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MAGNESIUM AND MICROVASCULAR ENDOTHELIAL CELLS: A ROLE IN INFLAMMATION AND ANGIOGENESIS

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1. ABSTRACT

Microvascular endothelial cells are protagonists in inflammation and angiogenesis. Since magnesium (Mg) deficiency promotes inflammation and impairs angiogenesis *in vivo*, we evaluated the effect of different concentrations of the cation on microvascular 1G11 cells. We found that low Mg inhibits endothelial growth and migration, while it increases some inflammatory markers. In particular we show that low Mg stimulates the synthesis of interleukin 1α and 6, of nitric oxide, a mediator of inflammatory responses, and of VCAM, which mediates monocyte/endothelial interactions. On the contrary, high Mg stimulates proliferation and migration and sensitizes microvascular cells to migratory signals, thus inducing crucial events in angiogenesis.

Our results demonstrate a direct role of Mg in modulating microvascular functions and provide a molecular explanation to the link among Mg, angiogenesis and inflammation observed in *in vivo* models.

2. INTRODUCTION

Magnesium (Mg) is the second most abundant intracellular cation and plays a key role in such important reactions as ATP hydrolysis, transphosphorylation, DNA and protein synthesis (1). A decrease of Mg has been documented in animal models or clinical settings of cardiovascular diseases or metabolic disorders, and whole-organism Mg supplementation has proven of some efficacy in ameliorating such diseases (2-3). A link between low Mg and inflammation has been demonstrated. Indeed, Mg deficiency in rats results in the activation of macrophages and in the elevation of plasma concentration of interleukin (IL) 6, a known mediator of acute phase response (4). In Mg-deficient rodents the inflammatory response is accompanied by the activation of a number of cells including macrophages, neutrophils, mastocytes and endothelial cells (5-6). Indeed, endogenously activated macrophages have been isolated in the peritoneal cavity of Mg-deficient rats (4). In addition, we have recently demonstrated that low Mg promotes dysfunctions in human endothelial cells derived from the umbilical vein (HUVEC) by generating pro-inflammatory and pro-thrombotic molecules (7).

Endothelial cells (EC) are implicated in atherogenesis, thrombosis and angiogenesis (8). Far from being homogeneous, the endothelium represents a consortium of groups of cells located within blood vessels of different tissues (9). Several factors influence this heterogeneity including: i) morphological and functional differences between large and small vessels and between cells derived from various microvascular endothelial beds; ii) different response to growth factors; iii) organ specificity reflecting the cumulative expression of post-translation modifications and also the expression of unique genes under the control of organ-specific regulatory elements; and iv) pathological conditions, such as tumor growth, which is accompanied by the development of a characteristic tumor vasculature.

We have shown that HUVEC are very sensitive to different concentrations of extracellular Mg (7, 10). Because of the aforementioned heterogeneity, we studied the effects of different concentrations of Mg on microvascular endothelial cells, which are protagonists in angiogenesis

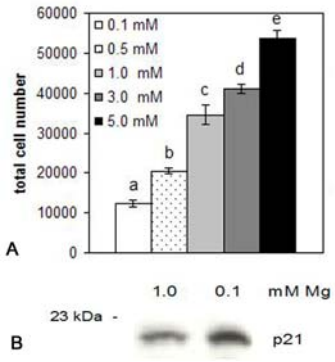

Figure 1. Modulation of 1G11 cell proliferation by different concentrations of Mg. (A) 1G11 cells were cultured in media containing 0.1, 0.5, 1.0, 3.0, 5.0 mM Mg. After 5 days, the cells were harvested by digestion with trypsin and viable cells counted using a Burker chamber. Results are the means ± SD. Values not sharing a superscript letter are significantly different ($P < 0.05$). (B) 30 µg of lysates of cells cultured in 0.1 and 1.0 mM Mg for 3 days were utilized for a western analysis with an anti-p21 antibody as described.

and inflammation. Since i) proliferation and migration are crucial steps in angiogenesis and ii) cytokines and nitric oxide have a role both in the formation of new vessels and in inflammation, we evaluated these parameters in microvascular endothelial cells cultured in concentrations of Mg ranging from 0.1 to 5.0 mM.

3. MATERIAL AND METHODS

3.1. Cell culture, proliferation and migration

Murine microvascular endothelial 1G11 cells were a gift from Drs. A. Mantovani and A. Vecchi (Istituto Mario Negri, Milano, Italy) (11). The cells were serially passaged in DMEM containing 10% FCS, ECGF (150 µg/ml) and heparin (5 U/ml) on 2% gelatin coated dishes. Cells were subcultured using 0.05% trypsin, 0.02% EDTA solution. The cells were routinely evaluated for the expression of endothelial markers, i.e. VE-cadherin and CD34, and utilized for 5-6 passages. All culture reagents were from Gibco. Mg-free medium was purchased by Bio Media (Boussens, France) and utilized to vary the concentrations of Mg by the addition of $MgSO_4$. In all the experiments the cells were seeded at low density in growth medium; after 24 h, the medium was changed to expose the cells to various concentrations of Mg. After 5 days, the viable cells were counted using a Burker chamber. Migration of 1G11 cells cultured in the presence of different concentrations of Mg was determined using an *in vitro* model of wound repair as previously described (7). Briefly, after 3 days culture in different concentrations of Mg, confluent endothelial cells were wounded and treated with Hepatocyte Growth Factor (HGF) (Tebu-Bio) (20 ng/ml) for 18 h. The number of cells migrating from the wound origin was counted with a light microscope at 100X magnification using a grid.

Statistical analysis was performed using the GraphPad Instat software package (GraphPad, San Diego, CA). Results were expressed as the means ± SD. All data were subjected to the One-way Analysis of Variance (ANOVA) followed by the Tukey-Kramer Multiple Comparison test to determine differences ($P<0.05$) among the groups. Values not sharing a superscript letter are significantly different ($P < 0.05$).

3.2. Western blot

1G11 cells were lysed in 10 mM Tris-HCl (pH 7.4) containing 3 mM $MgCl_2$, 10 mM NaCl, 0.1% SDS, 0.1% Triton X-100, 0.5 mM EDTA and protein inhibitors, separated on SDS-PAGE and transferred to nitrocellulose sheets. Western analysis was performed using antibodies against p21, VCAM, or c-Met (Santa Cruz: sc6246, sc1504 and sc8057, respectively). The antibody against murine IL-6 was kindly provided by Drs. Mantovani and Vecchi. Secondary antibodies were labelled with horseradish peroxidase (Amersham Pharmacia Biotech). The SuperSignal chemiluminescence kit (Pierce) was used to detect immunoreactive proteins (7).

3.3. Interleukin (IL)-1α synthesis

IL-1α concentration in cell extracts (50 µg) was detected using Quantikine mouse IL-1α immunoassay according to the manufacturers' instructions (R&D Systems). The experiments were performed in triplicate. Statistical analysis was performed as described above.

3.4. Protein array

Confluent cells were cultured in 0.1 or 1.0 mM Mg containing media for 5 days. 5 ml of conditioned media were centrifuged and utilized to incubate the membranes on which 21 cytokines were spotted in duplicate (RayBiotech Inc, mouse cytokine array I). The assay was performed in duplicate according to the manufacturer's instructions.

3.5. Nitric oxide synthase (NOS) activity

NOS activity was measured in the conditioned media of 1G11 cells by using the Griess method for nitrate quantification according to the manufacturer's instructions. Briefly, 1:5 diluted media were exposed to nitrate reductase (250 mU/ml) and NADPH (100 mM) for 30 min at 37°C to reduce nitrate to nitrite. The samples were then treated with L-glutamine dehydrogenase and mixed with an equal volume of freshly prepared Griess reagent. The absorbance was measured at 540 nm. The concentration of NO in the samples was determined using a calibration curve generated with standard $NaNO_2$ solutions. The experiments were performed in triplicate. Statistical analysis was performed as described above.

4. RESULTS

4.1. Modulation of endothelial proliferation by Mg

1G11 cells were grown in the presence of different concentrations of extracellular Mg (0.1, 0.5, 1.0, 3.0 and 5.0 mM) for 5 days. 1.0 mM Mg is considered the physiological concentration of the cation. In rodents, after 8 days of experimental diet, a decrease in plasma Mg to a 0.14 mM can be detected (4). 3-5 mM concentrations in the plasma could be observed after acute Mg administration (12), and 5 mM has been previously utilized in *in vitro* studies (10, 13). In agreement with results obtained in other cell types (7, 13), Mg modulated endothelial proliferation in a dose dependent manner. Culture in low Mg inhibited 1G11 cell proliferation, while high concentrations of the cation stimulated it (Figure 1A). These effects were reversible

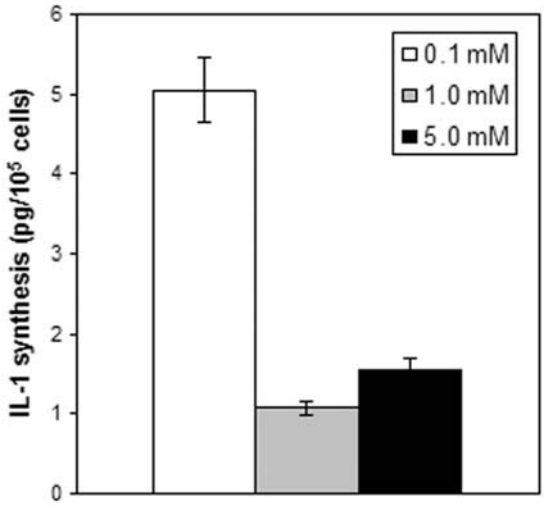

Figure 2. IL-1 synthesis in 1G11 cells cultured in different concentrations of Mg. Cell extracts were utilized to measure the levels of IL-1α as described in the methods. Results are the means ± SD. Values not sharing a superscript letter are significantly different ($P < 0.05$).

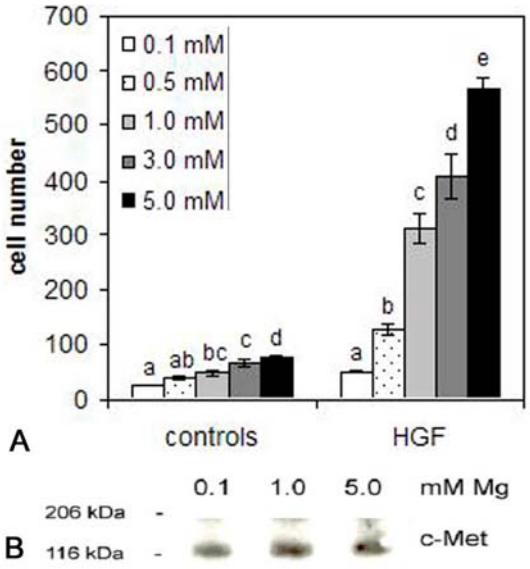

Figure 3. Modulation of 1G11 cell migration by different concentrations of Mg. (A) Confluent 1G11 cells were cultured in media containing 0.1, 0.5, 1.0, 3.0, 5.0 mM Mg. After 3 days, the cells were wounded and treated with HGF (20 ng/ml) for 18 h. The number of cells migrating from the wound origin was counted with a light microscope at 100X magnification using a grid. Results are the means ± SD. Values not sharing a superscript letter are significantly different ($P < 0.05$).

(B) Western blot was performed on lysates of 1G11 cells cultured for 3 days in 0.1, 1.0 and 5.0 mM Mg using antibodies against c-Met.

upon return to normal culture conditions (not shown). Interestingly, we found that that the growth inhibition by low Mg correlated with the down regulation of p21 (WAF1), an inhibitor of cyclin-dependent kinase, as detected by western blot (Figure 1B). Analogously to the results obtained in HUVEC (7), 3 days culture in medium containing 0.1 mM Mg upregulated the synthesis of interleukin (IL) 1α as detected by ELISA, whereas no modulation of IL-1α levels were observed between the controls and the cells grown in high Mg (Figure 2).

4.2. Modulation of endothelial migration by Mg

We evaluated the response of 1G11 cells to Hepatocyte Growth Factor (HGF), an angiogenic factor. 1G11 cells were cultured in medium containing various concentrations of Mg for 3 days before the addition of HGF (20 ng/ml) for 18 additional h. Figure 3A shows that low Mg concentrations impaired endothelial migration in response to HGF, while high concentrations of the cation stimulated it. It also shows that Mg alone can modulate endothelial migratory response. Low Mg inhibited cell motility while high Mg enhanced it, in accordance with the results by Lapidos *et al*. (14). The modulation of the sensitivity to HGF was not due to differences of the total amounts of the proto-oncogene c-Met, which is the receptor for HGF. Indeed, by western analysis, c-Met expression was comparable in cells cultured in 0.1, 1.0 or 5.0 mM Mg (Figure 3B). We could not detect any alteration in the tyrosine-phosphorylation of c-Met after binding the growth factor (not shown).

4.3. Modulation of the synthesis of cytokines by Mg

Since i) low Mg concentrations lead to dysregulated levels of some cytokines in rodents and ii) we describe an upregulation of IL-1α, we performed a protein array to obtain a broader profile of cytokine synthesis. 1G11 cells were cultured for 3 days in 0.1 or 1.0 mM Mg. The medium was then utilized for the proteomic analysis. Table 1 shows an overview of the cytokines synthesized by 1G11 cells. Out of 21 cytokines evaluated, we found a significant upregulation of IL-6 and a modest increase of MCP-1 in low Mg cultured cells (Table 1). Interestingly, no modulation of TNF α was detected. We confirmed the increased levels of IL-6 by western blot using anti-murine IL-6 antibodies (Figure 4).

4.4. Modulation of nitric oxide synthase activity and VCAM expression by Mg

Controversial reports are available about Mg and NO synthesis in endothelial cells. We evaluated NOS activity in cells cultured for 3 days in 0.1, 1.0 or 5.0 mM Mg. NOS activity was high in cell cultured in 0.1 mM Mg, while it markedly decreased in controls and in 1G11 exposed to 5.0 mM containing medium (Figure 5). Since VCAM is an adhesion molecule involved in inflammation and also in metastasis, it is noteworthy that we observed an increase of VCAM in cells exposed to 0.1 mM Mg for 3 days vs controls (Figure 6).

5. DISCUSSION

Influences of Mg on cell behavior have been suggested by a number of reports. Low Mg reduces the proliferation rate of non transformed and spontaneously

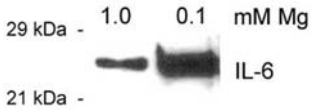

Figure 4. Modulation of IL-6 levels in 1G11 cells cultured in low Mg. Western blot was performed as described on lysates of 1G11 cells cultured for 3 days in 0.1 and 1.0 mM Mg using anti-murine IL-6 antibodies.

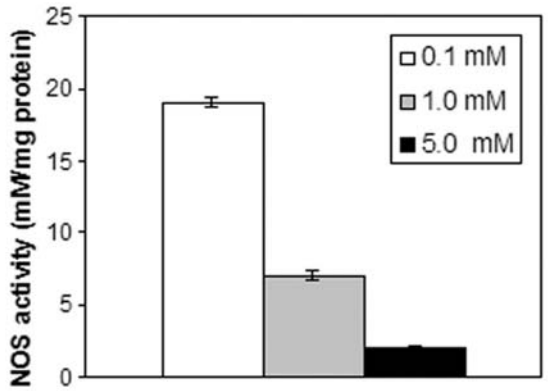

Figure 5. NOS in 1G11 cells cultured in different concentrations of Mg. The activity of NOS in 1G11 cells cultured for 3 days in 0.1, 1.0 and 5.0 mM Mg was measured by the Griess method as described. Results are the means ± SD. Values not sharing a superscript letter are significantly different ($P < 0.05$).

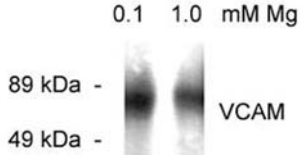

Figure 6. Modulation of VCAM in 1G11 cells cultured in low Mg. Western blot was performed on lysates of 1G11 cells cultured for 3 days in 0.1 and 1.0 mM Mg using anti-VCAM antibodies.

transformed Balb/c 3T3 cells (15), of human fibroblasts (16), of HL60 leukemia cells (13), among others. Recently, the transient receptor potential melastatin (TRPM) 7 has been reported to be critical to Mg entry in eukaryotic cells (17). To further support the role of Mg in driving cell proliferation, it is noteworthy that cells in which TRMP 7 was genetically deleted are Mg depleted and growth arrested (17). Endothelial cells are also affected by different concentrations of Mg. Indeed, extracellular Mg alters both migration and proliferation of bovine capillary cells (18). Accordingly, Mg has been proposed to serve as a chemotactic factor for HUVEC (14). We have recently demonstrated that low Mg inhibits the growth of HUVEC and promotes pro-inflammatory events, while high Mg induces growth and sensitizes the cells to migratory signals (7, 10).

Because endothelial cells are highly heterogeneous, we have investigated the response of murine microvascular endothelial cells to different concentrations of Mg. Since microvascular cells are protagonists in angiogenesis, inflammation and repair, we pointed our attention on events that are critical in the aforementioned conditions, i.e. proliferation, migration and cytokine synthesis. We found that low Mg inhibits microvascular endothelial cell growth and this correlates with the upregulation of p21, which preferentially binds to cyclin/CDK2 complexes necessary for the transition from the G1 to the S phase, thus inducing growth arrest. Since IL-1α is a potent inhibitor of endothelial proliferation (19), it is noteworthy that cells cultured in low Mg overexpress this cytokine that could play a role in braking cell growth. The inhibition of cell proliferation is in part depending on Mg deficiency-induced metabolic adaptations, such as a decrease in high energy phosphates and a decline in protein and carbohydrate synthesis. In addition, since Mg is a natural calcium antagonist (20), it is conceivable that an imbalance between Ca and Mg may play a role in modulating cell growth.

In agreement with previous reports (10, 18), high Mg stimulates the proliferation of 1G11 cells and this is in part mediated by the regulation of energy production and the stimulation of the activity of enzymes which require Mg (13). These metabolic events may also play a role in the modulation of endothelial migration observed in 1G11 cells cultured in different concentrations of Mg. Since Mg is a cofactor of adenylate cyclase and cAMP-dependent phosphodiesterase, we speculate that the sensitization to HGF in high Mg as well as the reduced response in low Mg might be dependent on the fact that different Mg levels could alter cAMP, which is implicated in endothelial migration (21). Moreover, it is noteworthy that Mg is required for the assembly of actin polymers and for myosin ATPase activity, two key components of the motor responsible for cell migration (18).

Migration and proliferation are crucial steps in angiogenesis (22). We therefore propose that monitoring Mg levels could be important in all those situations where angiogenesis is pathological. To this purpose, it is noteworthy that in a wound Mg is elevated (23) and this promotes endothelial migration and growth, thus facilitating repair. Since Mg deficiency is common in diabetic patients (2), it is conceivable that low Mg could be involved in retarding wound healing, a common and serious complication of the disease. We also propose that impaired angiogenesis could be involved in inhibiting the tumor growth observed in Mg deficient animals (24).

Cytokines are fundamental both in angiogenesis and inflammation (8). We found an upregulation of IL-1α and this is in agreement with previous results in HUVEC (7). IL-1α is a pro-inflammatory molecule and it is well accepted that Mg deficiency promotes inflammation. In addition, IL-1α modulates the synthesis of several other cytokines. Based on previous studies in animal models showing alterations in cytokine network after Mg-free diet, we used a proteomic approach - i.e. protein array - to have a profile of cytokine synthesis in 1G11 cultured in low Mg. We detected a marked upregulation of IL-6, which was confirmed by western blot. Since IL-6 levels are high in animals fed with Mg-deficient diet (4), it is conceivable that microvascular cells are an important source of this cytokine under these conditions.

Table 1. Protein array on conditioned media collected after 5 days of culture in 0.1 or 1.0 mM Mg

	0.1 mM Mg	1.0 mM Mg
GCSF	+	+
GMCSF	+	+
IL-2	-	-
IL-3	-	-
IL-5	-	-
IL-6	++++	+
IL-9	-	-
IL-10	-	-
IL-12	-	-
IL-12 p70	+	+
IL-13	-	-
IL-17	-	-
Interferon gamma	-	-
MCP-1	++	+
MCP-5	+/-	+/-
RANTES	+/-	+/-
SCF	-	-
sTNF-R1	-	-
TNF alpha	-	-
TPO	+/-	+/-
VEGF	-	-

Conditioned media were utilized to evaluate the levels of 21 cytokines spotted in duplicate on the filters. The spots were quantitated by densitometry. The results are shown as arbitrary units ranging from (-) negative to (++++) highly expressed

IL-6 is a pleiotropic cytokine implicated in acute phase responses and inflammation, both events being evident in Mg deficient rodents (4). In addition, the endothelial barrier dysfunction detected in this animal model can be ascribed, at least in part, to IL-6, which reversibly increases endothelial permeability via alterations in the ultrastructural distribution of tight junctions and morphologic changes in cell shape (25). It is noteworthy that IL-6 has been reported to inhibit endothelial growth *in vitro* and VEGF-induced rabbit corneal angiogenesis (26). On these bases, we hypothesize that the high amounts of IL-6 in low-Mg cultured 1G11 cells may represent another contributing factor to the inhibition of their growth. Under these culture conditions we also observed an overexpression of VCAM, a marker of inflammation, which belongs to the superfamily of immunoglobulin adhesion molecules. VCAM is induced on the endothelial surface by cytokines and binds VLA-4, expressed by monocytes and most of the lymphocytes. Focal adhesion of leukocytes to the microvasculature is a key step in inflammation and immune response. In addition, VCAM is involved in metastasis. Recently, we demonstrated an increase in the number of metastases in Mg-deficient diet mice inoculated with Lewis lung carcinoma cells (24) and we hypothesize an involvement of VCAM. These data may be relevant since cancer patients are often affected by low Mg status.

We also show that nitric oxide synthesis is modulated by the concentrations of Mg. We detected high amounts of NO in 1G11 cells cultured in low Mg when compared to controls or cells grown in high Mg containing media. This finding is in keeping with the pro-inflammatory environment induced by culture in low Mg. NO was originally discovered as a vasodilator product of endothelium (27). Indeed, NO acts in a paracrine fashion to stimulate smooth muscle cells guanylyl cyclase to produce 3',5'-cyclic monophosphate which causes relaxation of the blood vessels (28). The fact that low Mg stimulates NO synthesis may explain the vasodilatation which occurs in Mg deficient rabbits. Interestingly, HUVEC show a totally different behavior, since an enhancement of NOS activity was observed in cells cultured in high Mg (10). We propose that the different results reported in the literature may be due to the type of endothelial cells used. We argue that high Mg induces the synthesis of NO in macrovascular endothelial cells, thus providing an explanation to the usefulness of the cation in preventing development and severity of hypertension (29). On the other hand, since i) NO is a mediator of inflammatory responses and ii) microvascular endothelial cells are protagonists in the vascular changes during inflammation, the different behavior of 1G11 vs HUVEC is not surprising.

In conclusion, our findings provide a molecular explanation to the link among Mg, angiogenesis and inflammation observed in *in vivo* models.

6. ACKNOWLEDGEMENT

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7. REFERENCES

1. Wolf, F. I. and A. Cittadini: Chemistry and biochemistry of magnesium. *Mol Aspects Med* 24, 3-9 (2003)

2. Touyz, R. M: Magnesium in clinical medicine. *Front Biosci* 9, 1278-1293 (2004)

3. Shechter, M: Does magnesium have a role in the treatment of patients with coronary artery disease? *Am J Cardiovasc Drugs* 3, 231-239 (2003)

4. Malpuech-Brugère, C., W. Nowacki, M. Daveau, E. Gueux, C. Linard, E. Rock, J. Lebreton, A. Mazur and Y. Rayssiguier: Inflammatory response following acute magnesium deficiency in the rat. *Biochim Biophys Acta* 1501, 91-98 (2000)

5. Mak, I. T., B. F. Dickens, A. M. Komarov, T. I. Wagner, T. M. Phillips and W. B. Wegliki: Activation of the neutrophils and loss of plasma glutathione during magnesium deficiency – modulation by nitric oxide synthase inhibition. *Mol Cell Biochem* 176, 35-39 (1997)

6. Maier, J. A. M., C. Malpuech-Brugère, E. Rock, Y. Rayssiguier and A. Mazur: Serum from magnesium-deficient rats affects endothelial cells in culture: role of hyperlipemia and inflammation. *J Nutr Biochem* 9, 17-22 (1997)

7. Maier, J. A. M., C. Malpuech-Brugère, W. Zimowska, Y. Rayssiguier and A. Mazur: Low magnesium promotes endothelial cell dysfunction: implications for atherosclerosis, inflammation and thrombosis. *Biochim*

Biophys Acta 1689, 13-21 (2004)

8. Cines, D. B., E. S. Pollak, C. A. Buck, J. Loscalzo, G. A. Zimmerman, R. P. McEver, J. S. Pober, T. M. Wick, B. A. Konkle, B. S. Schwartz, E. S. Barnathan, K. R. McCrae, B. A. Hug, A. M. Schmidt and D. M. Stern: Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 91, 3527-3561 (1998)

9. Lang, I., M. A. Pabst, U. Hiden, A. Blaschitz, G. Dohr, T. Hahn T and G. Desoye: Heterogeneity of microvascular endothelial cells isolated from human term placenta and macrovascular umbilical vein endothelial cells. *Eur J Cell Biol* 82, 163-73 (2003)

10. Maier, J. A. M., D. Bernardini, Y. Rayssiguier and A. Mazur: High concentrations of magnesium modulate vascular endothelial cell behaviour *in vitro*. *Biochim Biophys Acta* 1689, 6-12 (2004)

11. Dong, Q. G., S. Bernasconi, S. Lostaglio, R. W. De Calmanovici, I. Martin-Padura., F. Breviario, C. Garlanda, S. Ramponi, A. Mantovani and A. Vecchi: A general strategy for isolation of endothelial cells from murine tissues. Characterization of two endothelial cell lines from the murine lung and subcutaneous sponge implants. *Arterioscler Thromb Vasc Biol* 17, 1599-1604 (1997)

12. Malpuech-Brugere, C., W. Nowacki, E. Rock, E. Gueux, A. Mazur and Y. Rayssiguier: Enhanced tumor necrosis factor- α production following endotoxin challenge in rats is an early event during magnesium deficiency. *Biochim Biophy Acta* 1453, 35-40, (1999)

13. Wolf, F. I. and A. Cittadini: Magnesium in cell proliferation and differentiation. *Front Biosci* 4, D607-617 (1999)

14. Lapidos, K. A., E. C. Woodhouse, E. C. Kohn and L. Masiero: Mg(++)-induced endothelial cell migration: substratum selectivity and receptor-involvement. *Angiogenesis* 4, 21-28 (2001)

15. Rubin, H: Effect of Magnesium content on density-dependent regulation of the onset of DNA synthesis in transformed 3T3 cells. *Cancer Res* 42, 1761-1766 (1982)

16. Mckeehan, W. L. and R. G. Ham: Calcium and magnesium ions and the regulation of multiplication of normal and transformed cells. *Nature* 275, 756-758 (1978)

17. Schmitz, C., A.-L. Perraud, C. O. Johnson, K. Inabe, M. K. Smith, R. Penner, T. Kurosaki, A. Fleig and A. M. Scharenberg: Regulation of vertebrate cellular Mg homeostasis by TRPM7. *Cell* 114, 191-200 (2003)

18. Banai, S., L. Haggroth, S. E. Epstein and W. Casscells: Influence of extracellular magnesium on capillary endothelial cell proliferation and migration. *Circ Res* 67, 645-650 (1990)

19. Maier, J. A. M., M. Statuto and G. Ragnotti: Endogenous interleukin 1 alpha must be transported to the nucleus to exert its activity in human endothelial cells. *Mol Cell Biol* 14, 1845-1851 (1994)

20. Iseri, L.T. and J. H. French: Magnesium: nature's physiologic calcium blocker. *Am Heart J* 108, 188-193 (1984)

21. Hackett, S. F., Z. Friedman and P. A. Campochiaro: Cyclic 3',5'-adenosine monophosphate modulated vascular endothelial cell migration *in vitro*. *Cell Biol Int Rep* 11, 279-287 (1987)

22. Folkman, J: Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Med* 1, 27-31 (1995)

23. Grzesiak, J. J. and M. D. Pierschbacher: Shifts in the concentrations of magnesium and calcium in early porcine and rat wound fluids activate the cell migratory response. *J Clin Invest* 95, 227-233 (1995)

24. Nasulewicz, A., J. Wietrzyk, F. I. Wolf, S. Dzimira, J. Madej, J. A. M. Maier, Y. Rayssiguier, A. Mazur and A. Opolski: Magnesium deficiency inhibits primary tumor growth but favors metastasis in mice, *Biochim Biophys Acta* in press (2004)

25. Desai, T. R., N. J. Leeper, K. L. Hynes and B. L. Gewertz: Interleukin 6 causes endothelial barrier dysfunction via the protein kinase C pathway. *J Surg Res* 104, 118-123 (2002)

26. Hatzi, E., C. Murphy, A. Zoephel, H. Rasmussen, L. Morbidelli, R. Ahorn, K. Kunisada, U. Tontsch, M. Klenk, K. Yamauchi, M. Ziche, L. Schweigerer and T. Fotsis: N-myc oncogene overexpression down-regulates IL-6; evidence that IL-6 inhibits angiogenesis and suppresses neuroblastoma tumor growth. *Oncogene* 21, 3552-3561 (2002)

27. Moncada, S., R. M. Palmer and E. A. Higgs: The discovery of nitric oxide as the endogenous nitrovasodilator. *Hypertension* 12, 365-372 (1988)

28. Ignarro, J. J: Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annu Rev Pharmacol Toxicol* 30, 535-560 (1990)

29. Touyz, R. M: Role of magnesium in the pathogenesis of hypertension. *Mol Aspects Med* 24 107-136 (2003)

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