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Reduced Lectin Stimulation of Lymphocytes from Magnesium-Deficient Rats

By *T. Günther*

Institut für Molekularbiologie und Biochemie and

R. Averdunk

Institut für Klinische Chemie und Klinische Biochemie Freie Universität Berlin

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Summary: Spleen cells and thymocytes from Mg-deficient rats, fed a Mg-deficient diet for 10–13 weeks, incorporate [^{3}H]thymidine at half the rate of normal cells.

The cells from Mg-deficient rats are less stimulated by lectins than those from normal animals.

These effects correspond to the reduced content of γ-globulins in serum in Mg deficiency. They are caused by the altered electrolyte content of the Mg-deficient cells.

Verminderte Lectin-Stimulierung der Lymphocyten von Magnesium-Mangel-Ratten

Zusammenfassung: Lymphocyten aus Milz und Thymus von Ratten, die 10–13 Wochen Mg-arm ernährt wurden, bauen [^{3}H]Thymidin nur mit halb so hoher Geschwindigkeit ein wie die Zellen von normal ernährten Ratten.

Die Lymphocyten von Mg-arm ernährten Ratten werden durch Lectine weniger stimuliert als normale Zellen.

Dieses Verhalten erklärt den verminderten Gehalt des Serums an γ-Globulinen im Mg-Mangel. Es wird durch den veränderten Elektrolytgehalt der Lymphocyten im Mg-Mangel ausgelöst.

Introduction

Mg-deficient *Yoshida* ascites tumor cells (1) or fibroblasts (2) (as well as Mg-deficient animals) exhibit a reduced rate of growth and biosynthesis of DNA, RNA and protein. At the same time, there is an alteration in the intracellular concentrations of Na^{+}, K^{+}, Ca^{2+}, Mg^{2+} and cAMP (3). Mg deficiency results in increased cell permeability, which leads to a decrease in intracellular [K^{+}] and [Mg^{2+}] and to an increase in intracellular [Na^{+}] and [Ca^{2+}] (1, 4).

The increased [Na^{+}] releases stored Ca^{2+} from mitochondria and produces a further increase in cytosolic [Ca^{2+}]. The elevated cytosolic [Ca^{2+}] leads to a further enhancement of permeability and to a stimulation of adenyl cyclase; this, in turn, causes an increase in intracellular [cAMP], which also increases permeability. The altered concentrations of Na^{+}, Ca^{2+}, and especially K^{+} may cause the inhibition of DNA-, RNA- and protein biosynthesis (4).

In agreement with the reduced protein biosynthesis, a reduction in immunoglobulins and antibody formation is found during Mg deficiency (5–8).

A transformation of lymphocytes to lymphoblasts preceeds antibody formation. In this process the membrane permeability is altered, resulting in an increased leakiness of the cell membrane and increased turnover of K^{+} and an enhanced uptake of Ca^{2+} (for lit. see l. c. (9, 10)). These alterations correspond to those in Mg deficiency. We therefore studied intracellular concentrations of Na^{+}, K^{+}, the rate of DNA synthesis, and the stimulation of thymus and spleen cells from normal and chronical Mg-deficient rats by lectins.

Methods

30 male Wistar rats with a body weight of 100 g were given low-magnesium feed (Altromin C 1035, Mg content 1.6 mmol/kg) and distilled water ad libitum for 10–13 weeks.

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The control animals were 10 Wistar rats of the same body weight which were fed Altromin (Mg content, 83 mmol/kg) and tap water (Mg content of the water, 0.4 mmol/l) ad libitum for the same time period.

The animals were killed under ether anaesthesia.

The number of erythrocytes and leucocytes, the proportions of granulocytes and lymphocytes, the hemoglobin content, the hematocrit values, the Hb content per erythrocyte and the mean cell volume were determined in a clinical chemical central laboratory by the usual routine methods.

The concentrations of Na^+ and K^+ in the serum were measured by flame photometry, and the concentrations of Ca^{2+} and Mg^{2+} by atomic absorption spectrometry.

For the determination of the Na^+ and K^+ contents of the thymus and spleen, the organs were weighed immediately after their excision and ashed at about 700 °C. The ashes were taken up in 1.0 ml 0.1 mol/l HCl and the contents of Na^+ and K^+ were determined in a flame photometer (Eppendorf).

For the determination of the rate of DNA synthesis and lectin stimulation, one thymus or spleen was taken from a control animal, or 2–3 from Mg-deficient rats, and homogenized in sterile RPMI medium with 10% fetal calf serum (Seromed, München). The homogenate was filtered under sterile conditions, and the filtrate was washed 3 × in the same medium by centrifugation at 200 *g*. The cell concentration was determined by counting in a *Neubauer* counting chamber.

Bacterial growth in the medium was inhibited by 100 μg/l streptomycin and 10^5 U/l penicillin.

The incubation mixtures in stoppered incubation vessels (Falcon) contained

1.8 ml RPMI - 10% calf serum
0.1 ml cells in RPMI - 10% calf serum
0.1 ml lectin in RPMI - 10% calf serum
10 μl [^3H] thymidine, radioactivity 37 kBq = 1.0 μCi (NEN Chemicals, spec. act. 740 TBq/mol = 20 Ci/mmol).

The cell concentration in the incubation mixtures was 2 × 10^6/ml.

The lectin concentrations were
Phytohemagglutinin (Difco) (mg/l): 0, 4, 8, 16, 32, 64
Concanavalin A (Serva, Heidelberg) (mg/l): 0, 4, 8, 16
Lipopolysaccharide (Difco) (mg/l): 0, 30, 40, 50, 60.

The mixtures were gassed with 5 % CO_2 containing air and incubated at 37 °C for 72 h in an incubation chamber under slow rotation.

To save cells, most experiments were carried out on a micro scale in a microtiter system (Falcon). The incubation mixtures for this system contained

0.1 ml cells in RPMI - 10% calf serum
0.1 ml lectin in RPMI - 10% calf serum
10 μl [^3H]thymidine with 3.7 kBq = 0.1 μCi (spec. act. 740 TBq/mol = 20 Ci/mmol).

The cell and lectin concentrations were the same as in the macro-mixtures. The micro-mixtures were incubated in an incubation chamber gassed with 5 % CO_2 containing air.

For experiments with low-Mg RPMI medium, 10% calf serum and the same substances as in normal RPMI, except for magnesium, were mixed and sterilized by filtration. (Mg content of the low-Mg RPMI medium = 0.1 mmol/l).

At the end of the experiment, the cells were centrifuged off for 10 min at 800 *g* and washed 3 × in cooled 0.15 mol/l NaCl. The cell sediment from the 2 ml incubation mixtures was taken up in 2 ml 0.1 mol/l NaOH, and the sediment from the 0.2 ml mixtures in 0.2 ml 0.1 mol/l NaOH. The radioactivity of a 0.1-ml aliquot was measured in 10 ml dioxan scintillation mixture in a Tricarb (Packard) counter.

The DNA was determined according to *Burton* (11), and the protein according to *Lowry* et al. (12).

Results

Electrolyte content in serum, spleen and thymus

During the period of Mg deprivation, the changes typical for Mg deficiency occured. The Mg concentration in the serum decreased to 0.32 ± 0.05 mmol/l ($\bar{x}$ ± SEM, n = 6), compared to a control value of 0.98 ± 0.14 mmol/l ($\bar{x}$ ± SEM, n = 6). The Na^+ and K^+ concentrations in the serum did not change, whereas the Ca^{2+} concentration was increased by 4%. In the thymus and spleen of the magnesium-deficient rats, the Na^+ content rose about 45%, and the K^+ content decreased about 67%.

Blood count

The various parameters of the blood count were determined in order to facilitate comparison with other experiments (tab. 1). The erythrocyte count, and thus the hematocrit values, decreased during the experiment. This corresponds to the previously observed changes in chronic Mg deficiency (13, 14, 15). The loss of Hb was somewhat less severe than the decrease in erythrocyte count, so that the Hb content per erythrocyte rose slightly. This effect does not result from a smaller cell volume of the Mg-deficient erythrocytes. They were found to be somewhat swollen (tab. 1). The number of leucocytes increased by 52%; granulocytes and lymphocytes were equally affected.

Tab. 1. Blood count from 5 normal and 7 Mg-deficient rats. Mean ± S. E. M.
MCH = mean cell hemoglobin, MCV = mean cell volume

	Erythrocytes [10^{12}/l]	Hb [g/l]	Hematocrit	MCH [pg]	MCV [fl]	Leukocytes [10^9/l]	Granulocyte fraction	Lymphocyte fraction
normal rats (n = 5)	7.15 ± 0.19	14.86 ± 0.28	0.376 ± 0.011	20.5 ± 0.5	0.52 ± 0.01	6.46 ± 0.18	0.085 ± 0.006	0.882 ± 0.016
Mg deficient rats (n = 7)	5.80 ± 0.21	13.70 ± 0.21	0.340 ± 0.005	22.3 ± 0.5	0.57 ± 0.01	9.82 ± 0.48	0.090 ± 0.006	0.870 ± 0.017

Immunoglobulins

In the present experiments, we found a decrease in the serum proteins, particularly the γ-globulins (not shown), in agreement with earlier results (5–8).

Lymphocyte stimulation

In the process of antibody formation, lymphocytes are transformed to lymphoblasts, and these multiply clonally. The blast transformation can be experimentally induced by stimulation with lectins.

Table 2 shows that the rate of incorporation of [^{3}H]thymidine into spleen and thymus cells of chronically Mg-deficient rats is reduced to half the control value. The same difference between normal and Mg-deficient cells is found when the cells are incubated in a low-Mg RPMI-medium with 10% calf serum. In this medium, the rates of incorporation were only 20% lower than in normal medium, in both Mg-deficient and normal cells.

The stimulation of spleen and thymus cells from normal rats by phytohemagglutinin, lipopolysaccharide or concanavalin A is 1.5 times higher than the stimulation of cells from Mg-deficient animals. In low-Mg RPMI medium, the stimulation by phytohemagglutinin, lipopolysaccharide or concanavalin A of normal and Mg-deficient spleen and thymus cells is in each case about 20% lower than in normal RPMI medium. The optimal lectin concentrations for cells from normal and Mg-deficient animals were not significantly different.

After the addition of lectin and subsequent incubation, some of the cells clump and die. After 2 days, all the surviving lymphocytes proliferate (16).

For a quantitative evaluation of the above results, it is therefore necessary to know whether the cells from Mg-deficient animals are destroyed at a higher rate by lectins, or whether they proliferate less vigorously. In some of the experiments, therefore, we determined the DNA and protein contents of the cells after the three-day incubation with lectins. We found that the protein and DNA contents of the cultures of spleen and thymus cells from normal rats were not significantly changed by the lectins and the incubation in low-Mg RPMI medium. With the cell cultures from Mg-deficient rats we again found the same DNA and protein contents, with and without lectins, and in both media. From this one can conclude that the cells from Mg-deprived animals are not significantly more sensitive to lectins than normal cells. However, the protein and DNA contents of cultures of spleen and thymus cells from Mg-deficient animals were 25% lower than the controls after 3 days incubation, although the number of cells at the beginning of the experiment was the same. This indicates that the cells from Mg-deficient rats have a lower proliferation rate.

Tab. 2. Effects of lectins on the incorporation of [^{3}H]thymidine in spleen cells and thymocytes from normal and Mg-deficient rats in normal and low-Mg medium. The lectin concentrations at which maximal stimulation occurred were 16 mg/l for phytohemagglutinin, 50 mg/l for lipopolysaccharide and 8 mg/l for concanavalin A. Mean ± S. E. M. from 7 different experiments each.

	Rate of [^{3}H]thymidine incorporation					
	Normal medium			Mg-deficient medium		
	unstimulated [counts/min · 10^6 cells]	Phytohemagglutinin stimulated/unstimulated	Lipopolysaccharide stimulated/unstimulated	unstimulated [counts/min · 10^6 cells]	Phytohemagglutinin stimulated/unstimulated	Lipopolysaccharide stimulated/unstimulated
Spleen						
Normal rats (n = 7)	536 ± 86	3.74 ± 0.28	1.87 ± 0.24	435 ± 75	3.08 ± 0.28	1.68 ± 0.23
Mg-deficient rats (n = 7)	294 ± 36	2.63 ± 0.26	1.22 ± 0.17	237 ± 50	2.00 ± 0.18	1.18 ± 0.10
	unstimulated [counts/min · 10^6 cells]	Phytohemagglutinin stimulated/unstimulated	Concanavalin A stimulated/unstimulated	unstimulated [counts/min · 10^6 cells]	Phytohemagglutinin stimulated/unstimulated	Concanavalin A stimulated/unstimulated
Thymus						
Normal rats (n = 7)	505 ± 125	2.23 ± 0.18	19.6 ± 2.0	360 ± 72	2.00 ± 0.13	16.0 ± 1.0
Mg-deficient rats (n = 7)	220 ± 61	1.96 ± 0.32	12.0 ± 0.5	177 ± 30	1.62 ± 0.29	9.5 ± 0.6

Discussion

Typical symptoms of chronic Mg deficiency appeared in our experimental animals during the 10 to 13 weeks of Mg-deficient diet. These included a decrease in the Mg concentration in the serum, mild anemia, leucocytosis and a decrease in the serum proteins, especially the γ-globulins. The leucocytosis was less pronounced than that found by other workers (15), who reported a 2.5-fold increase.

In one animal, an infiltratively growing lymphocytic thymoma had formed after 90 days. This corresponds to the results of others (14), who found a rate of such tumors between 5 and 20%, depending on the strain.

The most significant result was the reduced incorporation of thymidine in the spleen and thymus cells of Mg-deficient animals and the reduced responsiveness of these cells to lectins.

The rates of stimulation in the control cells, four-fold for phytohemagglutinin and 19-fold for concanavalin A, are relatively low compared to those in peripheral lymphocytes. One reason for this is the high cell density of $2 \cdot 10^6$/ml used in these experiments; with 10^5/ml the stimulation rates are ten times higher (16). Furthermore, in the thymus 10–20% of the cells are mitotically active, in contrast to the peripheral lymphocytes (18).

The spleen and thymus cells from Mg-deficient rats incorporated thymidine at half the control rates, but their DNA and protein contents were only about 25% lower. It follows that the spleen and thymus cells of Mg-deficient animals have a lower rate of DNA synthesis or proliferation than normal cells. A related phenomenon is the lower responsiveness of the Mg-deficient spleen and thymus cells to lectins.

After incubation in low-Mg RPMI medium, the DNA synthesis rates and the responsiveness of both control and Mg-deficient cells are about 20% lower. It is unlikely that this effect results from a reduced binding of lectins to their receptors at the cell membrane in the low-Mg medium, because there was no alteration in the optimal lectin concentrations in the low-Mg medium. The reduced responsiveness in cells from Mg-deprived animals and in Mg-poor RPMI medium explains the decrease in the γ-globulin fraction in the serum and the reduced immunizability of Mg-deprived rats (15).

The cause of this behaviour must lie in changes in the electrolyte and cell metabolism of Mg-deficient cells. In these cells, an increased permeability causes a drop in the K^+ concentration and a rise in the intracellular concentrations of Na^+, Ca^{2+} and cAMP, and an inhibition of the synthesis of protein and DNA (1, 3, 4). We also observed these changes in the Na^+ and K^+ concentrations in the spleen and thymus cells of the Mg-deficient rats.

The permeability of the cell membrane is also increased during lectin stimulation (9, 10). The effects of Mg deficiency and lectins on the membrane may be additive. If the changes in the concentrations of ions and substrates caused by increased permeability are not compensated by increased active transport rates, the intracellular K^+ concentration decreases, among other things. If this decrease exceeds 10–20% of the normal K^+ concentration, DNA and protein synthesis decrease in parallel to the K^+ concentration (19).

The decreased responsiveness of Mg-deficient lymphocytes to lectins corresponds to the reduction in the number of plaque-forming cells after i. v. injection of sheep erythrocytes into Mg-deprived animals (8), and explains the reduced antibody synthesis in Mg deficiency. The cause may be the reduced rate of protein synthesis due to the decrease in intracellular K^+ concentration. Due to the complex mechanism of the immune response, the details of which are still not completely clear, other effects may interfere in Mg deficiency. The Mg content decreases and the intracellular concentrations of cAMP and Ca, and probably also of Ca^{2+} ions, increase. The increase in the intracellular Ca^{2+} concentration plays a decisive role in the immune response. The Ca ionophore A 23187, which increases the intracellular $[Ca^{2+}]$, is by itself mitogenic. An increase in the intracellular Ca^{2+} concentration is also involved in the effects of other lectins. It may represent a common member in the reaction chain because the effects of suboptimal concentrations of A 23187 and phytohemagglutinin are additive (20). Nevertheless, the DNA synthesis and responsiveness of Mg-deficient lymphocytes to lectins were reduced.

The reason for this behaviour may be the severe reduction in the intracellular K^+ concentration in Mg deficiency which decreases the rate of protein synthesis. Apparently lymphocyte stimulation requires both an increase in the Ca^{2+} concentration and a sufficient K^+ concentration. This might also be an explanation for the fact that the less specific Ca ionophore X 537 A is not mitogenic (9). It apparently reduces the intracellular K^+ concentration too far. The inhibition of lymphocyte stimulation by ouabain, the specific inhibitor of Na^+-K^+ transport, can be explained in the same way (9).

Since the development of leukocytosis and thymomas is associated with a higher synthesis rate in these cells, another, presently unknown mechanism must be responsible for their formation.

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Prof. Dr. Th. Günther
Institut für Molekularbiologie
und Biochemie
der Freien Universität Berlin
Arnimallee 22
D-1000 Berlin 33