Sleep Medicine Reviews xxx (2011) 1–14

Contents lists available at SciVerse ScienceDirect

Sleep Medicine Reviews

journal homepage: www.elsevier.com/locate/smrv

THEORETICAL REVIEW

Restless legs syndrome – Theoretical roles of inflammatory and immune mechanisms

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ARTICLE INFO

Article history:
Received 27 May 2011
Received in revised form
22 September 2011
Accepted 26 September 2011
Available online xxx

Keywords:
Restless legs syndrome
Irritable bowel syndrome
Small intestinal bacterial overgrowth
Lactulose breath test
Inflammation
Immune
Iron
Dopamine
Opioids

SUMMARY

Theories for restless legs syndrome (RLS) pathogenesis include iron deficiency, dopamine dysregulation and peripheral neuropathy. Increased prevalence of small intestinal bacterial overgrowth (SIBO) in controlled studies in RLS and case reports of post-infectious RLS suggest potential roles for inflammation and immunological alterations.

A literature search for all conditions associated with RLS was performed. These included secondary RLS disorders and factors that may exacerbate RLS. All of these conditions were reviewed with respect to potential pathogenesis including reports of iron deficiency, neuropathy, SIBO, inflammation and immune changes. A condition was defined as highly-associated if there was a prevalence study that utilized an appropriate control group. Small case reports were recorded but not included as definite RLS-associated conditions.

Fifty four diseases, syndromes and conditions have been reported to cause and/or exacerbate RLS. Of these, 38 have been reported to have a higher prevalence than age-matched controls, 9 have adequate sized reports and have general acceptance as RLS-associated conditions and 7 have been reported in case report form. Overall, 42 of the 47 RLS-associated conditions (89%) have also been associated with inflammatory and/or immune changes. In addition, 43% have been associated with peripheral iron deficiency, 40% with peripheral neuropathy and 32% with SIBO. Most of the remaining conditions have yet to be studied for these factors.

The fact that 95% of the 38 highly-associated RLS conditions are also associated with inflammatory/immune changes suggests the possibility that RLS may be mediated or affected through these mechanisms. Inflammation can be responsible for iron deficiency and hypothetically could cause central nervous system iron deficiency-induced RLS. Alternatively, an immune reaction to gastrointestinal bacteria or other antigens may hypothetically cause RLS by a direct immunological attack on the central or peripheral nervous system.

Introduction

The etiology of restless legs syndrome (RLS) is unknown. Current evidence for the pathophysiology of RLS suggests dopaminergic dysfunction and altered control of iron homeostasis with central nervous system (CNS) iron depletion.[1] Data for RLS-associated genetic links that allow for iron deficiency and other pathophysiological changes is also emerging.[2–5] Peripheral neuropathy with or without clinical symptoms has been proposed as a secondary cause for RLS in a number of disorders.[6] Recently the endogenous opiate system has been suspected as having a role in the pathophysiology of RLS by stabilizing dopaminergic substantia nigra degeneration under conditions of iron deprivation.[7] In addition, human autopsy studies have shown decrements in the endogenous opioids beta endorphin and met enkephalin in the thalamus of RLS patients compared to controls.[8]

However, recent controlled studies demonstrating an increased prevalence of small intestinal bacterial overgrowth (SIBO), human immunodeficiency virus (HIV) infection, and systemic lupus erythematosis and cases of hepatitis C-, *streptococcus-, Mycoplasma-, and Borrelia*-associated RLS suggest a potential role for

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doi:10.1016/j.smrv.2011.09.003

Abbreviations

CNS	central nervous system
CSF	cerebrospinal fluid
ESRD	end-stage renal disease
GI	gastrointestinal
HCV	hepatitis C virus
HIF-1a	hypoxia inducible factor-1a
HIV	human immunodeficiency virus
IL	interleukins
IBS	irritable bowel syndrome
LBT	lactulose breath test
LPS	lipopolysaccharides
MS	multiple sclerosis
NOS	nitric oxide synthase
NO	nitric oxide
PLMD	periodic limb movements disorder
PLMS	periodic limb movements in sleep
RLS	restless legs syndrome
RA	rheumatoid arthritis
SIBO	small intestinal bacterial overgrowth
SLE	systemic lupus erythematosis
TfR	transferrin receptor
TNF-α	tumor necrosis factor-alpha

Glossary

RLS (restless legs syndrome) defined by 4 obligate criteria: a) an urge to move the legs usually but not always associated with leg discomfort; b) worsening of symptoms at rest, i.e., lying or sitting; c) at least partial and temporary relief by activity; and d) worsening of symptoms later in the day or at night.

SIBO (small intestinal bacterial overgrowth) syndrome defined as when there are more than 10^5 colony functioning units higher than the distal ileum that may lead to vitamin malabsorption, malnutrition and/or weight loss.

inflammation and/or immunological disorders in RLS.[9–14] Recent studies suggest roles for hypoxia inducible factor-1a (HIF-1a) and nitric oxide synthase (NOS) in RLS which also support the potential role for immune and inflammatory mechanisms in RLS.[15,16] In addition, nitric oxide is known to play a role in inflammation independent of its relationship to HIF-1a.[17] Three other gastrointestinal disorders have recently been linked to RLS: irritable bowel syndrome (IBS), Crohn's disease and celiac disease.[9,18–21] All three can be associated with systemic inflammation, immune alterations and SIBO.[22–25] All of these observations led us to consider whether inflammation and immune dysfunction might play a more general role in RLS.

To expand on this theory, we review all known causes for secondary RLS and risk factors that exacerbate RLS in general and report evidence for inflammation, immune disorders and SIBO as well as iron deficiency and peripheral neuropathy in each of these conditions. Hepcidin has also been found to be altered in RLS and thus this relationship was explored further.[26] The peptide hepcidin is the primary iron regulatory hormone and is up-regulated by inflammation as well as by increased iron stores in the liver.[27] The potential interaction of these potential pathogenic factors with RLS-related genes is also discussed.

Methods

A Pubmed search for disorders and risk factors highly-associated with RLS was carried out. These conditions included diseases commonly known as secondary RLS disorders and risk factors that are known to exacerbate RLS. The key words for the search started by using "restless legs syndrome" or "RLS" (alone) and RLS matched with etiology, pathogenesis, risk factors, risk and/or cause. This was supplemented by detailed analysis of review articles and texts in order to determine all of the disorders highly-associated with RLS. To be comprehensive, a similar search using "periodic limb movements", periodic limb movements disorder (PLMD) and periodic limb movements in sleep (PLMS) was done in order to look for other conditions associated with RLS. If there was controversy whether or not the condition could be considered an RLS mimic, the best available most recent controlled study was chosen and articles that expressed alternative conclusions were critically reviewed. When supporting documentation of a condition was determined to be on the basis of an observational study, an expert neurologist (AW) skilled in diagnosis of RLS decided whether or not it was generally considered to be RLS or a mimic. A condition was defined as highly-associated if there was a prevalence study that utilized an appropriate age-matched control group. Small case reports were recorded but not included as definite RLS-associated conditions. When a disease was recently observed to be associated with RLS as a single case report or small case series, it was not considered a highly-associated condition. However, these diseases are listed in the results section as well as part of this comprehensive review process in order to consider other possible mechanisms for RLS.

Each individual RLS disorder or risk factor were separately matched with each of the following key words: iron, iron deficiency, hepcidin, peripheral neuropathy, neuropathy, SIBO, bacterial overgrowth, inflammation, IL-1, IL-6, IL-8, IL-12, IL-17, IL-18, cytokines, tumor necrosis factor-alpha (TNF-α), lipopolysaccharides (LPS), antibodies, immune, immune disorders, immune alterations, inflammation, infection and iron regulatory genes. It was then determined if these factors might be common to a majority of the RLS-associated conditions. Reports of interaction of inflammation, infection and immune alterations with RLS and iron regulatory genes were then evaluated.

Studies were identified as being controlled studies, observational studies which included case series, laboratory studies which included measurements using established assays but without a control group and review articles which supported the condition with citation of multiple studies. When peripheral neuropathy was reported in case reports in conditions not normally well known to have neuropathy, this was denoted in Table 1 but was not included in the total number of conditions with peripheral neuropathy. The search for iron deficiency for each condition included systemic and CNS iron deficiency. Only five conditions had studies of CNS iron stores and are denoted on the table. In the remainder of the conditions, iron deficiency was denoted as "No" if systemic iron deficiency is generally known not to be present in the condition. This is in contrast to the literature search for SIBO and peripheral neuropathy where "NS" (not studied) was generally applied since these factors can be subclinical.

Results

The occurrence of iron deficiency, SIBO, inflammatory and/or immunological mechanisms and peripheral neuropathy discovered by the literature review of RLS disorders are compiled in Table 1. Determinations for the presence of CNS iron deficiency, SIBO, cytokines, immune disorders and hepcidin have not been performed in many of these RLS-associated conditions.

Please cite this article in press as: Weinstock LB, et al., Restless legs syndrome – Theoretical roles of inflammatory and immune mechanisms, Sleep Medicine Reviews (2011), doi:10.1016/j.smrv.2011.09.003

Table 1
Iron deficiency, small intestinal bacterial overgrowth (SIBO), inflammation and/or immunological alterations and peripheral neuropathy in conditions associated with restless legs syndrome (RLS). References are categorized as either: a controlled study (CS); an observational case series (OS); a laboratory study (LS) which uses defined assays but does not have a control group; or a review article (RA). Highly-associated conditions are defined as RLS conditions shown to have a statistically higher prevalence than controls. This table does not include seven single case reports associated with RLS (see result section).

Name and reference for conditions associated with RLS	Systemic iron deficiency[a]	SIBO[b]	Inflammation and/or immune alterations[c]	Peripheral neuropathy[d]
Total ($N = 47$)	29 (43%)	15 (32%)	42 (89%)	19 (40%)
Highly-associated conditions ($N = 38$)	14 (37%)	12 (32%)	36 (95%)	14 (37%)
Type of study (totals) CS = 38; OS = 11; RA = 1	CS = 10; OS = 10; RA = 2	CS = 12, OS = 2; RA = 1	CS = 18, OS = 2; LS = 20; RA = 11	CS = 6; OS = 5; LS = 4; RA = 4
Neurologic diseases				
Parkinson's disease[126 CS]	No[e]	Yes[45 CS]	Yes[127 LS,128 RA]	No[d]
Multiple sclerosis[64 CS]	No[e]	NS	Yes[129 CS]	No[d]
Essential tremor[130 CS]	NS	NS	NS	NS
Migraine[131 CS]	No[e]	NS	Yes[132 CS]	NS
ADHD[62 CS]	Yes[133 CS]	NS	Yes[134 LS]	NS
Narcolepsy[75 CS]	No	NS	Yes[135 LS]	NS
Post-stroke[118 CS]	No	NS	Yes[136 LS]	NS
Charcot-Marie-Tooth type 2 [137 CS]	No	NS	Yes [138 LS]	Yes [139 RA]
Spinocerebellar ataxia[140 CS]	No	NS	Yes[141 LS]	Yes[142 LS]
Chronic inflammatory demyelinating polyneuropathy[143 CS]	No	NS	Yes[144 RA]	Yes[144 RA]
Spinal cord injury[145 CS]	No	NS	Yes[146 LS]	NS
Amyotrophic lateral sclerosis[147 CS]	No[e]	NS	Yes[148 CS]	Yes[149 LS]
Alzheimer's disease[150 CS]	No[e]	NS	Yes[151 RA]	No
Post-polio syndrome[152 CS]	No	NS	Yes[153 LS]	No
Tourette's syndrome[154 OS]	Yes[155 CS]	NS	Yes[156,157 LS]	NS
Friedrich's ataxia[158 OS]	Yes[158 OS]	NS	NS	NS
Lumbar radiculopathy[159 OS]	No	NS	Yes[160 CS]	Yes[161 RA]
Gastrointestinal disorders				
Irritable bowel syndrome[9 CS]	No	Yes[162 CS]	Yes[163 CS]	NS
Celiac disease[19 CS]	Yes[21 OS]	Yes[164 CS]	Yes[22 LS, 25 CS]	Yes[165 LS]
Crohn's disease[18 CS]	Yes[166 CS]	Yes[167 RA]	Yes[23,168 RA]	Yes[169 CS]
Gastric resection[170 CS]	Yes[171 OS]	Yes[172 OS]	NS	No[d]
Chronic liver disease[173 OS]	No	Yes[174 CS]	Yes[175 LS]	Yes[176 OS]
Rheumatologic diseases				
Rheumatoid arthritis[72 CS]	Yes[177 CS]	Yes[43 CS]	Yes[178,179 RA]	Yes[180 OS]
Fibromyalgia[181 CS]	No	Yes[47 CS]	Yes[182,183 CS]	NS
Sjögren's syndrome[184 CS]	Yes[185 OS]	NS	Yes[186 CS]	Yes[187 CS]
Bruxism[188 CS]	No	NS	NS	NS
Systemic lupus erythematosis [10,189 CS]	No	NS	Yes [195 RA]	No[d]
Scleroderma[190 OS]	Yes[191 OS]	Yes[46 CS]	Yes[192 CS]	Yes[193 OS]
Cryoglobulinemia[63 OS]	No	NS	Yes[194,195 LS]	Yes[196 CS]
Metabolic conditions				
Renal disease[197 CS]	Yes[198 OS]	Yes[49 CS]	Yes[199 RA]	Yes[200 RA]
Diabetes[201 CS]	Yes[202 CS]	Yes[203 CS]	Yes[204 CS]	Yes[205 CS]
Pregnancy[206 CS]	Yes[207 OS]	NS	Yes[208 LS]	No[d]
Obesity[209 CS]	Yes[210 CS]	Yes[48 CS]	Yes[210 CS]	No[d]
Hypothyroidism[211 CS]	Yes[212 RA]	Yes[44 OS]	Yes[213 OS]	No[d]
Acromegaly[214 CS]	No	Yes[215 CS]	Yes[216 LS]	Yes[217 LS]
Pulmonary disorders				
Sleep apnea[218 CS]	No	NS	Yes[219 CS]	No[d]
Sarcoidosis[220 CS]	Yes[221 OS]	NS	Yes[222,223 LS]	Yes[224 OS]
COPD[225 CS]	Yes[226 OS]	NS	Yes[227,228 CS]	NS
Pulmonary hypertension[229 OS]	No	NS	Yes[227,230 CS]	NS
Lung transplantation[231 OS]	Yes[232 OS]	NS	Yes[233 LS]	NS
Other conditions				
Anemia[234 CS]	Yes[54 CS]	NS	Yes[235 CS]	No
Age[218 CS]	Yes[236 RA]	Yes[237 CS]	Yes[238 CS]	Yes[239 CS]
Depression[240 CS]	Yes[241 CS]	NS	Yes[242 CS]	No
Chronic venous disorder[243 CS]	No	NS	Yes[244 RA]	Yes[245 CS]
Erectile dysfunction[246 CS]	No	NS	Yes[247 RA]	NS
Drug-induced RLS[248 RA, 249 CS]	No	NS	NS	No
Post-infectious RLS[11 CS, 12 OS, 14 OS]	No	NS	Yes[11 CS, 12 OS]	Yes[13 OS]

Additional abbreviations: ADHD: attention-deficit/hyperactivity disorder; COPD: chronic obstructive pulmonary disease; NS: not studied.
[a] Includes decreased systemic (peripheral) iron and/or ferritin levels.
[b] Includes reports where there is a significant prevalence of SIBO by either culture or breath testing.
[c] Includes alterations in inflammation: increased levels of interleukins 1, 6, 8, 12 and/or 17 and/or tumor necrosis factor-alpha (TNF-α) and/or overall immune function.
[d] This denotes that there are case reports of peripheral neuropathy in conditions where neuropathy is not well known to occur.
[e] Studies show that CNS iron levels are normal.

A total of 54 conditions have been reported to be associated with RLS. These include 47 conditions with multiple patients in controlled studies or sizable case series (see Table 1 for references) and 7 disorders with single or small numbers of patients. Using controlled studies alone, 38 conditions were identified as being highly-associated with RLS. With respect to drug-induced RLS, in addition to a controlled study for serotonin reuptake inhibitor (SSRI) therapy, a review article is cited which included many other

drugs that have been reported in case report format. Seven of the 11 conditions supported by observational studies in the Table 1 had large numbers of patients: lumbar radiculopathy (21% of 243 consecutive sleep disordered patients with lumbosacral radiculopathy had RLS), Tourette's syndrome (10% of 144 patients had RLS), chronic liver disease (67% of 141 patients had RLS, although this was reduced to 16% of 23 patients when other RLS co-factors such as renal insufficiency were used as exclusions), pulmonary hypertension (44% of 55 patients had RLS), lung transplantation (48% of 42 patients had RLS), scleroderma (22% of 27 patients had RLS) and cryoglobulinemia (in a series of 88 patients with cryoglobulinemic neuropathy, there were 78 hepatitis C virus (HCV)-positive patients [37 with RLS] and 10 HCV-negative patients [2 with RLS]; Franco Gemignani, MD, unpublished data). One of the 11 observational studies was medium-sized: Friedrich's ataxia (50% of 16 patients had RLS). Some of the case series included in the post-infectious RLS condition were small although on the other hand a controlled study of HIV patients showed significant difference from controls. Finally, gastric resection was included in the Table 1 since it was one of the first secondary causes of RLS described.

Seven case reports of conditions occurring with RLS were not included in Table 1 or in the 38 conditions highly-associated with RLS. These included familial amyloidosis (4 siblings),[28] Morton's neuroma (1 patient whose symptoms improved after neuroma surgery),[29] Huntington's disease (1 patient),[30] hyperparathyroidism (1 patient with mild hypercalcemia that RLS resolved after parathyroid resection),[31] multifocal motor neuropathy (1 patient),[32,33] Chiari type 1 syndrome (5 patients)[34] and transverse myelitis (1 patient who developed myelitis immediately following mononucleosis).[35] The latter four conditions, however, are suggestive of the possible role of alterations in spinal sensorimotor circuits as an alternative explanation for RLS pathophysiology.[36]

Overall, 42 of the 47 (89%) RLS-associated conditions are also reported to be associated with inflammation and/or immune changes. When highly-associated conditions were analyzed, 36/38 (95%) are reported to have these changes. Inflammation was most commonly associated with elevated interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels. In the other 5 RLS-associated conditions, inflammation has not been studied. In addition, 20 (43%) are associated with systemic iron deficiency, 19 (40%) with peripheral neuropathy and 15 (43%) with SIBO (see Fig. 1). Many of the conditions have not been studied for CNS iron deficiency or SIBO. The level of confidence that each condition was associated with each pathological condition may be reflected in the type of studies that are available. For iron deficiency there were 10 controlled studies, 10 observational studies and 2 review articles. For SIBO there were 12 controlled studies, 2 observational studies and 1 review article. For inflammatory and/or immune disorders there were 18 controlled studies, 2 observational studies, 20 laboratory studies and 11 review articles. Finally, for peripheral neuropathy, there were 6 controlled studies, 5 observational studies, 4 laboratory studies and 4 review articles.

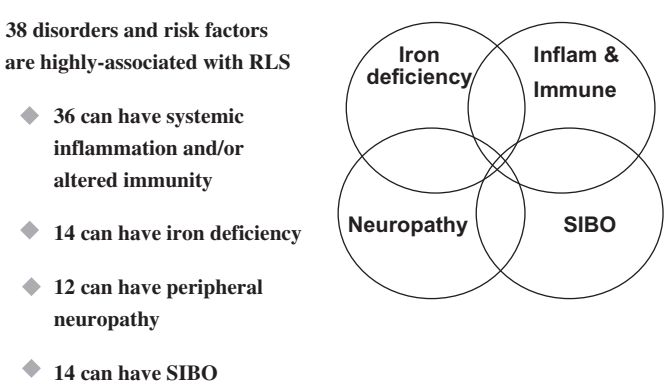

Fig. 1. Potential interplay of pathologic factors in secondary RLS. Abbreviations: RLS: restless legs syndrome; Inflam & Immune: inflammation and/or altered immunity; SIBO: small intestinal bacterial overgrowth; Neuropathy: peripheral neuropathy.

Of the 17 neurological disorders, 3 have been associated with iron deficiency, 1 with SIBO, 15 with inflammation/immune alterations and 5 with peripheral neuropathy. Five have been investigated to determine if there were disorders of CNS iron levels and the studies showed increased iron levels in the brain (see Table 1). Rare case reports of neuropathy for conditions whereby neuropathy is not part of the usual presentation are noted in the table but are not counted in the total number above or in Fig. 1. Of the 5 gastrointestinal disorders, 3 have been associated with iron deficiency, 5 with SIBO, 4 with inflammation/immune alterations and 3 with neuropathy. Of the 7 rheumatologic disorders, 3 have been associated with iron deficiency, 3 with SIBO, 6 with inflammation/immune alterations and 4 with neuropathy. In the 6 metabolic disorders, 5 have been associated with iron deficiency, 5 with SIBO, 6 with inflammation/immune alterations and 3 with neuropathy. Of the 5 pulmonary disorders, 3 have been associated with iron deficiency, 5 with inflammation/immune alterations and 1 with neuropathy. None have been studied for SIBO.

In the 7 other miscellaneous conditions, anemia has been associated with iron deficiency and inflammation/immune alterations; aging has been associated with all four factors; depression has been associated with iron deficiency and inflammation/immune alterations; chronic venous disorder has been associated with inflammation and neuropathy; and erectile dysfunction has been associated with inflammation. Post-infectious RLS has been associated with immune and neuropathic alterations. Drug-induced RLS has not been studied for these associated factors.

In 9 of the 47 RLS-associated conditions that have been studied for alterations in hepcidin activity, 5 have had elevated levels, 2 have had variable levels and 2 have had normal levels. The disorders with elevated hepcidin levels include rheumatoid arthritis (RA), end-stage renal disease (ESRD), pregnancy, obesity and iron deficiency anemia.[37–40]

Discussion

Since the etiology of RLS unknown, a central organizing concept is needed to explain the vast number of conditions that trigger this well defined syndrome, reasonable therapeutic improvement by various pharmacologic agents, the evolving role of iron regulation and the various genetic loci that have been associated with RLS in selected populations. In light of the fact that the same set of symptoms occurs in idiopathic RLS, familial RLS and RLS that is possibly caused or exacerbated by up to 54 conditions, it is reasonable to anticipate that there are multiple pathways involved in RLS pathogenesis. We have tried to find what potential pathogenic mechanisms and risk factors are in common in the major secondary RLS conditions. We have therefore examined iron deficiency, peripheral neuropathy, small intestinal bacterial overgrowth (SIBO) and inflammation/immune alterations. Our review of the RLS literature shows that the majority of conditions highly-associated with RLS have also been reported to be associated with systemic inflammation and/or immune disturbances. This connection has not been explored in the past for the pathogenesis of RLS.

Overall, 89% of RLS-associated conditions have also been associated with inflammation and/or immune changes, 43% with systemic iron deficiency, 40% with peripheral neuropathy and 32% with SIBO. When controlled studies were used to define a condition as a highly-associated RLS condition in this analysis, 95% of these

disorders have also been associated with inflammation and/or immune changes, 37% with systemic iron deficiency, 37% with peripheral neuropathy and 32% with SIBO. By using controlled studies with aged-matched controls to determine that a condition was highly-associated with RLS, the concern that age alone or age associated inflammatory conditions allowed for a chance occurrence was lessened. Many of the RLS conditions have not been specifically studied for these factors as shown in Table 1. Most secondary RLS conditions have not been examined for evidence of the CNS iron deficiency that is commonly seen in primary RLS. Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease and migraine are associated with RLS but have neither systemic nor CNS iron deficiency. Inflammation was most commonly associated with increased levels of pro-inflammatory interleukins – most commonly IL-6, but also interleukins 1, 2, 4, 12, 17 and 18. TNF-α was a commonly elevated cytokine in these conditions. A diagnosis of SIBO was prevalent in all 15 conditions whenever it was examined by either direct testing with intestinal cultures or indirect testing with gas chromatography carbohydrate fermentation breath tests. The SIBO syndrome is classically defined when there are more than 10^5 colony functioning units higher than the distal ileum that may lead to vitamin malabsorption, malnutrition and/or weight loss.[41] Emerging data show that IBS is directly associated with SIBO and both are associated with elevated levels of systemic inflammatory cytokines[42] and extra-intestinal disorders which may not show signs of nutritional deficiencies or significant gastrointestinal symptoms.[43–49] All of these data raise the possibility that some of so-called idiopathic RLS could also be mediated by inflammatory and/or immunological mechanisms. Our controlled study showing a higher prevalence of SIBO in RLS patients compared to controls and case reports of *streptococcal*-, *Mycoplasma*- and hepatitis C-induced RLS plus recent observations of up-regulation of HIF-1a and NOS in RLS further suggest potential roles for these mechanisms.[3,9,12,13,15,50]

Preliminary inflammatory and immunological studies in RLS

A preliminary study of serum cytokines in idiopathic RLS was negative.[51] A blinded search for changes in serum complements in a small RLS study was negative (AS Walters, MD, unpublished data). In both studies, however, the sample sizes were small and serum levels were obtained during the day which does not account for the diurnal variation of RLS. In a study of 167 RLS and/or PLMS patients, there was a statistically significant increase in the inflammatory marker C-reactive protein that was directly proportional to an increase in PLMS/hour.[52] Similar to the previous study of cytokines in RLS, however, there was no relationship of PLMS/hour to the cytokines IL-6 or TNF-α. Although this study suggested that PLMS severity causes inflammation, it does not preclude the possibility that RLS is triggered by inflammation.

The means by which inflammation and altered immunity could contribute to the cause and/or exacerbation of RLS includes three major theories: 1) inflammation causes CNS iron deficiency through alterations in hepcidin; 2) humoral or cellular immunological mechanisms cause a direct attack upon the central or peripheral nervous system; or 3) RLS gene variants interact with inflammatory disorders, immune alterations and/or chronic infections such as SIBO to potentiate them. Each potential mechanism is explored in detail.

Relation of inflammation and CNS iron deficiency to RLS

Inflammatory mechanisms and mediators include white blood cells, cytokines (including pro-inflammatory interleukins and TNF-α), nitric oxide, prostaglandins and substance P. In primary and familial RLS, central iron deficiency is well documented by cerebrospinal fluid, magnetic resonance imaging and autopsy studies.[53] In both primary and secondary RLS conditions systemic (peripheral) iron deficiency contribute to RLS symptoms.[54] Inflammation can lead to systemic iron deficiency and it therefore seems reasonable that inflammation could trigger CNS iron deficiency and subsequent RLS symptoms.

Hepcidin may be the primary link to explain this phenomenon. Fig. 2 illustrates how this could happen. Hepcidin is the main hormone involved in regulation of iron levels and has been shown to be produced by the liver in humans and the brain in animal models.[55,56] It is known that increased hepcidin levels can lead to decreased serum iron levels and perhaps decreased availability of the iron to the brain (see Fig. 2).[27,57] Increased hepcidin levels may occur in the face of inflammation as it is known that inflammation can lead to cytokine IL-6 production which can stimulate hepcidin production. In addition, in the face of infections, lipopolysaccharides form as a breakdown product of bacteria. It is known that lipopolysaccharides can also stimulate hepcidin production.[58] Hypoxia is an additional known cause of increased hepcidin production (Fig. 2).[59] Both IL-6 and LPS levels are elevated in SIBO and IL-6 is elevated in many inflammatory systemic disorders. Lipopolysaccharides are increased in SIBO because gram negative bacteria translocate through the gastrointestinal (GI) mucosa and the bacterial coating is shed and absorbed into the systemic circulation. In addition, it is known that ferroportin normally binds to iron to export iron from cells into the circulation. Hepcidin will bind to ferroportin which reduces iron trafficking out of cells in the duodenum and reticulocyte endothelial system thus reducing iron availability.[58] When hepcidin binds to ferroportin the ferroportin is degraded and thus iron is not exported from cells into the circulation (Fig. 2). In the face of increased hepcidin production, ferroportin is not available to bind to iron and iron deficiency could result (Fig. 2). Since all of the iron that is available to the body comes externally from eating, if iron is not absorbed from the GI tract into the circulation, anemia would result with subsequent decreased iron availability to all the body organs including the brain. Compatible with this is the observation that systemic iron deficiency correlates with decreased cerebrospinal fluid (CSF) iron levels.[53]

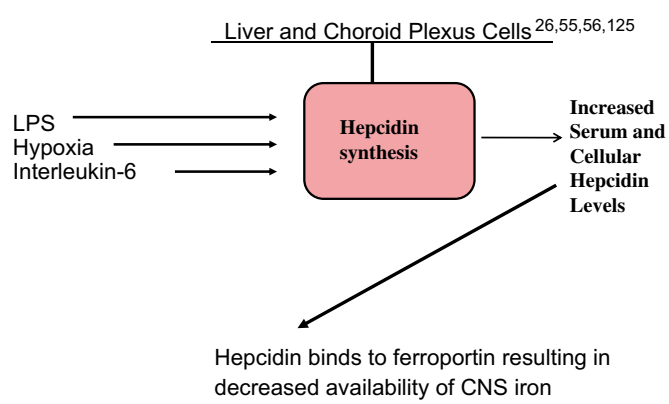

Fig. 2. Regulation of hepcidin synthesis in the setting of inflammation and theoretical consequences for developing CNS iron deficiency and subsequent RLS. Hepcidin is the main hormone involved in regulation of iron levels and has been shown to be produced by the liver in humans and in the brain in animal models. Increased hepcidin levels lead to iron deficiency. Interleukin-6 is the main cytokine that can increase hepcidin levels. Lipopolysaccharides which are breakdown products of gram negative bacteria stimulate hepcidin synthesis. Hypoxia also stimulates hepcidin synthesis. Hepcidin binds to ferroportin on human choroid plexus cells and decrease availability of iron for the CNS. Not shown – Bacteria may also utilize iron and cause iron deficiency.[57] Abbreviations: LPS: lipopolysaccharides.

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Direct up-regulation of hepcidin production in the choroid plexus by systemic inflammation and lipopolysaccharides as seen in murine models may help explain CNS iron deficiency.[55,56] In humans, a search for pro-hepcidin, a precursor to hepcidin, demonstrated increased levels in brain tissue of early onset RLS patients including neuromelanin cells, substantia nigra and putamen and decreased levels in the CSF.[26] This suggests a potential role of hepcidin in primary RLS patients. In this study ferroportin was demonstrated to be present on ependymal cells of the choroid plexus lining the ventricles. This is compatible with our theory in which chronic low grade systemic inflammation is responsible for increased circulating hepcidin levels. When hepcidin becomes bound to choroid plexus-associated ferroportin, this could decrease availability of iron for the CNS in RLS (Fig. 2).

Thus far the conditions highly-associated with RLS reported to have elevated hepcidin levels include RA, ESRD, pregnancy, obesity and iron deficiency anemia.[37–40] Inflammatory cytokines from RA, ESRD, pregnancy and obesity could explain these increased hepcidin levels. Further study of hepcidin in RLS patients with other conditions is warranted.

In the majority of small intestinal diseases associated with SIBO (i.e., small bowel pseudo-obstruction, jejunal diverticulosis), systemic iron deficiency anemia due to iron malabsorption from the duodenum is generally not a problem. Thus simple malabsorption is not a good explanation for how SIBO could lead to CNS iron deficiency. Cytokines or circulating lipopolysaccharides released by SIBO could induce hepcidin release with subsequent reduced transportation of iron into the choroid plexus and brain tissue and explain CNS iron deficiency.[55] In contrast to SIBO, a different type of chronic bacterial infection has been associated with iron deficiency. Helicobacter pylori reduces iron stores possibly by one or more of the following pathways: gastrointestinal blood loss, decrease in the absorption of dietary iron and enhanced uptake of the iron by the bacterium.[57]

Five conditions had studies of CNS iron stores and as denoted on Table 1, in each there was evidence for increased iron levels in the brain. These were all neurological diseases that have also been associated with inflammatory and immune disorders. This suggests that additional mechanisms may be responsible for RLS symptoms and that in some conditions there is no link between inflammation and iron levels.

Immunological disorders and RLS

Immunologic mechanisms focus on either humoral or cellular immunity and include studies of antibodies, cellular immune disturbances, macrophages, killer cells, nitric oxide and complement. Molecular mimicry is the most widely held belief how bacteria or viruses can trigger autoimmune diseases and this has roles in various diseases. The best example in neurological diseases is Guillain–Barré syndrome which can occur after *Campylobacter* enteritis especially in individuals with a specific genotype.[60] We theorize that antigenic stimuli by other gastrointestinal bacteria or other antigens could result in autoimmune nerve damage in the CNS, spinal cord or peripheral nervous system and result in RLS. Several diseases associated with RLS highlight this possible hypothesis.

Data to support that there is a direct attack upon the CNS and peripheral nervous system through humoral or cellular immune mechanisms are derived from post-infectious RLS studies. Six organisms have been reported to cause post-infectious RLS: streptococcus, *Mycoplasma, Borrelia* (Lyme's disease), cytomegalovirus, HIV and HCV.[12,50,61] In the study by Matsuo et al., antibodies against human caudate and putamen were identified in RLS patients with streptococcal and *Mycoplasma* infections.[12] Streptococcal infections can also trigger ADHD which has been associated with RLS.[62] Hepatitis C is more highly-associated with RLS perhaps mediated by neuropathy due to cryoglobulinemia, an immune vasculitis.[63]

Data to support that immune diseases may play a role in RLS include associations with systemic lupus erythematosis (SLE), multiple sclerosis (MS), RA, Sjögren's syndrome, scleroderma, celiac disease and Crohn's disease. SLE is a classic autoimmune disease which only recently has been studied to determine a higher prevalence of RLS than controls.[10]

MS is thought to be associated with RLS. There have been 3 studies documenting an increased prevalence of RLS in MS compared with controls, with a general prevalence rate of RLS in up to one third of MS cases.[64–66] A fourth study with a control group found no difference in RLS prevalence.[67] Restless legs syndrome may have unexplained relapses and remissions which is similar to MS and often is a pattern seen in other autoimmune disorders.[68] Evidence suggests that MS may be triggered by various infections and may do so through molecular mimicry.[69] Interestingly, hydrocortisone, a therapy for MS and other autoimmune disorders, has also been found to be effective against RLS symptoms in a double blind study of RLS patients without MS[70] suggesting a possible autoimmune diathesis in some cases of RLS.

RA is a systemic disease exhibiting both inflammation and immune disorders. Reynolds et al. initially noted that 30% of patients with RA have RLS.[71] Salih et al. subsequently noted that although 25% of patients with RA have RLS, only 4% of patients with osteoarthritis have RLS.[72] This suggests that the RLS seen in RA is just not based upon the discomfort from arthritis alone. In contradiction, another more recent study found an equal prevalence of RLS in patients with RA and osteoarthritis.[73] Nonetheless all of these studies are in agreement as to the high prevalence of RLS in RA. On the other hand, the prevalence of RA and RA serology in idiopathic RLS appears to be low.[74] These data in combination suggest that RA predisposes to RLS and not vice versa which is what one would expect if the immunological hypothesis is true.

Narcolepsy has recently been shown to have an increased prevalence of RLS.[75] Narcolepsy is highly-associated with gene loci on chromosome 6.[76] A positive blood test for HLA-DQB1*0602 is found in the majority of patients with narcolepsy.[77] These genes have immunologic functions. A relationship between immunogenicity in narcolepsy and RLS is thus possible.

Gastrointestinal disorders may shed light on additional potential immunologic alterations and RLS pathogenesis. Data regarding immune disorders associated with IBS and SIBO are emerging[24] and add to other mechanisms that have already been shown in celiac disease and Crohn's disease.

The prevalence of RLS in 85 celiac disease patients was 25% compared with 10% of their spouses ($P < 0.02$).[19] Another large study substantiated this observation in celiac disease.[78] Although it might be presumed that celiac disease causes RLS via malabsorption of iron there are alternative explanations based on immune or inflammatory disorders. In a small case series ($N = 4$) with apparent idiopathic RLS, iron deficiency led to the diagnosis of celiac disease and successful treatment.[21] In contrast, peripheral iron deficiency was correlated with RLS in only half of the patients in a study with 85 RLS patients.[19] Neither a gluten free diet or iron metabolism correlated with RLS in a similarly sized, controlled study.[78] Humoral immune mechanisms appear to have a role in the neurological complications of celiac disease. It was shown that 16/71 (22.5%) celiac patients had neurologic manifestations and presence of serum antibodies to neural antigens and antibody reactivity to neural antigens was detected in 30/71 (42%) patients.[79] Alterations in cellular and humoral immune dysfunction in response to gluten is well known and plays an important role in

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many disorders associated with celiac disease.[80] Thus, more than one mechanism (e.g., iron deficiency, inflammatory and/or immunological alterations) may be relevant causes of RLS in celiac disease.

The prevalence of RLS in 272 Crohn's disease patients was 30% compared with 9% of their spouses ($p < 0.001$).[18] The prevalence of RLS is high in patients with Crohn's disease.[18] Extra-intestinal manifestations of Crohn's disease are a complication of autoantibodies and inflammation.[81] Circulating antibodies to bacteria, autoimmune antibodies, altered immunity, phenotypic genetics and reaction to small intestinal bacteria are important in the pathogenesis of Crohn's disease.[82] The response to intestinal bacteria is well documented to cause alterations in T-cells, IgA response and induction of the inflammatory bowel disease associated gene TL1A in antigen presenting cells.[82] These data all suggest the possibility that RLS in Crohn's disease could also be mediated by immune disorders.

In a recent study, IBS and SIBO were reported to be highly-associated with RLS.[9] IBS was diagnosed in 28% RLS subjects compared to 4% controls ($p = 0.0317$). SIBO as determined by an abnormal lactulose breath test was diagnosed in 69% RLS subjects compared to 28% controls who were unselected for gastrointestinal symptoms ($p = 0.0033$) and compared to 10% of completely asymptomatic controls. In our studies of IBS patients who had SIBO and RLS and with our subsequent clinical experience, it was common to have a history of antecedent gastroenteritis prior to developing both IBS and RLS. Post-infectious IBS is a well known, common phenomenon and has been associated with SIBO and a number of inflammatory and immune alterations, has predisposing genetic risk factors and all of these associations further highlights the potential hypothesis of other post-infectious syndromes including RLS.[83,84] Finally, anaerobic gastrointestinal bacteria, which are increased in the setting of SIBO, are also known to generate nitric oxide which may damage the intestinal lining, enter the systemic circulation and cause inflammation or immune alterations which could exacerbate RLS.[85]

An additional potential explanation for a link between inflammation, immune alterations and RLS include the potential role of the pro-inflammatory cytokine interleukin-17.[86] This cytokine has been found to be involved inflammatory responses in inflammatory and autoimmune diseases of the nervous system and is increased in four secondary RLS disorders (MS, RA, Crohn's disease and transverse myelitis).[87–89] In these cited lab studies the level of IL-17 correlated both with disease activity of systemic inflammatory autoimmune diseases as well as neuroinflammation which has not been seen with other interleukins.

Inflammation, immunity, infection and the peripheral and central nervous systems

Peripheral neuropathy is a feature of 37% highly-associated conditions of RLS (see Table 1). RLS can be associated with subclinical, otherwise asymptomatic, peripheral neuropathy.[90] Some cases of RLS could be independent of CNS iron deficiency and do so by direct effect of immunologic and/or inflammatory mechanisms in the brain, spinal cord and/or peripheral nerves. Antibodies formed in response to gut bacteria in the setting of SIBO or other GI diseases could damage nerves and result in RLS via autoimmune mimicry. An example how this could occur in RLS include hepatitis C-induced cryoglobulinemia neuropathy *vis a vis* an immune-related vasculitis.[50] Examples of autoantibody formation against brain tissue in RLS due to other microorganisms has previously been discussed.[12]

The role of NOS in inflammation and immunity has been extensively studied.[91] NOS produces nitric oxide which is a free radical signaling molecule that plays a key role in the pathogenesis of inflammation. NOS has anti-inflammatory effects under normal physiological conditions. Nitric oxide, on the other hand, is considered as a pro-inflammatory mediator in a variety of abnormal situations in which its levels are increased. Nitric oxide can also serve as a potent neurotransmitter at neuronal synapses and contributes to the regulation of apoptosis both of which could contribute to RLS.[92] Finally, nitric oxide is involved in the pathogenesis of inflammatory disorders of the joint, gut and lungs and possibly in auto-immune neurological diseases such as MS.[92] Thus, there is a putative link between the immunological properties of NOS and the production of RLS symptoms. A more direct demonstration of the connection between NOS and RLS comes from autopsy studies where NOS was up-regulated in substantia nigra in RLS patients compared to controls and genetic allelic association studies where certain NOS gene variants were more frequently expressed in RLS than controls.[2]

An alternative mechanism for SIBO-induced inflammation and gas production playing a role in peripheral neuropathy-induced RLS include the roles of substance P and circulating hydrogen sulfide. These toxic products could sensitize nerve or muscle tissue (Henry Lin, MD, personal communication 2010). Substance P and hydrogen sulfide has been shown to affect neurotransmission and can act as nociceptive agents at nerve endings.[93] As an extension to this theory, if the largest meal of the day occurs at night it may increase these intestinal fermentation products or other inflammatory disturbances during the evening and play into the diurnal nature of RLS.

Inflammation, immunity, iron and RLS-related genes

Recent studies have pointed to a role for genetic factors in RLS. At present it is unclear how most genetic factors contribute directly to RLS. Linkage studies in familial RLS have pointed to several chromosomal regions statistically associated with RLS, but to date no RLS genes have been identified.[1] Using a different genetic approach, genome wide association studies have shown that RLS has been associated with variants in the *PTPRD*, *MEIS1*, *BTBD9*, and *MAP2K5/LBXCOR1* genes on chromosomes 9p23-24, 2p, 6p, 15q as well as the gene for nitric oxide synthase (NOS) located on chromosome 12.[1,2,5]

An important question to consider is whether systemic inflammation or immune disturbances interact with gene variants and affect the CNS or peripheral nervous system. One possibility is that some of these genes are susceptibility factors and both gene and environmental triggers such as immunological compromise or inflammation are required to increase the likelihood of getting RLS. A hypothetical example is that a genetic diathesis in RLS could make an individual at a greater risk for RLS as a direct or an indirect post-infectious complication. This type of interaction between gene and environment is well known in many disorders and hypothesized for many others (e.g., Crohn's disease and post-infectious IBS). At the present time it is unknown whether any of the candidate gene variants apart from NOS predispose to inflammation and infection. None are proven to increase the risk of an auto-immune attack in RLS although *PTPRD* appears to be important in the development of post-infectious Tourette's syndrome which in turn can be associated with RLS,[94] Streptococcal infections can also trigger ADHD and ADHD is also associated with RLS.[62]

A recent study indicates that there is up-regulation of the HIF-1a pathway in substantia nigra neurons in RLS as indicated by an autopsy study of 6 RLS patients and 6 controls.[15] Because of the intimate relationship of this pathway to inflammation, an immunological diathesis *vis a vis* RLS can reasonably be proposed. However, an alternate explanation put forth by Patten and

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colleagues is that activation of this pathway can result from or contribute to cellular iron deficiency.[15]

It is noteworthy that three of the five genes or gene products associated with RLS have been linked to iron deficiency – *BTBD9*, *MEIS1* and NOS (the latter by increased levels of nitric oxide).[2,4,95] The best known allele linked to iron deficiency in RLS is *BTBD9* where serum ferritin levels were decreased by 13% per allele (95% confidence interval, 5 to 20; $P = 0.002$) in RLS patients.[3,4] MEIS1 may also be linked to iron as shown by Silver et al.[96] Iron chelation with desferoxamine increased MEIS1 protein by 37%. Forty eight-hour inhibition of MEIS1 production increased transferrin receptor (TfR) mRNA by 35%, TfR2 mRNA by 146%, SLC40A1 (ferroportin) mRNA by 173% and decreased hepcidin mRNA by 68%. Thus, MEIS1 protein appears to be, in part, regulated by cellular iron status, while *MEIS1* itself may also feedback to regulate some component of cellular iron management. Iron deficiency could be directly linked in RLS by genetic means via up-regulation of the gene encoding for hepcidin in response to inflammation.[97] In the murine model, LPS trigger choroid plexus production of hepcidin via transient transcription of the gene encoding for hepcidin and expression of several other iron-related genes in choroid plexus cells (see Fig. 2).[55] A commonality between the RLS gene variants and other known genes involved in iron metabolism (*DMT1*, *TF*, *TFRC*, *ZIRTL*, *HAMP*, *HJV* and *TMPRSS6*) could not be found in the literature search.

Some of the genes known to be more highly-associated with RLS through the GWAS studies have also been shown to be altered, albeit in various ways, in some of the disorders that are more highly-associated with RLS. In studies that have examined specific secondary RLS disorders, the following links have been noted: *PTPRD* has been associated with ADHD and type 2 diabetes (in 2 ethnic groups)[98,99]; *BTBD9* has been associated with ADHD and Tourette's syndrome[100,101]; and NOS and *MAP2K5* have been associated with ADHD.[102]

Potential roles of RLS medicines in inflammation and immune mechanisms

To consider immunological mechanisms for the cause for RLS may be counterintuitive in light of the fact that the current RLS drug therapies which are directed at the CNS are effective.[1] There is evidence for direct and specific mechanisms of action involving specific neurotransmitter systems for the various RLS treatments including the dopaminergic agents, the anticonvulsants gabapentin and pregabalin, the opioids and the benzodiazepines. The principal mechanisms of these medications do not exclude additional mechanisms of these drugs that could interact with the immunologic and inflammatory mechanisms proposed herein. In addition there is evidence that all of these therapeutic agents, the neurotransmitter systems they affect or their receptors also modulate the immune system, alter inflammatory moieties in the body and, in turn, are affected by inflammation.[103–106] Furthermore, RLS may be triggered by multiple mechanisms including inflammation and immunological alteration which could affect neurotransmitters and receptors other than those affected by the known drugs. Although rapid action of dopamine agonists might argue against the role of inflammation, the complexity of RLS allows for the potential role of several factors.

Objective evidence of a generalized enhanced inflammatory/immunological response in RLS is currently lacking. This must first be shown. Subsequently, one important thing that will need to be tested in future work is whether or not therapeutic agents such as the dopaminergic agonists quell any inflammatory response in RLS which might be expected if the inflammatory/immunological hypothesis for RLS is correct. On the other hand, dopaminergic and other classes of agents might modulate the symptoms of RLS through independent mechanisms even in the face of an enhanced inflammatory/immunological response.

Role of iron and inflammation in ESRD patients with RLS

Renal disease is highly-associated with RLS. Although renal failure is not primarily triggered by inflammation in most cases, ESRD is associated iron deficiency, SIBO, peripheral neuropathy, inflammation and immune disorders (see Table 1). The most common causes of ESRD are hypertension and diabetes yet inflammation is generally increased.[107] It has been observed that RLS improves after transplantation.[108] Two theories for this improvement include improvement in iron availability and reduction of systemic inflammation after transplantation.[109] Hemodialysis patients also get iron deficiency owing to loss of blood in the catheters with each dialysis session and lack of erythropoietin because of diseased kidneys. Chronic inflammation also may play a role by causing anemia of chronic disease via the effect of hepcidin. In ESRD oral iron usually does not improve anemia – possibly owing to hepcidin preventing absorption or release from the reticuloendothelial system. There are few data to suggest a change in peripheral neuropathy after transplantation and studies of SIBO after transplantation have not been performed. Iron insufficiency associated with ESRD appears to be due to reticuloendothelial iron arrest (with high ferritin and low iron saturation) which could be due to increased hepcidin secondary to systemic inflammation associated with ESRD.[110] After transplantation, the response to iron improves which also supports the inflammatory theory as a mediator of RLS.[111]

Role of inflammation in cardiovascular disease and stroke in RLS

There is a growing body of literature showing a relationship of RLS and PLMS to hypertension, heart disease and stroke.[112,113] It is now appreciated that inflammation plays a larger role in the predisposition to cardiovascular disease than was previously recognized and it has long been known that stroke may be mediated by inflammatory mechanisms.[114,115] We raise here for future consideration the possibility that the increased prevalence of vascular disease in RLS may be mediated by an altered predisposition to inflammation and immunological compromise. Although vasculitic or anti-phospholipid antibody strokes, for example, represent a minority of strokes in the general population, perhaps they are over-represented in RLS if the inflammatory/immunological hypothesis is true. We point again to the alterations in HIF-1a and NOS as possible mediators of this predisposition.[2,15] The roles of both HIF-1a and NOS are complex and sometimes contradictory but are currently the subject of intense investigation in studies of stroke and animal models of hypoxia.[116,117]

Potential weakness of the present study's theories and methods

The inferences presented in the relationship between the pathogenesis of RLS and the pathological conditions which have been associated with them is a starting ground for theories that need direct evaluation in particular patient groups with secondary RLS. It is hazardous to jump to conclusions that when pathologic mechanisms exist in a secondary RLS condition that this mechanism is responsible for the RLS. The data available should only be considered as indications for possible future research. The alternative possibility that the RLS-associated condition itself causes or is associated with inflammation or immunologic disorders which are not indirectly or directly responsible for RLS is obvious. By example, both MS and post stroke states are associated with immune alteration/inflammation, yet the RLS in each could be

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directly related to damage to certain structures in the brain/spinal cord and thus not result from the immune alteration/inflammation itself.[64,118] On the other hand, in our study of Crohn's disease and RLS, patients noted that their RLS symptoms lessened when they had less gastrointestinal symptoms suggesting a stronger role for inflammation.

An alternative concern in the issue of inflammation is whether RLS itself can cause inflammation or immunological change. This could occur through nonspecific mechanisms such as RLS associated insomnia or, more specifically, through the arousals associated with the PLMS in RLS.[119] However, this latter concern may not be an issue in the inflammatory conditions such as RA and Crohn's disease where controlled studies have shown in the former that RLS is more common in RA and not vice versa and in the latter where activity of the intestinal disease correlates with the severity of the RLS.

One may raise the question of accurate diagnosis of RLS in the 47 conditions cited. More recently it has been shown that positional discomfort, leg cramps and arthritis may meet all 4 criteria for RLS and, therefore, other questions are needed to distinguish these disorders from RLS. We cannot exclude the possibility that some studies may have inadvertently included such mimics.[120] Whenever possible, to be inclusive yet accurate, we cite the best controlled study that supports the association of RLS to the condition in question. We are also restrained by limitations on the number of references and hence do not present a full discussion regarding the association of every particular disorder with RLS. Finally with respect to the controlled studies of the 38 highly-associated RLS conditions themselves, the majority of the studies were large and had appropriate control groups. The eleven other associated conditions included 2 small reports as noted in the results section.

Conclusion

The etiology and pathophysiology of RLS is yet to be determined. Although specific genetic links are prevalent and CNS iron deficiency plays an important role in the pathophysiology, we do not know what triggers the iron deficiency or how most gene links are directly involved in the pathophysiology of RLS. In this unique syndrome there are many potential ways for the symptoms of RLS to occur including alternative causes such as peripheral neuropathy. Inflammation and immune alterations are prevalent in 95% of the highly-associated RLS conditions. This suggests the possibility that RLS may be mediated through these mechanisms. There are three hypothetical mechanisms by which this can occur: 1) inflammation can trigger iron deficiency and this in turn is a well known trigger for RLS; 2) immunological reactions to bacteria or other unknown antigens may hypothetically trigger RLS by direct immunological attack on the central or peripheral nervous system; and 3) altered host defenses based on genetic variants may predispose individuals to inflammation or an altered immunological response leading to RLS.

To confirm the inflammatory/immune theory, individuals with secondary or primary RLS would need to be specifically tested for SIBO, inflammatory and/or immunological changes and have reduction of RLS symptoms by treating these factors. This has been suggested by two lines of investigation: 1) treatment of RLS with hydrocortisone led to reduction of RLS symptoms in a double-blind, placebo-controlled study[70]; and 2) treatment of SIBO with a non-absorbed antibiotic alone or along with treatment directed at improving intestinal immune function and permeability led to improvement of symptoms of RLS in two open-label studies[121,122] and one double-blind, placebo-controlled study.[123] It is interesting that there is also a precedent for this in the treatment of another extra-intestinal condition. Eradication of SIBO (and presumed reduction of systemic inflammation and/or immune alterations) led to remission of rosacea in a large double-blind, placebo-controlled study.[124]

Other areas for future research in RLS include: 1) determine whether there is an increase in inflammatory cells in peripheral nerve, brain and spinal cord in RLS patients versus controls; 2) measurements of general antibody levels directed against peripheral nerves, brain and spinal cord; 3) histological evaluation for immune complex deposition induced by gut bacteria and inflammatory cells in peripheral nerves and select brain regions; 4) measurement of hepcidin or pro-hepcidin in all RLS associated disorders and determination of whether reduction of inflammation by treating the underling systemic disorder could improve RLS symptoms and alter CNS and/or peripheral hepcidin and iron level; 5) exploring immunological and inflammatory properties of NOS, nitric oxide and the hypoxia inducible factor pathway in RLS; and 6) performing prospective studies of iron deficiency, SIBO, inflammation/immune disorders and peripheral neuropathy in both primary and secondary RLS patients.

Practice points

- The same set of symptoms occurs in idiopathic RLS and familial RLS, and RLS caused or exacerbated by numerous medical conditions and risk factors. Multiple mechanisms are likely involved in the RLS pathogenesis including the possibility that RLS is induced in some cases by inflammatory and immunological mechanisms. Evidence in favor of the hypothesis is as follows:
- RLS patients show an increased prevalence of SIBO in controlled studies and there have been case reports of post-infections RLS induced by streptococcus, *Mycoplasma, Borrelia* (Lyme's disease), cytomegalovirus (mononucleosis), HIV and HCV. Of the 38 conditions highly-associated with RLS, 95% have also been associated with inflammation and/or immune changes, 47% with systemic iron deficiency, 37% with peripheral neuropathy and thus far, 32% with SIBO.
- Recent evidence of the role of nitric oxide, the hypoxia inducible factor pathway and CNS iron deficiency also support a possible role of systemic inflammation and/or immune changes in RLS.

Research agenda

- Determine whether there is an increase in inflammatory cells in peripheral nerve, brain and spinal cord in RLS patients versus controls.
- Determine whether there is a general increase in antibody levels directed against peripheral nerves, brain and spinal cord in RLS patients versus controls.
- Determine possible immune complex deposition induced by gut bacteria and inflammatory cells in peripheral nerves and select brain regions.
- Measure hepcidin or pro-hepcidin in all RLS-associated disorders and determine whether reduction of inflammation by treating the underling systemic disorder could alter CNS and/or peripheral hepcidin and iron levels.
- Explore immunological and inflammatory properties of NOS and nitric oxide in RLS.
- Perform prospective studies of iron deficiency, SIBO, inflammation/immune disorders and peripheral neuropathy in both primary and secondary RLS patients.

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Financial disclosures

Dr. Walters has received research funding from the National Institutes of Health. Dr. Walters has served as a consultant to UCB Pharma and was a speaker at a CME symposium sponsored by UCB Pharma. Dr. Weinstock has received research funding from Salix Pharmaceuticals, Inc. Dr. Paueksakon does not have any financial relationships to disclose. Editorial assistance for preparation of the table and references was provided under the direction of the authors by MedThink Communications via funding from Salix Pharmaceuticals, Inc.

Acknowledgments

We wish to thank Franco Gemignani, MD and Paul Goodfellow, PhD for reviewing this manuscript and Juliane Winkelmann, MD, Erica Unger, PhD, and James Connor, PhD for their helpful comments on the manuscript. This was an investigator initiated study.

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Please cite this article in press as: Weinstock LB, et al., Restless legs syndrome – Theoretical roles of inflammatory and immune mechanisms, Sleep Medicine Reviews (2011), doi:10.1016/j.smrv.2011.09.003

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