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REVIEW

Possible roles of magnesium on the immune system

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During the last few years, magnesium (Mg) has been subject of research due to its functionality in the organism. It is one of the most important micronutrients, and therefore its role in biological systems has been extensively investigated. Particularly, Mg has a strong relation with the immune system, in both nonspecific and specific immune response, also known as innate and acquired immune response. The aim of this paper is to review the state of the art about the interactions between Mg and the immune system. We discuss the link between dietary Mg and inflammation, apoptosis and alterations in number and function of innate immune cell populations, described in animal models. Furthermore, the immune system can be compromised in human populations under certain circumstances, including athletes and elderly people. The importance of a balanced Mg homeostasis and its interaction with the immune system in these groups has also been reviewed. Although emerging data support the relevant role of Mg in the immune response, further research is needed; and special efforts should be made to establish the most adequate dose in nutritional supplements to reach beneficial effects on health.
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Introduction

Magnesium (Mg) is the second-most abundant cation in cellular systems. It exerts a large variety of biological functions, ranging from structural roles by complexing negatively charged groups such as phosphates in nucleic acids, control role in enzyme activation or inhibition, and regulatory roles by modulating cell proliferation, cell cycle progression and differentiation. Even though less understood as compared to other ions such as calcium or phosphates, the intracellular Mg content appears to be regulated by Mg uptake, efflux and intracellular distribution, also in response to external stimuli. Regarding the relation between Mg and the immune system, several groups leading in Nutrition and Immunology have shown evidence that magnesium plays a key role in the immune response; that is, as a co-factor for immunoglobulin synthesis, C′3 convertase, immune cell adherence, antibody-dependent cytolysis, IgM lymphocyte binding, macrophage response to lymphokines and T helper–B cell adherence (Galland, 1988). Most of these studies have been designed in animal models, mainly focusing on what happens in Mg-depleted diet fed animals.

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Effects of Mg deficiency: evidence derived from animal model experiments

Inflammatory response

Several studies evidence close relations between Mg and the inflammatory response in rodents. Increased levels of proinflammatory cytokines (IL-6, TNF-α) have been reported in animals under Mg deprivation for 3 weeks (Weglicki *et al*, 1992). These authors have detected a plasma substance P (SP)—a well-known cytokine production stimulator (Weglicki & Phillips, 1992; Kabashima *et al*, 2002)—during the first week of Mg deficiency. In fact, the secretion of certain cytokines such as IL-2, IL-4, IL-5, IL-10, IL-12, IL-13 and IFN-γ has been shown to be induced by SP treatment (Weglicki *et al*, 1996). The secretion of these cytokines can be maximal at either 5 days (IL-4 and IL-5) or 7 days (IL-2, IL-10 and INF-γ) after Mg deficiency. After incubating peripheral blood T lymphocytes from Mg-deficient fed animals and control animals with medium containing 10^{-10} or 10^{-5} M SP, the secretion of cytokines appears to be related to an increased expression of SP receptors on the surface of T lymphocytes during the first week of Mg deficiency. According to these results, SP might play a key role in regulating T lymphocyte cytokine production during Mg deficiency, especially those cytokines regulating mast cells and the immune response leading to the onset of an immunopathological state. Nevertheless, these authors recognize that the association between the early neuropeptide release and the subsequent

inflammatory response is unclear. Malpuech-Brugère *et al* (2000) have also found elevated IL-6 levels just 4 days after Mg depletion, but without a significant increase of SP circulating levels and TNF-α. However, it is important to highlight that the time course of the inflammatory response is different in these studies.

Even though it seems that there must be evidence to establish a relation between Mg deficiencies and the inflammatory response, the underlying process for the activation of inflammatory mechanisms remains unknown. Other studies suggest that the inflammatory response induced by Mg deficiencies could contribute to atherosclerosis by modification of lipoprotein metabolism, inflammatory cell recruitment (Maier *et al*, 1997) and by the release of several growth factors that induce cell migration and proliferation (Bussière *et al*, 1995).

Nonspecific immunity

Mg deficiency might be accompanied by the activation of cells such as macrophages, neutrophils and endothelial cells (Mak *et al*, 1997; Malpuech-Brugère *et al*, 2000). In fact, macrophages have been found in the peritoneal cavity of Mg-deficient rats (Malpuech-Brugère *et al*, 2000). These macrophages seem to be activated endogenously—taking into account histological examination and reactive oxygen production, detected by chemiluminescent activity—and could contribute, at least in part, to the increased production of proinflammatory cytokines.

Polymorphonuclear (PMN) cell number and function has also been shown to be altered in rats fed an Mg-deficient diet for 8 days, together with the characteristic inflammatory response. In fact, an increased PMN number in neutrophils, related to an increased activity of phagocytosis, has been found in Mg-deficient rats compared with control rats. Moreover, both increased lipid peroxidation and higher plasma IL-6 and nitric oxide concentrations have been found in hearts from Mg-deficient rat, as compared with those from control rats (Bussière *et al*, 2002). These authors have also observed a decreased activity of PMN at high levels of extracellular Mg, whereas a suppression of phagocytosis has been described in the presence of low, but not high, Mg concentration in alveolar macrophages from rats (Ishiguro *et al*, 2000).

Apoptosis

The role of Mg in apoptosis is another approach on which several studies have been conducted. Mg mobilization in B cells undergoing Fas-initiated apoptosis has been examined (Chien *et al*, 1999). Fas molecule binding expressed on the cell surface initiates multiple signaling pathways that result in apoptotic cell death; these cells have increased cytosolic-free (noncomplexed) Mg^{2+} levels. Furthermore, the higher the concentration of anti-Fas antibody, the higher the percentage of these cells mobilizing Mg, fragmenting DNA, or externalizing phosphatidylserine. Some authors have suggested that increases in Mg^{2+} function not only as a co-factor for Mg-dependent endonucleases (Ginniakis *et al*, 1991; Widlak & Garrad, 2001) but also to facilitate cytochrome *C* release from mitochondria, which drives many of the postmitochondria, caspase-mediated events in apoptotic cells. From this point of view, Mg could be considered as a second intracellular messenger for these downstream events in apoptosis.

Effects upon immune system-related organs

Mg deficiency also seems to accelerate thymus involution. One of the most remarkable results, regarding effects of Mg deficiency on the organism, is the higher level of apoptosis shown in thymuses from Mg-deficient rats as compared with controls (Malpuech-Brugère *et al*, 1999).

Clinical signs of inflammation, splenomegalia and leukocytosis have also been presented in rats given an Mg-deficient diet after 8 days. There is also a higher number of adherent cells in the spleen cell suspensions from Mg-deficient rats, which provides an additional confirmation of the increased number of macrophages in the spleens of these rats. Moreover, a reduced proportion of CD8 + T cells has been shown under these conditions, which could be related to a decreased IFN-γ concentration in spleen homogenates (Malpuech-Brugère *et al*, 1998).

Finally, a different but very interesting approach has evidenced changes in gene expression in rat thymocytes in early Mg deficiency (Petrault *et al*, 2002). Potential targets of Mg deficiency have been identified using cDNA expression arrays. Several changes in gene expression, including upregulation of TNF receptor 1 and IL-1 receptor type I, have been demonstrated. It is important to highlight that all these changes in gene expression have been found very early during Mg depletion, only 2 days after the deficient diet, and even before inflammatory symptoms and perceptible modifications in cell functions appear. Reviewed roles of Mg in rodent immune system are summarized in Figure 1.

Insights from human-derived studies

Studies conducted in human populations are not so extended as those using animal models. These studies have been mainly focused on athletes, elderly people, and other risk groups such as pregnant women and children, seeking the role of Mg in the immune response and on different pathologies, where the immune system plays an important role.

Asthma

Mg is involved in several pathophysiological reactions connected with asthma, an illness that links with several systems, including specific immune reactions. Inhibition of

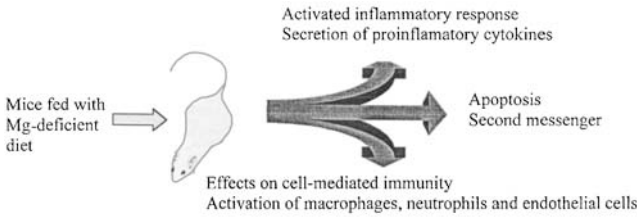

Figure 1 Effect of Mg depletion on the immune system of mice.

contraction of vascular and bronchial smooth muscles (*in vitro*), inhibition of acetylcholine and histamine release from cholinergic nerve terminals and mast cells, respectively, promotion of nitric oxide synthesis and prostacycline generation are some responses that are associated with changes in intracellular Mg concentrations (Fantidis *et al*, 1995; Hill *et al*, 1997).

Several studies have shown that intravenous Mg application could alleviate symptoms in acute and chronic asthma (Monteleone & Sherman, 1997). The therapeutic effect of Mg in asthma results from its involvement in modulating smooth muscle contractility (Rolla *et al*, 1987) and in promoting mediator release through its antagonism with calcium (Levine & Coburn, 1984). The participation of basophils and mast cells by releasing chemical mediators upon appropriate antigen stimulus in asthma is well-known (Ishizaka *et al*, 1970). One of the first events in the release of chemical mediators from basophils and mast cells is a rise in cellular calcium concentration (Yamamoto *et al*, 1999).

Mircetic *et al* (2001) have reported an increase of total plasma Mg concentration on the first day of asthma attack in children. This increase remains at the same level during 5 days after salbutamol treatment. In contrast, these authors have noticed a significant decrease in total intracellular Mg concentration from leukocytes on the first day of attack, but after 5 days of salbutamol therapy these values acquire normal levels. On the first day of attack, Mg urine excretion decreases about 30%, although it is restored after 5 days to healthy control values. These authors suggest that the increased Mg concentration found in plasma could be a result of Mg release from leukocytes with a subsequent increase in target cells of the attack, and, at the same time, the organism might save Mg through renal mechanisms.

Athletes

On the other hand, the evaluation of the immune system in athletes is frequently a target of research. Moderate and regular exercise can partially stimulate the immune response, whereas intense exercise may cause immunosuppression (Sharp & Koutedakis, 1992; Pedersen *et al*, 1999), which could increase the risk for occurrence of infectious diseases (Nova *et al*, 2001), especially after intense and prolonged training sessions. This immunosuppressed situation is characterized by a decreased activity of NK cells, neutrophils, T and B lymphocytes and saliva's IgA concentration (Nieman, 1998). Nevertheless, some athletes can withstand intense training periods without health problems while others are prone to infections. Thus, it has been postulated that other factors may interfere with immunoregulation. The notion that macro- and micronutrients are involved in the regulation of immunological processes and the ability to cope with muscular and systemic exercise stress have been gaining attention. Particularly, trace elements have been related to cell-mediated humoral immunity and nonspecific immunity such as T- and B-cell functions, NK-cell activity and cytokine release (Konig *et al*, 1998). Decreased concentrations of trace elements have been found in blood and tissues after training and competition (Speich *et al*, 2001). However, the magnitude of micronutrient losses is highly dependent on the type and intensity of exercise, the individual regulatory state, and, most important, on the nutritional status.

Apoptosis

There are also studies confirming the involvement of Mg in human cell apoptosis, in accordance with those performed in animal models. Black *et al* (2001) have found that physiologic levels of magnesium (0.8 to 1.2 mM) cause placental degeneration. These authors cultured placental tissue for 15 and 30 h in culture medium with 10% fetal calf serum supplemented with physiologic levels (1 mM) of $MgSO_4$. After incubation with 1 mM $MgSO_4$ an increase in apoptotic DNA fragmentation was evident after 15 h and it was more intense after 30 h, but little or no apoptosis was observed in placenta cultures without Mg treatment. They conclude that this placental degeneration is caused by magnesium based on the findings that magnesium can stimulate three hallmarks of the apoptosis process: by increasing oligosomal DNA fragmentation, cleavage of substrates associated with caspase activation, and tissue shrinkage associated with syncytial knot formation. They also found that Mg-induced placental apoptosis was attenuated by coincubation with antioxidants. Vitamin C, vitamin E and acetylcysteine were able to prevent this process in more than 50% of cases, indicating that oxidation–reduction reactions are involved in transducing the extracellular signal into an apoptotic event. This potential *in vivo* stimulation of placental apoptosis by extracellular magnesium is an important clinical issue. Although changes in Mg levels may normally be tolerated, it is possible that, in some individuals, a failure of Mg homeostasis may occur and perturb placental function, resulting in a pathologic impact on the fetal biological system's development.

Aging

In the case of aging, Mg status may be compromised for two reasons: insufficient intake or alterations in Mg metabolism. Furthermore, Mg deficiency is thought to contribute to the aging process and to the vulnerability to age-related diseases.

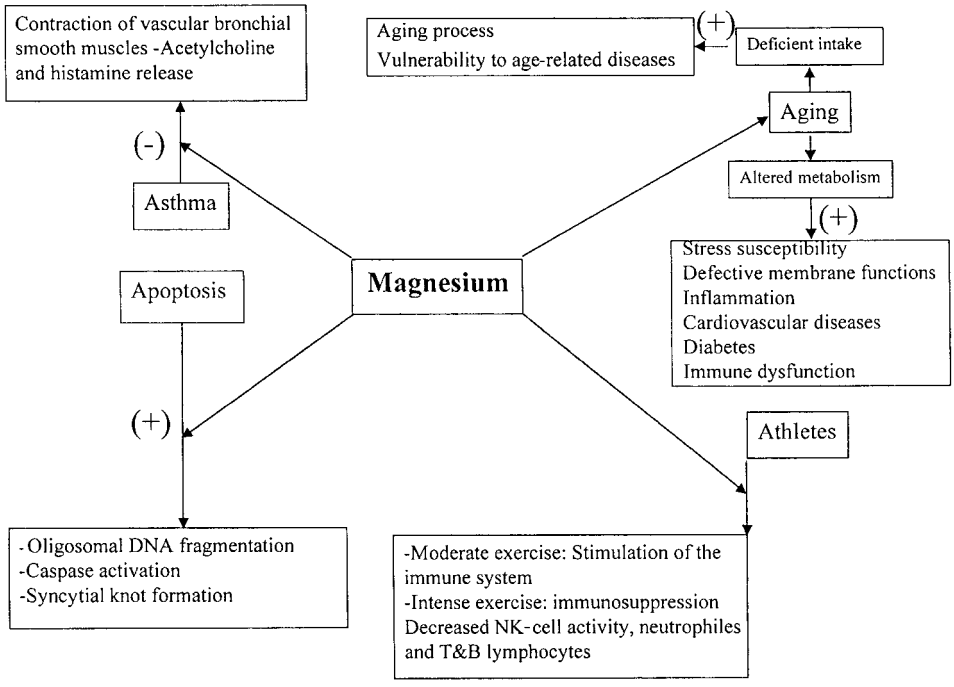

Figure 2 Scheme of the relation between magnesium and some aspects related to the immune system in humans.

The consequences of Mg imbalance in elderly people are related to stress susceptibility, defective membrane functions, inflammation, cardiovascular diseases, diabetes and immune dysfunction (Rayssiguier *et al*, 1993).

It is very common to find a nutrient intake less than optimum in elderly people, and this includes Mg and vitamin D. In fact, strong interactions between both nutrients have been demonstrated as well as their implication in the immune system mechanisms (McCoy & Kenney, 1996), calcium and other biosystems being also linked to these interactions. According to these authors, fundamental sites for possible interaction within the immune system include cell transformation, cell cycle regulation, nuclear DNA/chromatin stabilization, reactive oxygen species production and effects on enzymatic and hormonal actions, some of them closely related to the immune system status. Direct and indirect involvement of Mg in human immune system is summarized in Figure 2.

Conclusions

There is a strong relation between Mg and the immune system. We have reviewed different studies showing the role of Mg in different aspects of the immune response, both in animal models and in human systems. Mg involvement on inflammation, apoptosis, thymocyte gene expression and even in histological and cytological effects in animal models, as well as its relation with asthma, the immune system in athletes, aging processes and apoptosis in humans have been discussed. However, a number of questions are still waiting for more integrated and multidimensional experimental designs. Further research is necessary to find out the role played by Mg in other different biological processes related directly or indirectly with the immune system, since it still remains unclear.

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