

**BURNING MAGNESIUM, A SPARKLE IN ACUTE INFLAMMATION: GLEAMS FROM
EXPERIMENTAL MODELS**

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ABSTRACT

Magnesium contributes to the regulation of inflammatory responses. Here we focus on the role of magnesium in acute inflammation. Although present knowledge is incomplete to delineate an accurate scenario and a schedule of the events occurring under magnesium deficiency, it emerges that low magnesium and, to some instances, its transporters favor the induction of acute inflammation by sensitizing sentinel cells to sense the noxious agent, and then by participating to the orchestration of the vascular and cellular events that characterize the process.

Keywords

Magnesium, acute inflammation, leukocytes, endothelial cells

INTRODUCTION

Inflammation has been observed since the beginning of documented medical knowledge [1], but the disclosure of its significance and complexity is rather recent. It is now clear that inflammation is the automatic response of living tissues to damage. Through a series of interconnected events involving blood vessels and leukocytes, it defends from damages and paves the way to the repair of injured tissues and organs. Inflammation is activated by the release of chemical mediators that induce vascular and cellular events with the objective to recruit inflammatory cells, and in particular innate immune cells such as neutrophils and macrophages. These cells, in turn, phagocytize the noxious agent and produce additional chemical mediators that eventually lead to the activation of the adaptive immune response.

A link between inflammation and magnesium deficiency has been established long ago [2]. Magnesium (Mg) is an essential cation, which maintains vital cellular functions, since it is involved in all major cellular processes, including the regulation of energy metabolism, metabolic cycles and signaling pathways [3]. Also Mg transporters are part of a large array of physiological and pathological processes, including the regulation of immune response [3-7]. TRPM7, which possesses an ion transport domain and an active kinase domain and is responsible for cellular Mg homeostasis, phosphorylates phospholipase C γ 2, crucial in intracellular signaling after the activation of B lymphocyte, and is implicated in T cell migration [7,8]. MAGT1, a highly selective transporter for Mg, has a key role in T cell-mediated immune responses [9].

MAGNESIUM DEFICIENCY AND ACUTE INFLAMMATION

Mg deficiency impairs adaptive immune response, while it induces inflammation *in vivo* and *in vitro* [10]. In rodents, a severely Mg-restricted diet rapidly results in a dramatic drop of magnesemia which leads to characteristic inflammatory responses, such as hyperemia and edema, accompanied by leukocytosis and a significant increase of the plasma levels of interleukin (IL)-6 and acute phase proteins, including complement component C3 [11,12]. These events correlate with an impaired redox capacity characterized by a substantial increase in thiobarbituric acid-reactive substances associated with a significant reduction of the activity of superoxide dismutase and catalase [13]. Moreover, Mg deficiency reduces the synthesis of anti-oxidant glutathione, a reaction which is Mg dependent [14]. Short-term Mg deficiency induces also *de novo* synthesis of ceramide, which activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), the master regulator of inflammation, and induce the release of some inflammatory cytokines and chemokines [15].

Experimentally induced hypomagnesemia is associated with altered calcium (Ca) homeostasis [16]. To this purpose, it is noteworthy that Mg is considered the natural Ca antagonist [17] and, accordingly, Ca deficiency attenuates the pro-inflammatory effects of dietary Mg restriction [16, 18]. Moreover, circulating substance P, a pro-inflammatory neuropeptide, increases early in experimental dietary Mg deficiency. Substance P as well as other mediators contribute to induce the production of reactive oxygen and nitrogen species, which ultimately promote inflammation [19]. Since a link exists between inflammation and the composition of the microbiota, which educates the immune system [20], it is noteworthy that a Mg deficient diet is associated with a lower content of bifidobacteria in the intestine, an impairment of gut barrier and high levels of tumor necrosis factor (TNF) α and IL-6 mRNA in the liver and intestine. After 21 days of such a dietetic regimen, the bifidobacteria content increases, the performance of the intestinal barrier is restored and inflammation declines [21]. These findings suggest that a dynamic adaptive response occurs in animals fed a Mg poor diet.

On these bases, several mechanisms seem to be involved in Mg deficiency induction of inflammation: i) an altered symbiotic relationship of the host with the gut microbiota; ii) oxidative stress generated by the excessive production of free radicals; iii) the activation of neurogenic inflammation; and iv) an imbalance Ca/Mg (figure 1).

On the other hand, under stressful conditions, and inflammation certainly is a stress [22], the concentrations of magnesium decrease [23]. Recently, a reduction of Mg has been described in acutely inflamed tissues and this is caused by the activation of the IL-33/ST2 axis [24]. These results suggest that a decrease of Mg concentrations in the inflammatory site is secondary to inflammation itself and might contribute to the exacerbation of inflammatory response to immune challenge in Mg deficient animals. Indeed, a poor Mg diet increases the vulnerability to lipopolysaccharide (LPS) *in vivo* and enhances the response of neutrophils and macrophages *ex vivo* [25]. That Mg is directly implicated in this hypersensitivity to LPS is demonstrated by the prevention of these effects with Mg supplementation. In Mg-deficient animals the addition of magnesium before endotoxin significantly increases survival and lowers plasma values TNF α [26]. We will here summarize the involvement of Mg deficiency in the principal steps of acute inflammation, i.e. the recognition of the noxious agent, the delivery of leukocytes to the damaged tissue to eliminate it and the termination of the process.

MAGNESIUM DEFICIENCY AND ACUTE INFLAMMATION: SENSING THE DAMAGE

If inflammation is due to external pathogens invading a tissue, two sets of signals trigger the whole process. The first one sparks from the pathogen itself; the second one originates from the cells that have been damaged. In the case of sterile inflammation, the signals that alert the organism are endogenous and derive from the injured cells. Sentinel cells in the tissues, i.e. mast cells, dendritic cells and fibroblasts, perceive the offending agent and then alert neighbor cells, thus initiating inflammation. Mast cells are very abundant in the skin and in the mucosal tissues where they represent a first line of defense against external insults. In rats, Mg deficiency increases the degranulation of mast cells [27]. Since Mg antagonizes Ca [17], Mg deficiency rises cytosolic Ca, which facilitates degranulation by destabilizing membranes and activating trimeric G proteins [28-29]. It is interesting to note that mice heterozygous for a TRPM7 kinase deletion are hypomagnesemic and hyperallergic [30], thus mimicking the phenotype of animals fed a low Mg diet. Therefore, the kinase domain of TRPM7 assures proper Ca-induced exocytosis and regulates the Ca and Mg sensitivity of G protein-coupled receptor-mediated mast cell degranulation by modifying granular mobility and/or histamine content. Also dendritic cells are present in tissues that are in contact with the external environment where they sample the surrounding environment for pathogens [31]. If activated by the recognition of a pathogen, dendritic cells engulf and process it, and migrate to the regional lymph nodes where they present the antigen to T lymphocytes, thereby shaping immune response. Mg deficiency does not significantly impact on dendritic cell function in a model of co-culture with lymphocytes [32]. However, it is known that high extracellular Mg significantly suppresses the antigen-presenting capacity of the Langerhans cells, because it reduces expression of HLA-DR and costimulatory B7 molecules by the dendritic cells [33]. To our knowledge, no data are available about Mg and its transporters on the function of fibroblasts. Since injury and mechanical stress induce the release from fibroblasts of biologically active IL-33 [34], which acts as an alarmin, it would be interesting to evaluate whether Mg deficiency modulates IL-33 release in these cells. In general, studies about the effects of low Mg on fibroblasts should be fostered, since these cells respond to tissue injury by conditioning the production of cytokines and the recruitment of leukocytes in areas of inflammation [35].

MAGNESIUM DEFICIENCY AND ACUTE INFLAMMATION: THE VASCULAR EVENTS

Important vascular events characterize the early phases of acute inflammation. Vasodilation leads to an increase of blood flow, and is quickly followed by the raise of capillary permeability, with the

aim of boosting the accumulation of plasma proteins in the site of damage. These events are driven by the interconnected action of several mediators, initially vasoactive amines and then lipid products. In the beginning, histamine locally released by mast cells induces a rapid vasodilation and augments endothelial permeability producing intra-endothelial gaps. As mentioned above, Mg deficiency facilitates the degranulation of mast cells and, therefore, the release of preformed mediators, among which histamine. Meanwhile, mast cells, endothelial cells and other cell types present in the site of inflammation begin to synthesize prostaglandins and prostacyclin, vasodilators and vaso-permeabilizing agents, via cyclooxygenase. Also leukotrienes, which increase endothelial permeability, begin to be produced via lipoxygenase. Magnesium availability modulates the synthesis of several of these mediators (figure 2). The biosynthesis of eicosanoids, mainly prostacyclin, is stimulated in Mg deficiency [36,37]. Accordingly, magnesium suppresses the activation of phospholipase A2 and the production of arachidonate metabolites in macrophages [38] and inhibits lipoxygenase activity in human leukocytes [39]. Moreover, Mg deficiency induces the production of platelet activating factor, a vasodilator and vaso-permeabilizing factor [40] and the synthesis of nitric oxide, another potent inflammatory mediator which induces vascular permeability. In the plasma of Mg-deficient rats high concentration of nitric oxide were found because of the activation of inducible oxide synthase [41].

Endothelial cells are crucial in orchestrating the vascular reactions of acute inflammation. They secrete various molecules mediating vasodilatation and permeabilization such as prostacyclin and nitric oxide, and release cytokines and chemokines that facilitate the recruitment of leukocytes. In response to low extracellular Mg, cultured microvascular endothelial cells activate NF- κ B, which induces the expression of a large array of pro-inflammatory proteins [42]. Mg deficiency upregulates cell-surface adhesion molecules, which renders the endothelium adhesive for leukocytes [43], and the chemokines IL-8 and monocyte chemoattractant protein-1 (MCP-1/CCL2) [42]. IL-8 attracts neutrophils, which predominate in the inflammatory infiltrate during the early phases, and stimulates their degranulation with the consequent release of various enzymes that may contribute to tissue damage. MCP-1 is a potent chemotactic factor for many inflammatory cells including monocytes and T cells, which accumulate in the late phase of inflammation. Also C-Reactive Protein (CRP), a modulator of innate immunity and an marker of inflammation which increases in Mg deficiency [11,44], exerts potent pro-inflammatory actions on endothelial cells, i.e. by inducing the expression of adhesion molecules [45]. It is likely that high levels of CRP cooperates with Mg deficiency to activate endothelial cells.

MAGNESIUM DEFICIENCY AND ACUTE INFLAMMATION: THE CELLULAR EVENTS

Once attached to the endothelium, the leukocytes transmigrate, pierce the basal membrane by secreting metalloproteases and accumulate in extravascular sites. While no direct data are available about the effects of Mg on leukocyte transmigration, it is reported that low Mg induces the synthesis and the activity of metalloproteases [46], which facilitates the entry in the inflamed tissues. After exiting the blood, leukocytes migrate following the chemical gradient generated by locally produced chemoattractants, among which leukotrienes, chemokines and components of the complement system. In the site of injury, leukocytes are functional for eliminating the offending agents. In particular, the leukocytes capable of phagocytosis - neutrophils and macrophages - are the principal players, since they ingest and destroy microbes, foreign substances and necrotic tissues. The activation of these cells is triggered by elevation of intracellular Ca with the consequent involvement of phospholipase A2 and protein Kinase C. Since low extracellular Mg concentration leads to the increase of intracellular Ca and the activation of protein kinase C [47], it is not surprising that neutrophils and macrophages isolated from Mg deficient rats show higher phagocytosis than control animals [48]. However, extracellular Mg concentration has no significant impact on phagocytosis of cultured bone marrow-derived antigen-presenting cells [32]. The discrepancy between *ex vivo* and *in vitro* results might be ascribed to the systemic response that accompanies Mg deficiency, namely the activation of the hypothalamo-pituitary adrenal cortex axis and the renin-angiotensin-aldosterone system, both contributing to alterations of the immune response, as well as the increased plasma levels of substance P and cytokines, which are important priming agents [49]. Because killing of microbes is accomplished by reactive oxygen species (ROS), it is noteworthy that phagocytes from Mg deficient rats produce more ROS under basal conditions and are hyper-responsive to immune challenge when compared to control animals [26, 48]. Remarkably, human neutrophils from healthy donors incubated in low Mg concentration showed an increased respiratory burst in response to activating agents than controls in normal Mg concentration [50].

MAGNESIUM DEFICIENCY AND ACUTE INFLAMMATION: THE RESOLUTION

When no longer needed, inflammation is actively terminated to prevent unnecessary damage to tissues and restore their integrity and function. Resolution begins early through the coordinated synthesis of various anti-inflammatory mediators, among which IL-10, transforming growth factor (TGF) β , and pro-resolving lipid mediators such as lipoxins, resolvins and maresins [51]. Even

though little is known about the contribution of Mg to this process, some evidence is available that supports that Mg deficiency also has anti-inflammatory actions. By increasing the synthesis of metalloproteases [46], which cleave chemokines, low Mg reduces the infiltration of leukocytes. Moreover, cells cultured in low extracellular Mg produce high levels of IL-10 [47], which represses pro-inflammatory responses and limits unnecessary tissue disruptions. In microvascular endothelial cells, low extracellular Mg rapidly activates NF- κ B partly through the overproduction of ROS, but later it also activates peroxisome proliferator-activated receptor (PPAR) γ [42], probably through the induction of eicosanoids. PPAR γ inhibits NF- κ B by complexing with NF- κ B subunits and shuttling them from the nucleus to the cytoplasm [42]. We argue that the activation of PPAR γ in low Mg tips the balance towards the resolution of inflammation (figure 3). Turning our attention to Mg transporters, it is noteworthy that TRPM7 channel activity is implicated in macrophage polarization towards the anti-inflammatory M2 phenotype [52].

CONCLUSIONS

From the studies *in vivo*, *ex vivo* and *in vitro*, it is clear that, under Mg deficiency, pro-inflammatory events predominate over its anti-inflammatory actions. Accordingly, physiologic or high extracellular concentrations of Mg exert anti-inflammatory properties. In cultured endothelial cells high Mg inhibits NF- κ B and prevents the release of inflammatory mediators and cytokines [42], while in neutrophils and macrophages Mg inhibits oxidative burst [53,54]. Moreover, high extracellular Mg reduces the release of inflammatory cytokines from leukocytes [55] and the levels of Toll-like receptor (TLR) 4 in sebocytes [56]. Short-term exposure to Mg *in vitro* substantially reduced the frequency of neonatal monocytes producing TNF- α and IL-6 under constitutive and TLR-stimulated conditions, without influencing cell viability or phagocytic function. Upon TLR stimulation, Mg anti-inflammatory properties are due to the inhibition of NF- κ B activation through the increase of I κ B α levels [57]. It is likely that many of the effects of high Mg are due to its antagonisms with Ca and to its anti-oxidant action. Recently, Chandrasekaran and colleagues proposed a novel potential mechanism by which Mg inhibits inflammation. Mg could be involved in the activation of the thiamine pyrophosphate-dependent riboswitch, resulting in the increased synthesis from thiazole pyrophosphate of thiazole, which inhibits cyclooxygenase and hinders the formation of prostanoids [58].

This review underscores that there are many questions still open and highlights the need for more research to delineate a clear picture of how Mg, its transporters and sensors contribute to the modulation of acute inflammation.

FIGURES

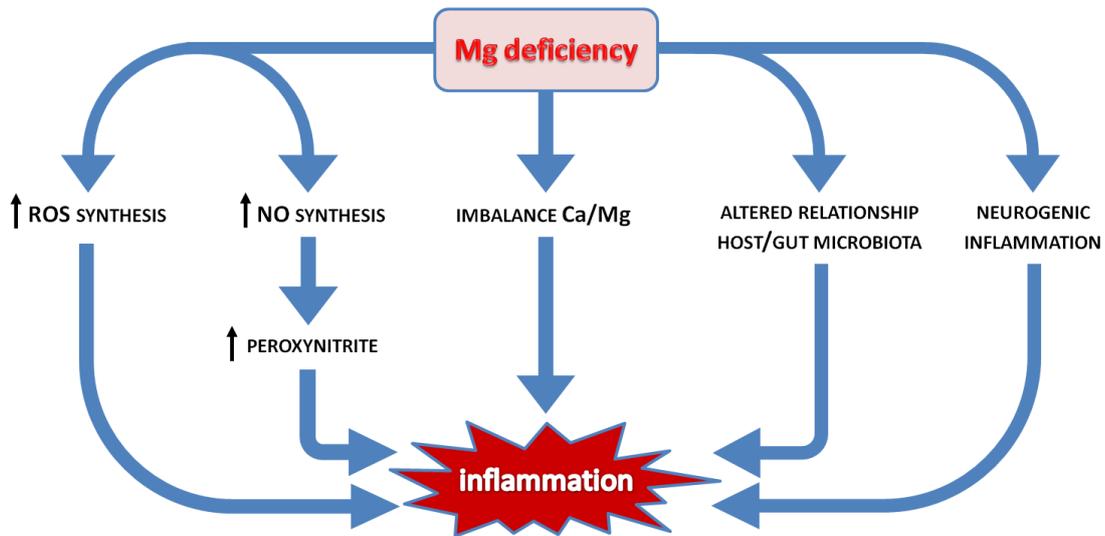


Figure 1. Possible mechanisms implicated in low-Mg related inflammation.

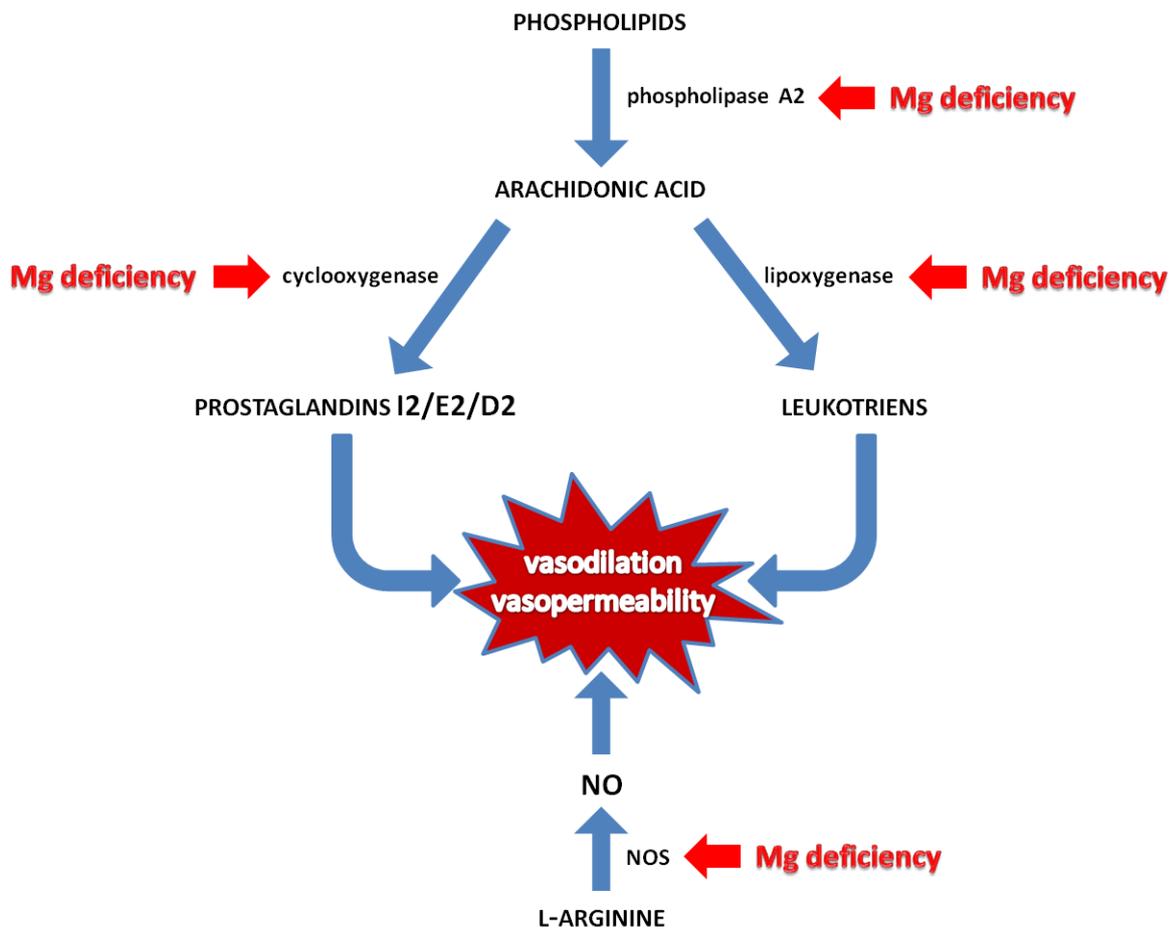


Figure 2. The modulation of the synthesis of eicosanoids by Mg deficiency.

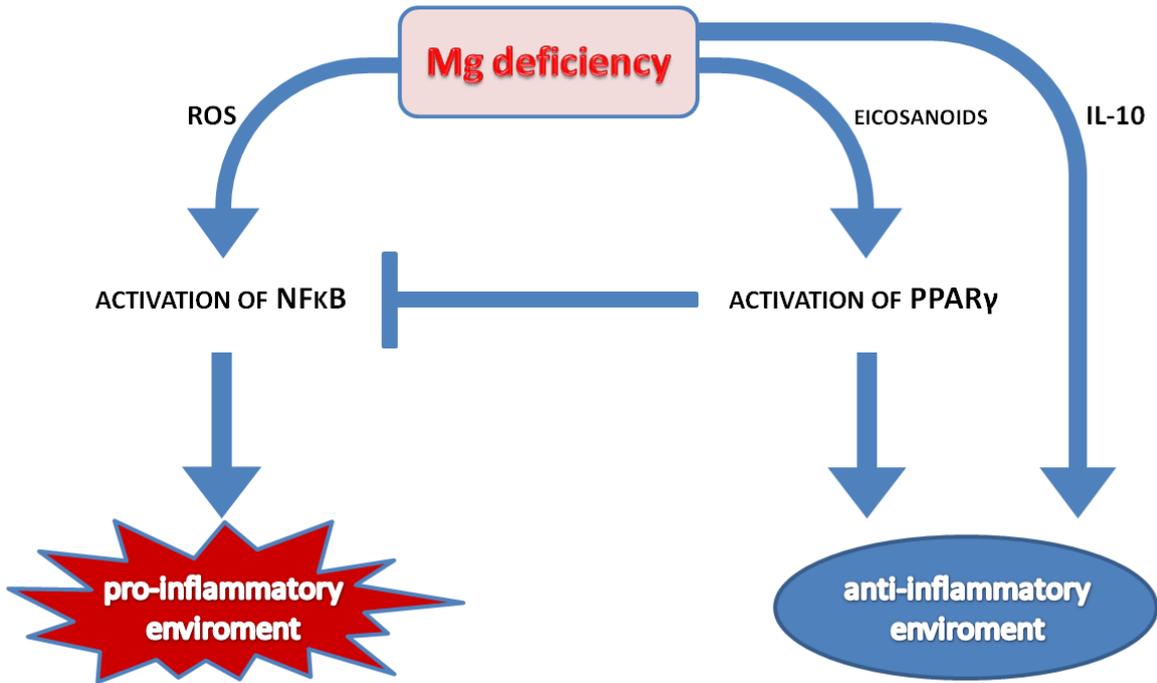


Figure 3. A summary of pro- and anti-inflammatory actions of Mg deficiency.

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