MSU crystals in vitro, suggesting that the therapeutic effect of this agent may be, at least in part, due to inhibition of NALP3 inflammasome activation.

The essential role of IL-1β has been further emphasized by work showing that MyD88, an intracellular adaptor protein involved in IL-1 receptor (IL-1R) signalling, is required for the inflammatory response to MSU crystals [46, 47]. Mice deficient in MyD88 or the MyD88-dependent IL-1 receptor show reduced neutrophil influx in response to MSU crystal injection, and blockade of IL-1 by neutralizing antibodies also attenuates the inflammatory response in the urate peritonitis model [47, 48]. The relevance of these observations has been confirmed by a proof-of-concept open study of 10 patients with acute gout, which demonstrated rapid response to IL-1 inhibition by anakinra [48].

These studies provide new insights into the pathways that lead to acute gouty inflammation, showing that MSU crystals activate highly conserved pathways of innate immunity to induce the acute inflammatory response in gout. Together, these reports point to a new paradigm of disease pathogenesis with IL-1 inflammatory response in the urate peritonitis model [47, 48].

Mechanisms of bone erosion in gout

In the presence of persistent hyperuricaemia, some patients with gout develop tophi and erosive joint disease. RANK ligand (RANKL)-mediated osteoclastogenesis has been identified as a process in the development of bone erosion in other forms of inflammatory arthritis, such as RA and PsA [49, 50]. However, the mechanisms of bone erosion in gout may differ from those in autoimmune inflammatory arthritis, as gouty erosions usually occur in the context of IA deposition of tophi [51]. Disordered osteoclast development has recently been implicated in the pathogenesis of the gouty erosion [52]. Peripheral blood mononuclear cells from patients with severe erosive gout have preferential ability to form osteoclast-like cells following stimulation with RANKL and M-CSF, and high numbers of these cells are cultured from SF from gouty knee effusions. Numerous osteoclast-like cells are localized within tophi and at the interface between soft tissue and bone in patients with gout. Although MSU crystals do not directly promote osteoclast formation from precursor cells in vitro, these crystals do alter the RANKL/osteoprotegerin (OPG) balance within stromal cells, and thus indirectly promote osteoclastogenesis. These data provide a clear rationale for the study of agents that target the RANKL/OPG axis or the osteoclast for prevention of erosion progression in chronic gout.

Conclusion

Despite the well-recognized role of hyperuricaemia and MSU crystal formation in the pathogenesis of gout, many patients with this disease continue to experience recurrent flares, chronic pain and disability [53–55]. The major developments in the understanding of the basic science of all stages of this disease, from hyperuricaemia to recurrent acute gout attacks to chronic erosive disease, should lead to development of novel therapeutic approaches to diagnosis, treatment and monitoring of this disease.

References


