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Magnesium Ions Inhibit the Antigen-Presenting Function of Human Epidermal Langerhans Cells *In Vivo* and *In Vitro*. Involvement of ATPase, HLA-DR, B7 Molecules, and Cytokines[1]

Christoph M. Schempp, Henning C. Dittmar, Daniela Hummler, Birgit Simon-Haarhaus, Jürgen Schulte-Mönting,* Erwin Schöpf, and Jan C. Simon
Departments of Dermatology and *Medical Biometry and Statistics, University of Freiburg, Freiburg, Germany

The combination of seawater baths and solar radiation at the Dead Sea is known as an effective treatment for patients with psoriasis and atopic dermatitis. Dead Sea water is particularly rich in magnesium ions. In this study we wished to determine the effects of magnesium ions on the capacity of human epidermal Langerhans cells to stimulate the proliferation of alloreactive T cells. Twelve subjects were exposed on four subsequent days on the volar aspects of their forearms to 5% $MgCl_2$, 5% NaCl, ultraviolet B (1 minimal erythemal dose), $MgCl_2$ + ultraviolet B, and NaCl + ultraviolet B. Epidermal sheets were prepared from punch biopsies and were stained for ATPase and HLA-DR. Compared with untreated skin, the number of $ATPase^+/HLA\text{-}DR^+$ Langerhans cells was significantly reduced after treatment with $MgCl_2$ ($p = 0.0063$) or ultraviolet B ($p = 0.0005$), but not after NaCl ($p = 0.7744$). We next questioned whether this reduced expression of ATPase and HLA-DR on Langerhans cells bears a functional relevance. Six subjects were treated on four subsequent days with 5% $MgCl_2$, ultraviolet B (1 minimal erythemal dose), and $MgCl_2$ + ultraviolet B. Epidermal cell suspensions from treated and untreated skin were assessed for their antigen-presenting capacity in a mixed epidermal lymphocyte reaction with allogeneic naive resting T cells as responder cells. Treatment with $MgCl_2$, similarly to ultraviolet B, significantly reduced the capacity of epidermal cells to activate allogeneic T cells ($p = 0.0356$). Magnesium ions also suppressed Langerhans cells function when added to epidermal cell suspensions *in vitro*. The reduced antigen-presenting capacity of Langerhans cells after treatment with $MgCl_2$ was associated with a reduced expression by Langerhans cells of HLA-DR and costimulatory B7 molecules, and with a suppression of the constitutive tumor necrosis factor-α production by epidermal cells *in vitro*. These findings demonstrate that magnesium ions specifically inhibit the antigen-presenting capacity of Langerhans cells and may thus contribute to the efficacy of Dead Sea water in the treatment of inflammatory skin diseases.
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The combination of seawater baths and solar radiation at the Dead Sea is a successful treatment for patients with psoriasis and atopic dermatitis (Schamberg, 1978; Even-Paz and Shani, 1989; Abels *et al*, 1995). Recently, baths with salts from the Dead Sea have been introduced for the treatment of these inflammatory skin diseases (Gruner *et al*, 1990). Several mechanisms have been implicated to contribute to the therapeutic efficacy of this combined treatment modality. First, higher concentrated salt solutions (15–25%) have been shown to elute chemotactic and proinflammatory mediators from lesional skin (Wiedow *et al*, 1992). Second, it has been shown that salt water bathing increases skin sensitivity to ultraviolet (UV) B radiation (Boer *et al*, 1982; Schempp *et al*, 1997, 1999). Increased photosensitivity may contribute to the efficacy of salt water baths followed by UV irradiation (Boer *et al*, 1982). Finally, immunomodulatory effects of single salt components on cell cultures and skin have been described. Dead Sea brine and some of its salts were found to inhibit cell proliferation *in vitro* (Shani *et al*, 1987). Bathing in magnesium-rich Dead Sea water, but not in NaCl solutions significantly reduced the number of $ATPase^+$ Langerhans cells in psoriatics and in healthy persons (Gruner *et al*, 1990). Dead Sea water is particularly rich in magnesium ions (300 g $MgCl_2$ per kg Dead Sea water) (Schempp *et al*, 1997). Therefore, we wished to determine the effects of magnesium ions on the expression of surface molecules by human epidermal Langerhans cells, on their allostimulatory capacity and on cytokine production by epidermal cell suspensions. *In vivo*, a 5% concentration of $MgCl_2$ was used to

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Reprint requests to: Dr. Christoph M. Schempp, Department of Dermatology, University of Freiburg, Hauptstr. 7, D-79104, Freiburg, Germany. Email: schempp@haut.ukl.uni-freiburg.de

Abbreviations: ATPase, adenosine triphosphatase; MECLR, mixed epidermal cell lymphocyte reaction.

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avoid osmotic effects of the salt solution. *In vitro*, a nontoxic $MgCl_2$ concentration (1%) was used.

MATERIALS AND METHODS

Media and chemicals $MgCl_2$ hexahydrate, NaCl, NH_4SCN, Trisma maleat, and sucrose were supplied from Sigma (St Louis, MO). Acetone, HCl, NaOH, and $NaHCO_3$ were obtained from Merck (Darmstadt, Germany). HEPES buffer solution, phosphate-buffered saline (PBS) and Hanks balanced salts solution (HBSS) w/o Ca^{2+}/Mg^{2+} were purchased from Life Technologies (Paisley, Scotland). DNAse I, dispase, and adenosine triphosphate (ATP) were obtained from Boehringer (Mannheim, Germany). Trypsin was obtained from Gibco (Eggenstein, Germany). Complete RPMI 1640 (c-RPMI) was supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin (all from Gibco). Formaldehyde 36.5%, $MgSO_4$, and $Pb(NO_3)_2$ were supplied from Riedel-de-Haen (Seelze, Germany). Cacodylate acid $(NH_4)_2S$ and Tween were obtained from Fluka (Buchs, Switzerland). Tris was purchased from Paesel & Lorei (Frankfurt, Germany).

Monoclonal antibodies (**MoAb**) Anti-HLA-DR (clone HB 145) and anti-CD11b MoAb (clone OKT-6) (ATCC, Rockville, MD) were used for negative selection enrichment of T cells. Sheep anti-mouse IgG MoAb coupled with Dynal beads were from Dynal (Hamburg, Germany). Mouse anti-human HLA-DR MoAb (α-chain, clone TAL.1B5) and the labeled streptavidin–biotin staining kit system 500 (Dako, Glostrup, Denmark) were used for *in vivo* staining of Langerhans cells. For *in vitro* staining of Langerhans cells, the following MoAb and isotype controls were used: phycoerythrin (PE) -conjugated anti-HLA-DR (clone L243, mouse IgG2a) and PE-conjugated IgG2a control MoAb; fluorescein isothiocyanate (FITC) -conjugated anti-B7–1 (clone BB1, mouse IgM) and anti-B7–2 (clone 2331, mouse IgG1) and FITC-conjugated IgM and IgG1 control MoAb (all from Pharmingen, San Diego, CA).

Irradiation UV radiation between 270 and 400 nm, peaking at 310 nm was delivered from 10 fluorescent UVB lamps, Philips TL20W/12 (Philips GmbH, Hamburg, Germany), housed in a UV 800 unit (Waldmann GmbH, VS-Schwenningen, Germany). UVB irradiance (280–320 nm) at the surface of the test areas was measured with a calibrated radiometer equipped with a SCS 280 photodetector (International Light, Newburyport, MA) and was 2.5 mW per cm^2 at a tube to target distance of 40 cm. For determination of the minimal erythema dose (MED) UVB irradiation was administered in gradually increasing doses (15, 30, 60, 90, 115, and 145 mJ per cm^2). Erythema was determined by visual assessment 24 h after irradiation as described (Schempp *et al*, 1999).

Subjects The protocol of the controlled prospective study was approved by the local ethics committee and written informed consent was obtained from all subjects who participated in the study. In part 1 of the study (enumeration of epidermal Langerhans cells in epidermal sheets) 12 healthy volunteers (age range 18–56 y; skin types II and III) with no history of skin disease or photosensitivity were enrolled. Six subjects (age range 20–37 y; skin types II and III) participated in part 2 of the study (mixed epidermal cell lymphocyte reaction, MECLR). The skin type was determined according to the classification of sun reactive skin types by Fitzpatrick (1988). Split skin from dermatologic surgery was used for the *in vitro* experiments [antigen-presenting assays, fluorescence-activated cell sorter (FACS) analysis, cytokine release].

Staining of Langerhans cells *in vivo* Circular test areas (2 cm in diameter) on the volar aspects of the forearms of 12 volunteers were treated on four consecutive days as follows (Schempp *et al*, 1997): 1 = 5% $MgCl_2$ solution (15 min); 2 = 5% $MgCl_2$ solution (15 min) followed by UVB irradiation (1 MED); 3 = 5% NaCl solution (15 min); 4 = 5% NaCl solution (15 min) followed by UVB irradiation (1 MED); 5 = UVB irradiation only (1 MED); 6 = untreated skin. Twenty-four hours after the last treatment punch biopsies (6 mm in diameter) were obtained from the center of the test areas. Biopsies were incubated in dispase working-solution (dispase, 5.0 g; HBSS, 1000 ml; HEPES buffer, 25 ml; pH 7.0 1 M $NaHCO_3$) for 2 h at 37°C, the epidermis was removed and washed in ice-cold PBS for 30 min. Epidermal sheets were divided into two halves. One half was stained for adenosine triphosphatase (ATPase) and the other half for HLA-DR expression of Langerhans cells.

Adenosine triphosphatase staining for Langerhans cells The ATPase staining of Langerhans cells was a modification of the procedure described by Juhlin and Shelley (1977). Briefly, epidermal sheets were washed three times in Tris buffer (Trisma maleat, 23.72 g; aqua dest. 500 ml; sucrose 34.35 g; pH 7.3 NaOH) and fixed for 20 min at 4°C in 0.05 M cacodylate–formaldehyde solution (0.2 M cacodylate acid; sucrose 6.85 g; 36.5% formaldehyde, 10 ml; aqua dest., 50 ml). After fixation, samples were washed three times in Trisma buffer and incubated at 37°C for 60 min in a ATP-Pb-containing solution (ATP, 10 mg; Trisma buffer 42 ml; 5% $MgSO_4$, 5 ml; 2% $Pb(NO_3)_2$, 3 (ml). The samples were then washed again in Trisma buffer and immersed for 5 min in 22% ammonium sulfide solution. The stained specimens were mounted in Kaiser's glycerol gelatine (Merck).

HLA-DR staining for Langerhans cells Epidermal sheets were fixed in acetone for 30 min at 4°C, were then washed at 4°C three times in 1 M PBS and incubated in Tris-buffered saline (0.05 M Tris, 100 ml; 0.85% NaCl, 900 ml) for 5 min. The specimen were then incubated for 90 min at 37°C in a 1:100 dilution of the primary anti-HLA-DR antibody. After washing three times in Tris-buffered saline the labeled streptavidin–biotin staining was performed according to the manufacturer's instructions (labeled streptavidin–biotin kit, Dako, Hamburg, Germany). The stained specimens were mounted in Kaiser's glycerol gelatine (Merck).

Enumeration of Langerhans cells The numbers of $ATPase^+$ and $HLA\text{-}DR^+$ epidermal cells were determined by the same investigator using a Olympus T2 microscope (Olympus Optical Co., Tokyo, Japan). Four randomly selected fields from each specimen were counted at ×200 magnification using a WHK 10 × 20 L-H optical grid (Olympus) to determine the number of Langerhans cells per mm^2. Blinded control samples were enumerated by a second investigator. This control enumeration yielded identical results when compared with the first observer (not shown). Analysis of variance (ANOVA) (BMDP statistical software, Los Angeles, CA) was performed, and single variables were compared using the Wilcoxon signed rank test with Bonferoni–Holm correction. $p \leqslant 0.05$ were considered significant. Each point in **Fig 2** represents the mean Langerhans cells frequency per mm^2 epidermis per volunteer.

Antigen-presenting cell assay

Epidermal cell suspensions Epidermal cell suspensions were generated by limited trypsinization of epidermal sheets as described (Weiss *et al*, 1995; Schempp *et al*, 2000). Trypsin was used in a concentration of 0.25% in PBS supplemented with 80 U per ml DNAse to dissociate the epidermis of suction blisters. Epidermal cell were washed twice in PBS (4°C, 1200 U per min) and were further cultured in c-RPMI.

Preparation of T cells Allogeneic resting T cells were enriched from heparinized blood by plastic adherence and immunomagnetic depletion with anti-HLA-DR and CD11b primary antibodies and second step sheep anti-mouse IgG MoAb coupled to Dynal beads (Dittmar *et al*, 1999). The resulting T cells were > 85% $CD3^+$ as determined by FACS analysis. T cells were cultured in c-RPMI.

MECLR Epidermal cells (5×10^4) were cocultured in triplicates with allogeneic T cells (1×10^5) in c-RPMI in 96-well round-bottom microtiter plates (Costar, Cambridge, MA) for 6 d (5% CO_2, 37°C, 168 h). [^{3}H]-thymidine (1 μCi) was added to each well for the final 16 h of coculture. Plates were harvested with a Canberra Packard Filter Mate (Canberra Packard, Frankfurt, Germany) and incorporation of [^{3}H]thymidine was determined by liquid scintillation spectroscopy using a Top-Count (Canberra Packard).

MECLR *in vivo* Circular test areas (2 cm in diameter) on the volar aspects of the forearms of six volunteers were treated on four consecutive days as follows: 1 = 5% $MgCl_2$ solution (15 min); 2 = 5% $MgCl_2$ solution (15 min) followed by UVB irradiation (1 MED); 3 = UVB irradiation only (1 MED); 4 = untreated skin. Twenty-four hours after the last treatment suction blisters (1.5 cm in diameter) were raised on the centers of the test areas as described (Kiistala and Mustakallio, 1967). Epidermal cell suspensions were prepared and an MECLR was performed as described above. Background values (T cells only, epidermal cell only) were subtracted from epidermal cell + T cell values. The influence of variables on proliferation rates was assessed with analysis of variance (ANOVA). $p \leqslant 0.05$ were considered significant.

MECLR *in vitro* Epidermal cell suspensions were prepared from split skin by incubation in dispase working solution as described above. Epidermal cells were preincubated for 24 h in the presence or absence of 1% $MgCl_2$. This $MgCl_2$ concentration was not toxic *in vitro* as determined by propidium iodide staining (not shown). Epidermal cell suspensions were washed and MECLR assays were further performed as described above.

Immunostaining and flow cytometry Epidermal cell suspensions were incubated in the presence or absence of 1% $MgCl_2$ for 24 h. After washing two times in PBS (4°C, 1200 U per min), the cells were stained for two color FACS analysis in PBS (4°C) with the following MoAb: PE-labeled anti-HLA-DR MoAb, FITC-labeled anti-B7–1 or B7–2 MoAb, and appropriate isotype control MoAb. Epidermal cell suspensions (5×10^4 cells per sample) were analyzed by FACScan using the CellQuest software (Becton Dickinson). The percentage of gated HLA-DR^+/$B7^+$ cells was determined with the CellQuest software.

Cytokine detection Epidermal cell suspensions (1×10^6 cells per ml) were incubated in the presence or absence of 1% $MgCl_2$ for 24 h. Supernatants were collected and were stored at –80°C. Specific cytokine enzyme-linked immunosorbent assays with recombinant human primary MoAb for TNF-α, interleukin (IL) –1β, IL-10, and IL-12 were performed according to the manufacturer's instructions (R&D Systems, Wiesbaden, Germany).

RESULTS

$MgCl_2$ but not NaCl reduces the expression by Langerhans cells of ATPase and HLA-DR *in vivo* We investigated if the number of epidermal $ATPase^+$ and HLA-DR^+ Langerhans cells was influenced by *in vivo* application of a 5% $MgCl_2$ solution. For comparison the skin was treated with a 5% NaCl solution, with UVB irradiation and with salt solutions followed by UVB irradiation. An example of HLA-DR staining for Langerhans cells in epidermal sheets is shown in **Fig 1**. The results of all volunteers are illustrated in **Fig 2(*a*)** (ATPase) and in **Fig 2(*b*)** (HLA-DR). Pairwise comparison of variables with the Wilcoxon test (Bonferoni-Holm corrected) revealed a significant reduction of both $ATPase^+$ and HLA-DR^+ Langerhans cells by UVB alone or $MgCl_2$ alone, whereas NaCl had no effect (**Table I**). The combination of $MgCl_2$ + UVB or NaCl + UVB also significantly

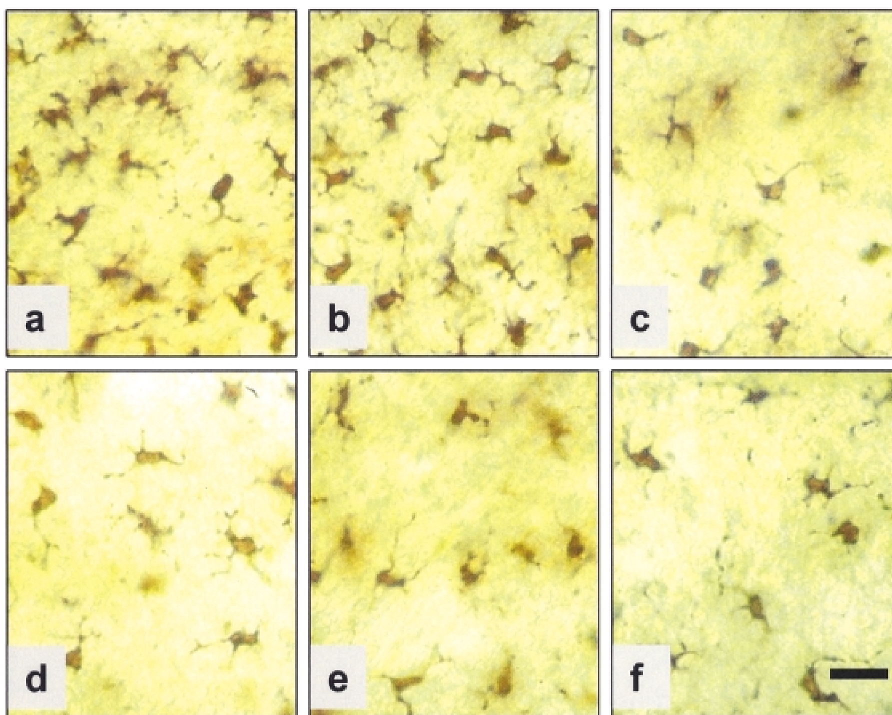

Figure 1. Effects of magnesium ions and UVB on the expression by Langerhans cells of HLA-DR molecules *in vivo*. The skin was treated on four consecutive days with a 5% $MgCl_2$, 5% NaCl solution (15 min), UVB irradiation (1 MED) alone or with salt solutions followed by UVB irradiation. The epidermis was separated from punch biopsies by dispase treatment and the sheets were stained for HLA-DR expression on epidermal Langerhans cells. The number of Langerhans cells is similar in untreated (*a*) and NaCl-treated (*b*) skin. By contrast, HLA-DR^+ Langerhans cells are reduced by $MgCl_2$ (*c*), UVB (*d*), $MgCl_2$ + UVB (*e*), and NaCl + UVB (*f*). *Scale bar*: 20 μm.

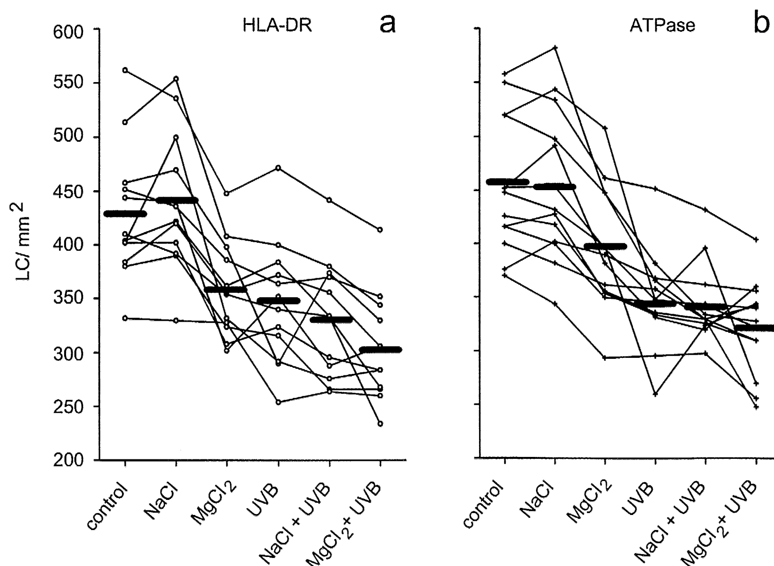

Figure 2. Magnesium ions reduce $ATPase^+$ and HLA-DR^+ Langerhans cells *in vivo*. The skin was treated and epidermal sheets were stained for HLA-DR (*a*) or ATPase (*b*) expression by epidermal Langerhans cells and the number of Langerhans cells per mm^2 was determined as detailed in **Fig 1** and *Materials and Methods*. Each point represents the mean Langerhans cells frequency per mm^2 epidermis per volunteer (n = 12). Langerhans cells numbers are reduced by $MgCl_2$, UVB, $MgCl_2$ + UVB, and NaCl + UVB, but not by NaCl only. For statistical analysis see **Table I**.

reduced the number of epidermal Langerhans cells, but only $MgCl_2$ + UVB had an additive effect when compared with UVB alone (**Table I**).

Magnesium ions inhibit the allostimulatory capacity of Langerhans cells *in vivo* To investigate whether the depletion of Langerhans cells by *in vivo* $MgCl_2$ application was related to effects on the antigen-presenting cell function of Langerhans cells we applied 5% $MgCl_2$ with or without subsequent UVB irradiation (1 MED) to the sun-protected forearm of six volunteers on four consecutive days. Untreated skin and skin treated with UVB alone served as controls. Twenty-four hours after the last application, epidermal cell suspensions were prepared from suction blisters and were analyzed for their capacity to stimulate the proliferation of alloreactive naive T cells. Epidermal cell suspensions from untreated skin were fully capable of stimulating the proliferation of allogeneic T cells. By contrast, epidermal cells showed a significantly reduced capacity to stimulate alloreactive T cell responses following $MgCl_2$ ($p = 0.006$), UVB ($p = 0.0001$), and $MgCl_2$ + UVB ($p = 0.0001$) treatment (ANOVA) (**Table II**).

Magnesium ions inhibit the allostimulatory capacity of Langerhans cells *in vitro* Next we questioned whether the reduced allostimulatory capacity of epidermal cell suspensions after *in vivo* $MgCl_2$ application was primarily caused by the emigration of Langerhans cells from the epidermis. To address this issue we preincubated epidermal cells with $MgCl_2$ *in vitro* before coculture with allogeneic T cells – a model that does not allow the Langerhans cells to emigrate. Again, epidermal cell suspensions from untreated skin stimulated the proliferation of alloreactive T cells. In contrast, epidermal cells pretreated with $MgCl_2$ showed a significantly reduced capacity to stimulate T cell proliferation (**Fig 3**).

Magnesium ions inhibit the expression by Langerhans cells of HLA-DR and B7 molecules *in vitro* To investigate whether the reduced allostimulatory capacity of epidermal cell suspensions was associated with an altered expression by Langerhans cells of HLA-DR or costimulatory B7 molecules *in vitro*, we incubated epidermal cell suspensions in the presence or absence of 1% $MgCl_2$ for 24 h. Two color FACS analysis with PE-labeled anti-HLA-DR MoAb and FITC-labeled anti-B7–1 or B7–2 MoAb of a representative experiment is shown in **Fig 4**. Untreated epidermal cell suspensions contained 0.8% HLA-DR^+ Langerhans cells (**Fig 4*b***). These HLA-DR^+ cells additionally expressed B7–1 (**Fig 4*c***) and B7–2 (**Fig 4*d***) molecules. Preincubation of epidermal cells with 1% $MgCl_2$ almost completely suppressed the expression of HLA-DR and B7 molecules on Langerhans cells (**Fig 4*e*, *f***).

Effects of magnesium ions on cytokine production of epidermal cell suspensions *in vitro* To elucidate further the mechanism by which $MgCl_2$ inhibits the allostimulatory capacity of epidermal cells we assessed the constitutive production of several cytokines by epidermal cells. Epidermal cell suspensions were incubated in the presence or absence of 1% $MgCl_2$ for 24 h. Subsequently, the supernatants were collected and analyzed for tumor necrosis factor (TNF)-α, IL-1β, IL-10, and IL-12 by specific

Table I. Influence of *in vivo* UVB, $MgCl_2$, NaCl, $MgCl_2$ + UVB and NaCl + UVB on the number of epidermal $ATPase^+$ and HLA-DR^+ Langerhans cells[a]

Compared parameters		p-values	
Parameter 1	Parameter 2	ATPase	HLA-DR
Normal Skin	UVB	0.0005	0.0005
Normal Skin	$MgCl_2$	0.0063	0.0005
Normal Skin	NaCl	0.7744	1.0000
Normal Skin	$MgCl_2$ + UVB	0.0005	0.0005
Normal Skin	NaCl + UVB	0.0005	0.0005
UVB	$MgCl_2$	0.0063	0.7744
UVB	NaCl	0.0005	0.0005
UVB	$MgCl_2$ + UVB	0.0386	0.0386
UVB	NaCl + UVB	0.1460	0.1460
NaCl	$MgCl_2$	0.0005	0.0005
NaCl	$MgCl_2$ + UVB	0.0005	0.0005
NaCl	NaCl + UVB	0.0005	0.0005
$MgCl_2$	$MgCl_2$ + UVB	0.0005	0.0063
$MgCl_2$	NaCl + UVB	0.0063	0.0010
$MgCl_2$ + UVB	NaCl + UVB	0.1460	0.0654

[a]Skin on the volar forearms of 12 volunteers was treated on four consecutive days and punch biopsies were obtained on day 5. Epidermal sheets were stained for ATPase and HLA-DR and Langerhans cells were enumerated as described in *Materials and Methods*. The mean Langerhans cell counts per mm^2 were compared using the Wilcoxon signed rank test with Bonferoni–Holm correction. p-values are indicated.

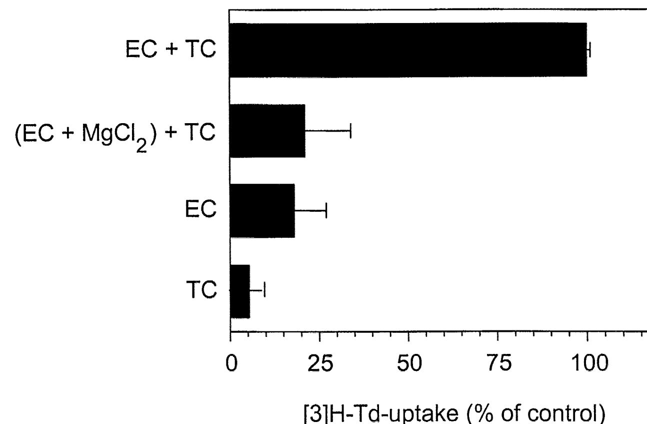

Figure 3. Magnesium ions inhibit the alloantigen-presenting function of Langerhans cells *in vitro*. Epidermal cells were generated from split skin and cells from the same cell preparation were preincubated in the presence or absence of 1% $MgCl_2$ *in vitro* for 24 h. Subsequently, the cells were washed and epidermal cell (5×10^4) were cocultured with allogeneic T cells (1×10^5) for 6 d. Cell proliferation was determined by $[^3H]$thymidine incorporation. The mean ± SD of three independent experiments is shown.

Table II. *In vivo* application of magnesium ions inhibits the alloantigen-presenting function of epidermal cells[a]

Subject no.	Untreated	$MgCl_2$ ($p = 0.006$)*	UVB ($p = 0.0001$)*	$MgCl_2$ + UVB ($p = 0.0001$)*
1	90.363 ± 1.920	58.619 ± 6.026	23.799 ± 3.584	8.089 ± 1.174
2	62.717 ± 7.978	29.753 ± 170	41.014 ± 5.588	24.414 ± 5.043
3	68.783 ± 916	60.660 ± 10.904	46.961 ± 8.724	2.603 ± 1.263
4	50.687 ± 3.621	32.395 ± 646	27.161 ± 3.369	17.405 ± 6.451
5	71.903 ± 5.761	75.381 ± 3.312	7.341 ± 1.259	3.999 ± 1.054
6	38.418 ± 9.903	24.466 ± 5.249	27.871 ± 2.296	24.284 ± 5.587

[a]Six subjects were treated on their volar forearms, epidermal cell suspensions were prepared and a mixed epidermal cell leukocyte reaction (MECLR) was performed as detailed in the Methods. Background values (epidermal cell alone, T cells alone) were subtracted from epidermal cell + T cells values and the mean ± SD of triplicate measurements is indicated. *p-value compared with untreated control (ANOVA).

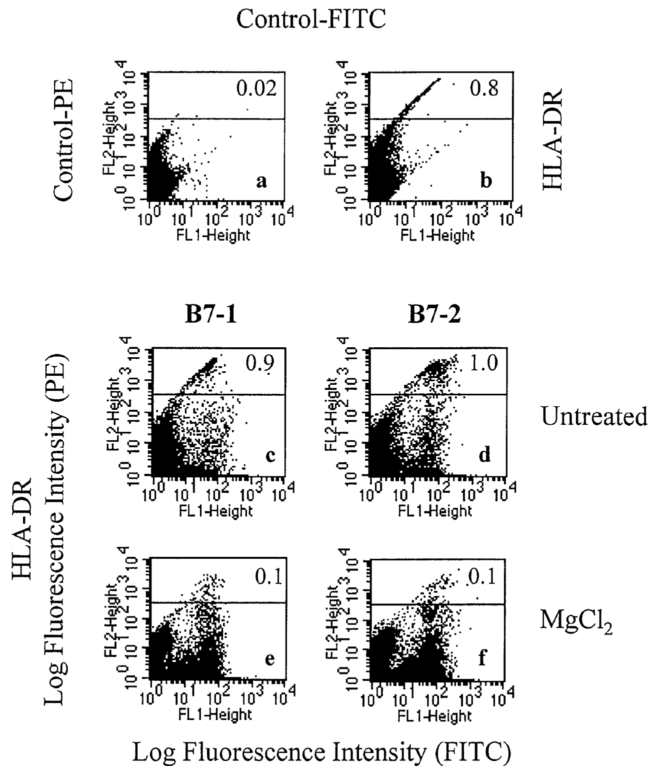

Figure 4. Magnesium ions inhibit the expression by Langerhans cells of HLA-DR and B7 molecules *in vitro*. Epidermal cells from the same cell preparation were preincubated in the absence (*a–d*) or presence (*e, f*) of 1% $MgCl_2$ *in vitro* for 24 h. Subsequently, the cells were washed and were labeled with MoAb against HLA-DR (PE) (*b–f*), IgG (*a, b*), B7–1 (FITC) (*c, e*), or B7–2 (FITC) (*d, f*). 5×10^4 cells per sample were analyzed by FACScan. The percentage of HLA-DR^+/$B7^+$ cells is displayed in the upper right corner. One of three independent experiments is shown.

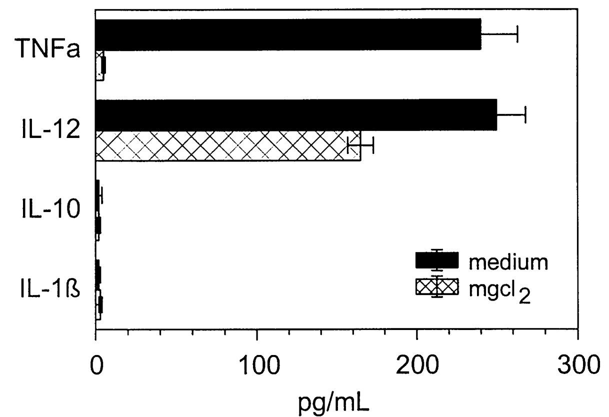

Figure 5. The constitutive secretion of TNF-α and IL-12 by epidermal cells is reduced by magnesium ions. Epidermal cells (1×10^6 cells per ml) were incubated in the presence or absence of 1% $MgCl_2$ for 24 h. Supernatants were collected and specific cytokine enzyme-linked immunosorbent assays with recombinant human primary MoAb for TNF-α, IL-1β, IL-10, and IL-12 were performed. The mean ± SD of triplicate measurements is shown. The experiment was repeated with similar results.

enzyme-linked immunosorbent assays. $MgCl_2$ almost completely suppressed the production of TNF-α and decreased the concentration of IL-12 (**Fig 5**). IL-1β and IL-10 were not detectable in this *in vitro* model.

DISCUSSION

This study investigated the effects of magnesium ions and low-dose UVB irradiation on epidermal Langerhans cells. In line with published reports (Aberer *et al*, 1981; Lynch *et al*, 1981; Koulu *et al*, 1985; Alcalay *et al*, 1989; Murphy *et al*, 1993), *in vivo* UVB irradiation (1 MED) resulted in a decrease of epidermal $ATPase^+$ and HLA-DR^+ Langerhans cells and a reduced allostimulatory capacity of epidermal cell suspensions. Similarly to UVB, the topical application of $MgCl_2$ but not of NaCl reduced the number of epidermal Langerhans cells. This reduced expression by Langerhans cells of ATPase and HLA-DR molecules *in vivo* was of functional relevance as demonstrated *in vivo* and *in vitro*: epidermal cell suspensions showed a reduced capacity to stimulate allogeneic T cell proliferation. The *in vitro* results suggest that the inhibitory effects of magnesium ions on epidermal cell suspensions are unlikely due to an emigration of Langerhans cells from the epidermis as Langerhans cells could not emigrate in this model. The impaired antigen-presenting function of Langerhans cells was associated with a reduced expression by Langerhans cells of class II and costimulatory B7 molecules, and with a suppression of the constitutive TNF-α production by epidermal cells *in vitro*.

Epidermal Langerhans cells are highly sensitive to the effects of UV radiation. *In vivo* low-dose UVB irradiation results in a decrease of Langerhans cells surface markers, i.e., ATPase, HLA-DR, and CD1a molecules (Aberer *et al*, 1981; Lynch *et al*, 1981; Koulu *et al*, 1985; Alcalay *et al*, 1989; Murphy *et al*, 1993). In parallel, the function of Langerhans cells isolated from UVB-exposed skin is impaired: epidermal cell suspensions from UVB-exposed skin show a reduced capacity to stimulate allogeneic or antigen-specific T cell proliferation *in vitro* (Cooper *et al*, 1985; Kremer *et al*, 1997; Dittmar *et al*, 1999), and in UVB-exposed skin, contact hypersensitivity responses are suppressed (Toews *et al*, 1980; Lynch *et al*, 1981). Also, epidermal cells irradiated *in vitro* with UVB have a reduced capacity to stimulate allogeneic or antigen-specific T cell responses (Stingl *et al*, 1981; Aberer *et al*, 1982).

UVB-exposed murine Langerhans cells have been shown to preferentially activate cells of the Th2 subset (Simon *et al*, 1990) and to induce specific clonal anergy in Th1 cells (Simon *et al*, 1991). These disturbed functions of UVB-exposed Langerhans cells may result from: (i) the appearance of $T6^-DR^+$ antigen-presenting cells in skin and subsequent activation of $CD8^+$ suppressor lymphocytes (Cooper *et al*, 1985; Baadsgaard *et al*, 1987, 1988); (ii) the increased production of suppressive mediators, such as IL-10 (Enk *et al*, 1993; Peguet-Navarro *et al*, 1994; Ullrich, 1994; Kang *et al*, 1998); or (iii) a disturbed expression of costimulatory molecules, such as B7–1 and B7–2, that are involved in T cell activation (Weiss *et al*, 1995; Rattis *et al*, 1996; Denfeld *et al*, 1998; Dittmar *et al*, 1999).

Most of the therapies used to treat psoriasis suppress cellular immune function and inflammation. These include UV irradiation, psoralen + UVA, cyclosporine, corticosteroids, methotrexate, anthralin, and retinoids. Most of these therapies have been shown to reduce Langerhans cell numbers and Langerhans cell function (Koulu and Jansen, 1982; Haftek *et al*, 1983; Morhenn *et al*, 1983; Koulu *et al*, 1985; Gottlieb, 1988; Alcalay *et al*, 1989). There is evidence for cell-mediated immune mechanisms in the pathogenesis of psoriasis, and it has been proposed that Langerhans cells in psoriasis plaques could activate dermal T cells in an autologous MECLR (Gottlieb, 1988). Alternatively, Langerhans cells could present an unknown autologous or exogenous antigen to T lymphocytes. T cell activation would then lead to the release of mediators of inflammation, and possibly of epidermal growth factors (Gottlieb, 1988; Bieber and Braun-Falco, 1989; Streilein, 1990).

Magnesium ions have been shown to suppress allergic contact dermatitis (Greiner and Diezel, 1990) and inhibit 5-lipoxygenase activity in human polymorphonuclear leukocytes (Ludwig *et al*,

1995). A possible mechanism by which Mg^{2+} ions exert their anti-inflammatory properties may be the competitive displacement of calcium ions from their binding sites (Tsien *et al*, 1987; Kramer *et al*, 1991; Graeff *et al*, 1995).

Consequently, competitive inhibition of the Ca^{2+}-sensitive phospholipase A_2 may occur, resulting in the suppression of cutaneous inflammation (Kramer *et al*, 1991). This is of importance because in psoriatic epidermis raised phospholipase A_2 activity has been demonstrated (Forster *et al*, 1985). Lanthanides, another group of Ca^{2+}-competitive elements, have been shown to inhibit Langerhans cell ATPase and contact sensitization with dinitro-fluorobenzene (Diezel *et al*, 1989; Gruner *et al*, 1991).

The findings from this study suggest that divalent magnesium ions may also inhibit Langerhans cell ATPase. Membrane ecto-ATPase protects Langerhans cells against the permeabilizing effects of extracellular ATP (Girolomoni *et al*, 1993) and its down-regulation is indicative of the reduction of Langerhans cell viability and antigen-presenting capacity (Aberer *et al*, 1981; Koulu *et al*, 1985; Gruner *et al*, 1991; Girolomoni *et al*, 1993).

Furthermore, the inhibition of calcium-dependent mechanisms by magnesium ions may be involved in the reduced expression by Langerhans cells of HLA-DR molecules. It has been shown that interferon-γ-induced HLA-DR molecule expression is associated with a rapid increase of calcium ions and that the induction of HLA-DR expression can be blocked by the inhibition of the interferon-γ-induced calcium influx (Klein *et al*, 1990; Ryu *et al*, 1993). Our finding of the concomitant downregulation of HLA-DR and B7 molecules by magnesium ions suggests that the regulation of B7 is also dependent on calcium-sensitive mechanisms.

Finally, the addition of magnesium ions to unstimulated epidermal cell suspensions resulted in a reduced production of TNF-α and, less pronounced, IL-12 *in vitro*. In the epidermis, TNF-α is primarily produced by human keratinocytes and not by Langerhans cells, although activated Langerhans cells may also secrete TNF-α (Schreiber *et al*, 1992; for review see Matsue *et al*, 1992; Kimber *et al*, 2000).

Thus, downregulation of the constitutive TNF-α production by epidermal cell suspensions suggests the involvement of keratinocytes in the modulation of the antigen-presenting function of Langerhans cells. The ability to present antigen is acquired by Langerhans cells during culture and is modulated by epidermal cytokines, that of greatest importance being TNF-α (reviewed by Kimber *et al*, 2000). TNF-α seems also to be involved in the induction and maintenance by Langerhans cells of major histocompatibility complex class II antigens and B7 costimulatory molecules (reviewed by Kimber *et al*, 2000). Thus, in addition to direct effects on Langerhans cells, i.e., blocking of ATPase and HLA-DR expression, magnesium ions may indirectly inhibit the antigen-presenting function of Langerhans cells via the reduction of keratinocyte-derived cytokines, i.e., TNF-α.

Taken together, this study demonstrates specific inhibitory effects of Mg^{2+} ions on epidermal Langerhans cell function that may account for the efficacy of Dead Sea water and $MgCl_2$ containing topical applications in the treatment of inflammatory skin diseases.

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