Phagocyte priming by low magnesium status: input to the enhanced inflammatory and oxidative stress responses

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Abstract. Epidemiological and experimental studies underline the role of magnesium in inflammation. Several data indicate an enhanced response of phagocytes (granulocytes, macrophages) derived from magnesium-deficient animals or cultured under low magnesium conditions to the inflammatory mediators’ stimulation. On the contrary, it was pointed out that high extracellular Mg²⁺ concentration might partially attenuate the activation of phagocyte leukocytes. Thus, it is likely that magnesium-deficient conditions lead to the priming (pre-activation) of phagocytic cells. Magnesium status is an important modulator of the phagocyte response to immune stimuli and consequently could be implicated in a wide range of pathophysiological issues, e.g. those related to the production of radical oxygen species (ROS). It is likely that magnesium directly modulates phagocyte priming by its calcium antagonism and indirectly by its effect on the immunoinflammatory processes, the source of the priming mediators.

Key words: magnesium status, respiratory burst, priming phenomenon, phagocyte leukocytes

Magnesium (Mg) is strongly involved in the metabolic networks and cellular functions, so that disturbances in magnesium homeostasis lead to multiple pathophysiological events, including an altered immune response, inflammation and oxidative stress [1]. The strongest evidence of a close connection between magnesium-deficiency and the inflammatory response is derived from experimental animal studies showing that magnesium deficiency leads to an exacerbated inflammatory response [1]. Epidemiological studies support this inverse relationship between magnesium status and inflammation [2, 3].

One of the inflammatory issues in experimental Mg deficiency is the enhanced recruitment of phagocytic cells and their effector functions [4-6]. This is particularly linked with the production of pro-inflammatory cytokines and the generation of oxygen species. On the other hand, increasing magnesium in vitro or in vivo can attenuate inflammatory responses and phagocyte activation [4, 7, 8].

Phagocytic cells play a pivotal role in the host defense against invading pathogens, combining anti-infectious and proinflammatory functions [9]. However, the strongly undesired effect of an excessive immune response during acute inflammation is tissue damage [10]. That is why the activity of these cells has to be rigorously controlled. The hallmark of phagocytes is their ability to produce and release radical oxygen species (ROS) in the multistep process termed respiratory burst [11]. The force response of phagocyte leukocytes (thought to be the major producers of oxidants) to a proinflammatory stimulus is largely determined by their former
presentation to so-called, “priming agents” such as cytokines (i.e. tumor necrosis factor alpha [TNF-α], interferon gamma [IFN-γ]), lipid mediators (i.e. platelet activating factor [PAF]) or bacterial products (i.e. lipopolysaccharide [LPS]) [12-15]. Priming refers to a process whereby a phagocytic cell changes from a quiescent to a “ready to go” state. The response of phagocytes to an activating stimulus is heightened by the previous exposure to a priming factor.

Priming agonists do not elicit effector functions on their own [16, 17] and according to the definition, they must be presented to the cell before exposition to a “real” activating factor. Priming is considered to be a reversible phenomenon [14, 18] and a “de-primed” cell usually retains its full capacity to be “re-primed” by this same or an alternative priming agent [14]. The time period of “being primed” and maximal priming is strictly dependent on the priming agent applied [16].

The priming effect (with reference to respiratory burst phenomenon), is characterized by the enhancement of superoxide anion production after stimulation by a variety of factors, including phorbol 12-myristate 13-acetate (PMA) and N-formyl-methionyl-leucyl-phenylalanine (fMLP). The production of reactive oxygen species (ROS) at meaningful levels, is not observed in unstimulated cells [19].

Efforts have been made to identify and to characterize a variety of priming agonists [13, 14, 16, 20, 21].

With regard to the exacerbated inflammatory response in magnesium deficiency conditions, the question arises: could low magnesium status be considered by itself as a priming factor?

The arguments of a link between magnesium and priming were first suggested by in vitro studies. It was demonstrated that, in the presence of 1 mM Ca²⁺, the production of oxygen metabolites by human neutrophils is suppressed by a variety of tri- and divalent cations, including Mg²⁺. The effect of these ions was competitive with Ca²⁺ since activation-induced cytosolic Ca²⁺ elevation was inhibited i.e. peak cytoplasmatic Ca²⁺ levels after fMLP stimulation were reduced to values found in the absence of extracellular Ca²⁺ [22]. It is important to underline, that the binding of fMLP to its cell surface receptor occurs independently of any divalent cations [23]. Bussière et al. [7], using various models of cell activation (PMA or fMLP), have shown that low extracellular concentrations of Mg²⁺ (0.2 mM vs 0.8 mM) markedly decreased oxyradical production by human polymorphonuclear cells. Also, high concentrations of Mg²⁺ strongly inhibited the oxidative activity of eosinophils, obtained from eosinophilic patients, in response to PMA stimulation [7]. However, recent ex vivo studies carried out on whole human blood have shown only limited effects of high magnesium concentrations on spontaneous and LPS-induced cytokine production [24]. It could be speculated that this inhibitory effect of magnesium could be dependent on the initial intensity of activation of phagocytes and on the initial Mg status of these cells.

The way that extracellular Mg²⁺ affects the optimal leukocyte activation is poorly defined and remains unclear. However, experimental data support the generally recognized action of Mg²⁺ as the natural calcium channel antagonist [25]. In fact, calcium is an important second messenger in the signaling pathway of respiratory bursts. A transient increase in free intracellular calcium concentration is itself sufficient to prime human granulocytes [23, 26]. Low extracellular Mg²⁺ concentration leads to the elevation of intracellular Ca²⁺ and results in phagocyte activation [5, 27].

Experimental studies, mainly on rodents, have shown that recruitment of phagocytic cells and their activity are altered by magnesium-deficiency [6, 28]. A supportive argument for the connection between magnesium and phagocyte priming is that neutrophils and macrophages from magnesium-deficient rats generated more ROS, even without any stimulation, than those from controls [6, 27]. The differences in the levels of ROS released by neutrophils and macrophages from magnesium-deficient animals and controls drastically increased after stimulation in vitro with PMA [6, 27]. In addition, an overexpression of vimentin was noticed in neutrophils from magnesium-deficient rats compared to those fed a magnesium-adequate diet [29]. Vimentin is a cytoskeletal protein component responsible for maintaining cell integrity [30] committed in a neutrophil adhesive ability, shape changing and motility, all considered as phenotypic markers of the primed cell.

Nevertheless, which mechanism(s) might be proposed to explain the priming effect of low magnesium status in vivo? As we discussed, the calcium antagonist properties of magnesium are undoubtedly a major factor responsible for the phagocytic cell response. However, in vivo, more complex mechanisms are certainly involved in phagocyte priming by low magnesium, and are also associated
with the generation of a wide range of mediators able to prime phagocyte leukocytes. Many previously published works have shown that experimental magnesium-deficiency in rodents leads to increased plasma concentrations of nitric oxide (NO) [31], proinflammatory cytokines and neuromediators [32, 33]. Several of these molecules are recognized as potential priming agents. For example, low doses of cytokines (picomolar range) do not cause activation of the respiratory burst. Conversely, they prime these responses in the context of formyl peptides, phorbol esters and opsonized particles [34-36].

Interestingly, Weglicki et al. [37] observed an increase in plasma concentration of substance P (SP) during the first week of magnesium deficiency in rats. SP is a tachykinin neuropeptide, involved in neurogenic inflammation and recognized as participating in the production of proinflammatory cytokines. In vitro studies [38, 39] established that SP strongly enhances ROS production by human neutrophils in response to immune stimuli. This priming occurred without effect on cytosolic-free Ca$^{2+}$ signaling and was independent of actin polymerization.

SP and cytokines are important priming agents but there is also reason to suppose that the inflammatory response is related to the general stressor effect of magnesium deprivation. Stress leads to the activation of the hypothalamo-pituitary adrenal cortex axis. There is also activation of the rennin-angiotensin system and hyperaldosteronism. Thus, the stressor effect and hyperaldosteronism could contribute to alterations of the immune response during magnesium deficiency. Moreover, stress responses induce the release of large quantities of excitatory amino acids, which are important players in the inflammatory response [40].

Taken together, magnesium status appears to be an important modulator of the phagocyte response to immune stimuli and thus to nonspecific immune responses. Magnesium modulates the priming of phagocytes directly by its calcium antagonism and indirectly by its effect on the immunoinflammatory processes. Because of the wide implications of Ca$^{2+}$ signaling in these processes, the calcium antagonist effect of extracellular Mg$^{2+}$ could be considered as the “primum movens” of the relationship between magnesium and inflammation.

**Disclosure**

None of the authors has any conflict of interest to disclose.

**References**


