Original Article

Effects of mycophenolic acid on human renal proximal and distal tubular cells in vitro

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Abstract

Background. Mycophenolic acid has been shown to be effective for the prevention and treatment of renal allograft rejection. Rejection episodes were found to be associated with an infiltration of lymphocytes and macrophages/monocytes into the diseased kidney. Expression of RANTES, HLA-DR and ICAM-1 may be important for the pathogenesis of this leukocyte infiltration. Therefore the aim of this study was to evaluate the effect of the antiproliferative and immunosuppressive agent mycophenolic acid (MPA) on cell growth and cytokine-induced expression of RANTES, HLA-DR and ICAM-1 of highly purified proximal (PTC) and distal tubular cells (DTC) from human kidney.

Methods. Human PTC and DTC were cultured in the presence of different concentrations of MPA (0.25–50 μM) or MPA plus guanosine (100 μM). Total cell number (DNA content) was determined after 4 days of cell culture by a non-radioactive fluorescence assay. Cells were stimulated by a combination of cytokines (IL1β + IFN+ TNFα = cytomix) or cytomix plus MPA. Secretion of RANTES protein was evaluated with an enzyme-linked-immunosorbent assay. Cell surface expression of HLA-DR and ICAM-1 was assessed by flow cytometric analysis.

Results. MPA inhibited cell growth of PTC and DTC in a dose-dependent manner. This effect was totally abolished by the addition of guanosine. Cytokine-induced RANTES expression was synergistically increased in the presence of MPA, an effect that was partially prevented by the addition of guanosine. Cytokine stimulation resulted in de novo expression of HLA-DR and a marked increase of ICAM-1 expression, which was partially inhibited by dexamethasone. Addition of MPA did not influence this stimulated expression.

Conclusions. We demonstrate that MPA has an effect on cell growth and chemokine release of tubular epithelial cells, and that these effects are dependent on the inhibition of cellular guanosine production. The clinical consequences of this possible pro-inflammatory effect of MPA on RANTES release may be abolished by a concomitant treatment with steroids.

Key words: distal tubule; epithelial cells; human; mycophenolic acid; proliferation; proximal tubule

Introduction

Mycophenolate mofetil (CellCept) has been shown to be a powerful immunosuppressant clinically used to prevent allograft rejection [1,2]. Mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil, is a potent, non-competitive, and reversible inhibitor of inosine-5'-monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme for de novo purine synthesis [3], and thus interferes with cellular biosynthesis of guanosine and deoxyguanosine nucleotides. Since proliferating B and T lymphocytes are more dependent on the de novo pathway for purine biosynthesis than on the salvage pathway [4], MPA is described to inhibit lymphocyte proliferation more effectively than proliferation of other cell types. Most cells are able to utilize the salvage pathway, which basically recycles free purine bases. One metabolic side-effect of guanosine depletion mediated by MPA is a decrease in the transfer of mannose and fucose to glycoproteins (e.g. adhesion molecules) [5,6]. Therefore, it has been suggested that by this mechanism MPA could also inhibit recruitment of leukocytes to sites of inflammation [7]. Yet, no data about the effect of MPA on renal tubular epithelial cells are available.

Renal tubular epithelial cells play a fundamental role in modulating inflammatory responses during inflammatory kidney diseases, autoimmune disorders and renal allograft rejection. Expression of chemokines, human leukocyte antigens, and adhesion molecules may be involved in renal infiltration by lymphocytes, monocytes and granulocytes, thus amplifying inflammatory diseases [8–10]. A family of mono-
cyte-specific chemoattractant and activating cytokines (chemokines) has been described to be upregulated during graft rejection, including the chemokine regulated upon activation, normal T cell expressed and secreted (RANTES) [8]. RANTES is a member of the superfamily of proinflammatory cytokines called CC chemokines that is a chemoattractant for memory T cells, monocytes, and eosinophils [11]. Moreover, recognition of foreign antigens by T lymphocytes during these immune responses requires the presence of human leukocyte antigens (HLA) on antigen-presenting cells [12]. The immunoglobulin-like molecule intercellular adhesion molecule-1 (ICAM-1) increases the avidity of T lymphocytes interactions with antigen-presenting cells bearing HLA antigens [12]. Proinflammatory cytokines like γ-interferon (γ-IFN), interleukin 1β (IL1β), and tumour necrosis factor α (TNFα) were found to be potent inducers of the expression of different cell surface antigens and soluble factors like RANTES, HLA-DR, and ICAM-1 in vitro [12–14]. Inhibition of this cross-talk between infiltrating cells and local renal cells by immunosuppressant drugs may result in an improvement in the treatment of transplant rejection.

Therefore the object of this study was to investigate the effect of MPA on proliferation and cytokine-induced antigen expression on highly purified proximal and distal tubular cells from human kidney.

Subjects and methods

Materials

Tissue culture media were obtained from Gibco (Eggenstein, Germany) and Sigma Chemicals (Deisenhofen, Germany), and MACS microbeads from Miltenyi (Bergisch-Gladbach, Germany). The monoclonal antibody (mAb) anti-HLA-DR, FITC and mAb anti-CD54-PE were purchased from Dianova (Hamburg, Germany). For the enzyme-linked immunoassay a mAb anti-RANTES, a bispecific rabbit anti-RANTES antibody and recombinant human RANTES were obtained from R&D Systems (Wiesbaden, Germany). Recombinant γ-IFN, IL1β and TNFα were provided by Strathman (Hamburg, Germany). MPA was from Hoffmann LaRoche (Grenzach-Wyhlen, Switzerland). Dexamethasone was from Ratiopharm (Ulm, Germany).

Isolation, culture and stimulation of human proximal and distal tubular cells

Human proximal (PTC) and distal tubular cells (DTC) were separated using antibody coated magnetic beads as described previously [15]. Cells were prepared after tumour nephrectomies from those portions of the human kidney not involved in renal-cell carcinoma. The tissue was minced into approximately 1 mm² pieces and digested with RPMI 1640 containing collagenase/disparse (1 mg/ml) at 37 °C for 1 h. The digested tissue fragments were passed through a 106 µm mesh and incubated for 45 min with RPMI 1640 supplemented with collagenase IV (1 mg/ml), DNase (100 µg/ml) and MgCl₂ (5 mM). After Percoll density centrifugation, the cell pellet was preincubated in 10 ml ice-cold RPMI 1640 for 20 min with human immunoglobulin G (hIgG; 2.5 mg/ml) added. Cells were incubated for 20 min on ice with the primary antibody (in RPMI plus hIgG; 5 µg/10⁷ cells). To enrich PTC we used a mAb against aminopeptidase M (CD13), specific for the proximal tubule. DTC were isolated through a mAb recognizing Tamm-Horsfall glycoprotein (THG), a specific antigen of the thick ascending limb of Henle’s loop and the early distal convoluted tubule. Then, cells were washed with PBS containing 5 mM EDTA and 0.5% BSA. Finally, cells were incubated with secondary antibody for 20 min, washed, passed through a fine mesh (pore size 40 µm), and isolated by immunomagnetic separation applying the Mini-MACS system [15].

After immunomagnetic isolation cells were seeded in six well plates precoated with human collagen IV (20 µg/ml). The cells were grown in medium 199 with 10% FCS at 37 °C and 5% CO₂ in a humidified atmosphere. The culture medium was supplemented with the antibiotic meropenem (100 µg/ml) during the first 2–3 days after isolation. Culture medium was changed every 3–4 days.

Proximal primary isolated cells were strongly positive for aminopeptidase M (98.6%); however, cells of the distal portion were strictly negative (98.7%). Ultrastructural analysis of PTC primary isolates revealed highly preserved brush border microvilli, well-developed endocytosis apparatus and numerous mitochondria, whereas DTC primary isolates showed smaller cells with basolateral invaginations and fewer apical microvilli. Characterization by immunofluorescence indicated the coexpression of cytokeratin and vimentin. Stainings for desmin, smooth-muscle actin, a fibroblast-specific marker, and von Willebrand factor to exclude the possibility of contaminating cells were negative [15]. In vitro differentiation of PTC and DTC was shown by scanning electron microscopy and flow cytometry [16, Baer et al, unpublished data]. Activity of brush border enzyme alkaline phosphatase was detectable in cultured proximal cells only, cultured distal cells revealed no activity [15]. A strong expression of the epidermal growth factor receptor in cultured DTC was shown, whereas the signal in cultured PTC was low [Baer et al., unpublished data]. Cultured cells displayed different adenylate cyclase responsiveness to hormonal stimulation. Parathyroid hormone (10⁻⁶ M) increased cAMP production in DTC up to 32.8-fold of the basal level, in PTC up to 3.5-fold only. Calcitonin (10⁻⁶ M) stimulated adenylate cyclase in DTC 4.3×, whereas only a low calcitonin response was found in PTC. Arginine-vasopressin (10⁻⁸ M) increased the distal cAMP production 1.9-fold of the basal level; the proximal cAMP production was negligible [15].

Assay for cytotoxicity

As an indicator of cytotoxicity, the activity of lactate dehydrogenase in cell culture supernatants of PTC and DTC was determined by a commercial assay, measuring the decrease of absorbance at 340 nm, resulting from conversion of NADH to NAD in the presence of pyruvate. Supernatants from cell cultures treated with MPA were collected and assayed for LDH release.

Fluorometric assay

For the measurement of the total cell number, a fluorometric assay with the DNA-intercalating fluorochrome DAPI (4,6-diamino-2-phenylindole) was used [17]. These measurements represent cell numbers at a specific time point of cell
culture in media supplemented with MPA or MPA plus guanosine. Cells (2 × 10^6) were seeded in medium 199 with 10% FCS and different concentrations of MPA (0.25 μM, 2.5 μM, 25 μM, 50 μM) in 96-well microtitre plates and cultured for 96 h. Medium 199 with 10% FCS served as a positive control, and medium 199 without FCS served as a negative control. The cells were washed twice with PBS, and lysed with 100 μl buffer (0.02% w/v SDS, 150 mM NaCl, 15 mM sodium citrate) at RT for 5 min. Finally 100 μl DAPI (2 μg/ml) was added to each well, and incubated at RT for 5 min. Fluorescence was measured in relative fluorescence units (FU) in a FluoStar fluorescence reader (BMG, Offenburg, Germany). A 355 nm excitation filter and a 460 nm emission filter were used. All results were expressed as mean values and standard deviations (n = 6).

**Stimulation of RANTES, HLA-DR, and ICAM-1 and treatment with mycophenolic acid**

A combination of proinflammatory cytokines, γ-interferon (γIFN; 200 U/ml), interleukin 1β (IL1β; 25 U/ml) and tumour necrosis factor α (TNFα; 10 ng/ml), was used for stimulation. Because our previous studies showed that a combination of cytokines resulted in a strong RANTES induction, we also used this cytokin in the current study.

Cells were grown in 24-well culture dishes until confluence. For stimulation of RANTES protein expression, cells were washed and stimulated with a mix of all three cytokines (cytomix) for 48 h in medium 199 without FCS. Stimulation of HLA-DR and ICAM-1 expression was done with cytokin for 96 h. To test the influence of MPA on the cytokinin-induced expression of RANTES, HLA-DR, and ICAM-1 cells were pre-treated with medium containing MPA (0.25 μM, 2.5 μM, 25 μM, 50 μM) at 37°C for 2 h. The cells were washed twice and incubated with the cytokin mixture together with MPA. As a control, cells were incubated with medium 199 alone (basal) or with cytokin mixture plus dexamethasone (10 μM). Supernatants were collected after 48 h for RANTES quantification, or cells were harvested after 96 h for flow cytometric analysis of HLA-DR and ICAM-1 expression.

For determinations of RANTES protein, supernatants were collected and assessed for the chemokine or stored at −20°C for later measurement. Cell number was assessed from trypsinized suspensions and counted in a Neubauer chamber. For flow cytometrical determination of HLA-DR and ICAM-1, cells were detached by trypsin/EDTA treatment for exactly 7 min, followed by immunostaining (see section ‘Flow cytometry’).

**Flow cytometry**

Confluent monolayers were washed twice with PBS. Suspensions were prepared by incubation with 0.05% trypsin and 0.02% EDTA for 7 min. The cells were washed twice with PBS. Aliquots of single cell suspension (1 to 2 × 10^5 cells) were incubated for 15 min at room temperature with directly labelled antibodies. Cells were washed with 2 ml PBS and pelleted by centrifugation at 300 g for 5 min. Cells were fixed in 1% paraformaldehyde. The labelled cells were analysed using a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany). All investigations included negative controls without antibodies and with non-relevant antibodies to evaluate the positive staining profile. The data of the level of fluorescence intensity, forwards and sideways (90°) scatter of 10^3 cells were processed using WinMDI Flow Cytometry Application Version 2.5 analysis software. Median of fluorescence intensity was calculated. Antigen expression of MPA-treated cells was correlated with the cytomix-stimulated antigen expression (100%). Each cell purification procedure was taken as a single, independent experiment, i.e. the number ’n’ in statistical analysis refers to the number of separate isolations.

**Statistical analysis**

The data of RANTES protein release were expressed as means ± SD. For statistical analysis, Student’s t-test for unpaired samples was used. P values < 0.05 were considered significant; P values < 0.01 were considered highly significant.

**Results**

**Cytotoxicity of MPA**

The measurement of lactate dehydrogenase (LDH) release into culture supernatant as a marker of cell death was incubated with horseradish–peroxidase–streptavidin for 1 h. After 1 h of incubation the plates were washed with PBS/0.05% Tween. Standard and samples were added to the wells and incubated for 2 h at room temperature. The plates were washed and incubated with biotinylated rabbit anti-RANTES for 2 h at room temperature, washed again and incubated with horseradish–peroxidase–streptavidin for 20 min. After washing, substrate reaction was followed by measurement of the optical density at 450 nm. Recombinant human RANTES was used as a standard.

**Quantification of RANTES expression**

The chemokine RANTES was quantified using sandwich enzyme-linked immunosorbent assay. In brief, wells of 96-well microtitre plates were coated with an anti-human RANTES mAb overnight at room temperature. Non-specific binding sites were blocked with PBS/1% BSA/5% sucrose. After 1 h of incubation the plates were washed with PBS/0.05% Tween. Standard and samples were added to the wells and incubated for 2 h at room temperature. The plates were washed and incubated with biotinylated rabbit anti-RANTES for 2 h at room temperature, washed again and incubated with horseradish–peroxidase–streptavidin for 20 min. After washing, substrate reaction was followed by measurement of the optical density at 450 nm. Recombinant human RANTES was used as a standard.

<table>
<thead>
<tr>
<th></th>
<th>PTC</th>
<th>DTC</th>
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<tbody>
<tr>
<td>Basal</td>
<td>9.0 ± 2.4</td>
<td>7.7 ± 2.5</td>
</tr>
<tr>
<td>Cytomix</td>
<td>19.5 ± 6.8</td>
<td>20.7 ± 11.6</td>
</tr>
<tr>
<td>+ MPA 50 μM</td>
<td>26.5 ± 2.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>+ MPA 50 μM + guanosine</td>
<td>23.5 ± 2.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>+ MPA 25 μM</td>
<td>23.7 ± 3.8</td>
<td>16.5 ± 0.7</td>
</tr>
<tr>
<td>+ MPA 25 μM + guanosine</td>
<td>28.5 ± 4.9</td>
<td>16.0 ± 1.4</td>
</tr>
<tr>
<td>+ MPA 2.5 μM</td>
<td>21.0 ± 3.5</td>
<td>14.0 ± 1.0</td>
</tr>
<tr>
<td>+ MPA 0.25 μM</td>
<td>16.5 ± 10.1</td>
<td>12.5 ± 0.7</td>
</tr>
<tr>
<td>MPA 25 μM (alone)</td>
<td>12.0 ± 1.4</td>
<td>10.5 ± 0.7</td>
</tr>
<tr>
<td>Cytomix + dexamethasone 10^-6 M</td>
<td>22.0 ± 5.6</td>
<td>18.5 ± 9.2</td>
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Data are presented as units LDH per litre medium. Means ± SD; n = 3; n.d., not done.
incubation with MPA. Cells (2 × 10^5) were seeded in media containing increasing concentrations of MPA or MPA plus guanosine. It should be noted specifically that these measurements represent total cell numbers at a specific time point of culture, not cell proliferation over a certain period of time [17].

After 4 days of cell culture, a significant decrease in total cell number (compared to normal cell culture medium as a control) was seen when MPA was added in a high concentration (50 μM: PTC −74.6%/DTC −74.8%; 25 μM: −71.8/−71.9; 2.5 μM −62.9/−51.9) (Figure 1A, B). This inhibition of cell growth was abolished by the addition of guanosine, indicating the inhibitory effect of MPA on IMPDH. The lower MPA concentration (0.25 μM) had no effect on tubular cell growth (Figure 1A, B).

**Effect of MPA on cytokine-stimulated RANTES secretion**

RANTES protein release into the culture supernatant of PTC and DTC was significantly increased by addition of cytomix for 48 h, as determined by enzyme-linked immunosorbsent assay (Table 2). As a control, cytomix, but no significant difference of cytomix for 48 h, as determined by enzyme-revealed a strong induction of both antigens by the cytokinin combination for 96 h. Flow cytometric analysis of the antigen expression of PTC and DTC (Table 2). This effect could be inhibited partially by additional incubation with guanosine. Incubation of cytomix in combination with a low MPA concentration (0.25 μM) induced no synergistic effect. MPA or guanosine given alone had no effect on RANTES secretion.

**Effect of MPA on cytokine-stimulated expression of HLA-DR and ICAM-1**

To study the effect of MPA on cytokin-induced expression of HLA-DR and ICAM-1, cells were cultured in the absence or presence of different concentrations of MPA together with the cytokine combination for 96 h. Flow cytometric analysis of the antigen expression revealed a strong induction of both antigens by the cytokinin combination for 96 h. Data were calculated by taking the cytokinin-stimulated condition as 100%. As a control, cells were incubated with the cytokinin plus dexamethasone. Antigen expression of HLA-DR was significantly inhibited by dexamethasone, whereas only a low and not significant reduction of ICAM-1 expression by dexamethasone could be detected (Table 3).

**Discussion**

Renal transplant rejection is characterized by a mononuclear cell infiltrate consisting mainly of monocytes, macrophages, and T cells in the graft. It has been shown that the immunosuppressive drug MPA prevents allograft rejection in animal experiments and clinical trials [18–20]. In general, MPA resulted in a better preservation of graft morphology, with less pronounced cellular infiltration and tubular atrophy [19].

Only limited data about the effects of MPA on renal tubular epithelial cells, a pivotal cell type involved in the rejection process, are available. Major side-effects of MPA therapy, i.e. gastrointestinal disorders with diarrhoea and eosinophilic infiltration indicate the possible effects of MPA on epithelial cells [21]. In this study, we investigated the effects of MPA on human renal proximal and distal tubular epithelial cells in vitro. In renal transplant recipients, therapeutically given MPA reaches the tubular epithelium in the graft at the basolateral cell membranes and therefore may affect tubular cell metabolism. The concentrations of MPA used in this in vitro study are in the range of blood concentrations obtained in vivo (C_max = up to 26 μg/ml) [22].

The effect of MPA on lymphocyte proliferation and the molecular mechanisms underlying this effect are
Table 2. Effect of MPA and MPA plus guanosine on cytokine-induced RANTES expression

<table>
<thead>
<tr>
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<th>PTC</th>
<th>Alteration</th>
<th>DTC</th>
<th>Alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>5.0 ± 3.7*</td>
<td>6.8 ± 5.1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytomix</td>
<td>536.7 ± 83.7</td>
<td>418.6 ± 102.8</td>
<td>838.1 ± 138.9*</td>
<td>+100.2%</td>
</tr>
<tr>
<td>+ MPA 50 µM</td>
<td>905.7 ± 218.8**</td>
<td>+68.7%</td>
<td>575.4 ± 81.7*</td>
<td>+37.5%</td>
</tr>
<tr>
<td>+ MPA 50 µM + gua</td>
<td>609.7 ± 108.1*</td>
<td>+13.5%</td>
<td>789.5 ± 105.4*</td>
<td>+88.6%</td>
</tr>
<tr>
<td>+ MPA 25 µM</td>
<td>923.6 ± 262.2*</td>
<td>+72.1%</td>
<td>546.6 ± 41.9*</td>
<td>+30.6%</td>
</tr>
<tr>
<td>+ MPA 25 µM + gua</td>
<td>622.0 ± 68.1**</td>
<td>+15.9%</td>
<td>826.5 ± 94.8*</td>
<td>+97.4%</td>
</tr>
<tr>
<td>+ MPA 2.5 µM</td>
<td>777.3 ± 113.6**</td>
<td>+44.8%</td>
<td>459.1 ± 25.3*</td>
<td>+18.5%</td>
</tr>
<tr>
<td>+ MPA 2.5 µM + gua</td>
<td>585.6 ± 147.8*</td>
<td>+9.1%</td>
<td>456.1 ± 38.6</td>
<td>+9.7%</td>
</tr>
<tr>
<td>+ MPA 0.25 µM</td>
<td>527.1 ± 139.1</td>
<td>−1.8%</td>
<td>482.8 ± 25.6</td>
<td>+8.9%</td>
</tr>
<tr>
<td>+ MPA 0.25 µM + gua</td>
<td>536.4 ± 50.1</td>
<td>−0.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPA 25 µM (alone)</td>
<td>7.5 ± 1.1**</td>
<td>6.1 ± 4.7**</td>
<td></td>
<td></td>
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<tr>
<td>Guanosine 100 µM</td>
<td>4.8 ± 0.7**</td>
<td>3.7 ± 2.2**</td>
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</tbody>
</table>

Data are represented as pg RANTES per 10^5 cells (means ± SD, n = 4–5, + P < 0.05 vs cytomix, ++ P < 0.01 vs cytomix, *P < 0.05 gua vs w/o gua). In addition data were calculated by taking the cytomix-stimulated condition as 100% (alterations in per cent vs cytomix), gua, guanosine, 100 µM.

![Chart](chart.png)

Fig. 2. Effect of dexamethasone on the cytokine-induced RANTES expression of PTC and DTC. Data are represented as pg RANTES per 10^5 cells (means ± SD, n = 3, * P < 0.01 vs basal, ++ P < 0.05 vs mix, +++ P < 0.01 vs mix). mix = cytomix, mix + dexa = cytomix plus dexamethasone.

Table 3. Effect of MPA on surface expression of HLA-DR and ICAM-1

<table>
<thead>
<tr>
<th></th>
<th>HLA-DR</th>
<th></th>
<th>ICAM-1</th>
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<tbody>
<tr>
<td></td>
<td>PTC</td>
<td>DTC</td>
<td>PTC</td>
<td>DTC</td>
</tr>
<tr>
<td>Basal</td>
<td>−88.1 ± 11.0%</td>
<td>−75.5 ± 5.0%</td>
<td>−71.3 ± 9.2%</td>
<td>−75.5 ± 5.0%</td>
</tr>
<tr>
<td>Cytomix</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>+ MPA 50 µM</td>
<td>+25.1 ± 28.9%</td>
<td>+5.7 ± 18.3%</td>
<td>+13.0 ± 13.7%</td>
<td>+12.8 ± 6.7%</td>
</tr>
<tr>
<td>+ MPA 25 µM</td>
<td>+12.3 ± 41.0%</td>
<td>+4.5 ± 13.1%</td>
<td>+9.0 ± 17.1%</td>
<td>+9.0 ± 13.2%</td>
</tr>
<tr>
<td>+ MPA 2.5 µM</td>
<td>+10.9 ± 27.4%</td>
<td>+8.4 ± 15.1%</td>
<td>+10.1 ± 18.1%</td>
<td>+9.0 ± 11.1%</td>
</tr>
<tr>
<td>+ MPA 0.25 µM</td>
<td>+3.3 ± 27.9%</td>
<td>+5.2 ± 18.2%</td>
<td>+4.3 ± 11.8%</td>
<td>+2.0 ± 8.2%</td>
</tr>
<tr>
<td>+ dexamethasone</td>
<td>−40.8 ± 11.2%</td>
<td>−53.5 ± 27.0%</td>
<td>−18.7 ± 6.2%</td>
<td>−10.7 ± 3.0%</td>
</tr>
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</table>

Median of fluorescence intensity in each experiment was calculated. The data were expressed by taking the cytomix-stimulated condition as 100% (means ± SD, n = 4–5). A strong induction of the antigens by cytomix compared to the basal level could be seen. No significant differences in antigen expression could be detected between cytomix and cytomix plus MPA. Dexamethasone significantly decreased the cytomix-stimulated expression of HLA-DR (P < 0.05) but not of ICAM-1.

An inhibitory effect on cell proliferation of smooth-muscle, endothelial, and mesangial cells has been described [25,26], whereas Eugui et al. found no inhibition of endothelial and fibroblast cell proliferation [27]. In mice, MPA prevented DNA synthesis in lymph nodes but not in testicular germinal cells and basal epithelial cells of the small intestine [7,28]. In our study, we could demonstrate that the total number of human tubular epithelial cells decreased after MPA treatment due to an inhibition of tubular cell proliferation. The abolition of this effect by guanosine supplementation conforms to the described action mode of MPA.

In renal and cardiac allografts, the chemokine RANTES is expressed at high levels 1 week after transplantation and correlates with the influx of T cells and macrophages during rejection [8,29–31]. The expression and secretion of RANTES by cytokine-treated tubular epithelial cells and other cell types, and the inhibition by glucocorticoids has been shown in different studies [14,32–34]. Therefore our interest was to evaluate the effect of MPA on this cytokine-induced chemokine expression. Incubation of tubular cells with cytokines and MPA resulted in a synergistic upregulation of RANTES secretion, whereas dexamethasone well described [7,23]. In case of the action mechanism of MPA by specific inhibition of purine de novo synthesis, the antiproliferative effect should be lymphocyte-specific. In-vitro studies showed the total inhibition of DNA synthesis in mitogen-stimulated lymphocytes [24]. MPA depletes GTP in human peripheral blood monocytes, but not in neutrophils [7]. Nevertheless,
inhibited the cytomix-induced RANTES production. This synergistic effect of cytokines plus MPA seems not to be restricted to tubular epithelial cells since it could be demonstrated in human endothelial cells (unpublished data). The mechanisms of the synergistic effect of MPA on RANTES expression are not currently known. Recent data revealed that an inhibition of nitric oxide (NO) results in an upregulation of LPS-stimulated RANTES expression [34]. Others described the inhibition of cytokine-induced NO production by MPA, and suggested that the activity of NO synthase is dependent on cellular GTP level [35]. We found a partial abolition of the synergistic effect by additional incubation with guanosine. We postulate that inhibition of tubular cell NO production by MPA is responsible for the synergistic upregulation of RANTES through MPA and the cytomix. The clearest benefit of MPA is in the post-transplantation period, where a combination therapy of MPA with cyclosporin A and steroids reduces biopsy-proven acute rejection [1].

A possible downregulation of RANTES expression during rejection episodes in patients treated with a combination therapy may be due to the action of steroids only. Therefore the synergistic effect of cytokines and MPA may not result in clinical implications during the treatment of rejection episodes. Furthermore, the main effect of MPA is on leukocytes and the inhibition of their proliferation results not only in a reduced infiltration but also in a reduced release of cytokines in the transplant.

Recognition of foreign antigens by T cells requires the presence of human leukocyte antigens (HLA) on antigen-presenting cells. The avidity of this interaction may be increased by the expression of other cell surface proteins such as intercellular adhesion molecule-1 (ICAM-1) [12]. Moreover, antigen-independent interactions between T cells or monocytes and their target cells are essential for generating and affecting the immune response [9]. This cellular cross-talk requires adhesion molecules like ICAM-1 on the membranes of the target cells [9,12,36]. The relationship between ICAM-1 and HLA (class II antigens) in immune responses is synergistic, and in cases of limiting HLA expression, ICAM-1 may be essential for antigen presentation [12]. De novo expression of HLA-DR and ICAM-1 by tubular epithelial cells during allograft rejection is well known [9,37,38]. Furthermore, the expression of these molecules by γIFN-stimulated tubular cells in culture has been described [9,12].

Since ICAM-1 and HLA-DR are co-expressed in transplant rejection and other inflammatory conditions in the human kidney, we studied whether MPA affects the stimulated expression of these antigens. In vitro, MPA has been shown to prevent the binding of activated human lymphocytes to activated endothelial cells, demonstrating its ability to inhibit recruitment and subsequent actions at the potential sites of rejection [7,40]. MPA has been described to block the glycosylation of glycoproteins involved in the adhesion of leukocytes to their target cells [5,28]. In contrast, MPA induced ICAM-1 expression on human endothelial cells, may be due to a marked GTP depletion and UTP increase, since both are responsible for the synthesis of glycoproteins [40]. MPA enhances TNF-α-induced VCAM-1 and E-selectin expression on endothelial cells by selectively increasing the mRNA-stability of these cell adhesion molecules [41]. In kidney-transplanted rats MPA reduced the number of cells positive for HLA-DR molecules [19]. Thomson et al. found no effect on the expression of cell activation molecules like HLA-DR on stimulated lymphocytes in vitro by MPA [42]. No significant effect of MPA on the cytokine-induced antigen expression of HLA-DR and ICAM-1 could be proven in our study. On the other hand, a significant inhibition of HLA-DR expression, but not ICAM-1 by dexamethasone was detectable. Other in vitro studies are in good agreement with these results, and showed that γIFN-induced HLA-DR expression can be inhibited by dexamethasone, whereas ICAM-1 expression was not significantly reduced [12,13].

These data demonstrate that apart from its immunosuppressive and antiproliferative effects on lymphocytes, MPA differentially affects the renal tubular epithelium by the inhibition of cell proliferation and the upregulation of cytokine-induced chemokine production. The clinical relevance of the synergistic upregulation of tubular RANTES synthesis seems to be minimized because MPA is used in combination with other immunosuppressive agents like steroids and is not as a single therapy. Furthermore, we showed that the elevated expression of HLA and ICAM-1 is not downregulated by MPA. In conclusion, the immunosuppressive actions of MPA appear to be independent from the effect on the tubular epithelium.

Acknowledgements. The authors thank Prof. Dr U. W. Tunn and Dr G. Nunez, Urological Department, Medical School Offenbach, Germany, for supply with human renal tissue, Marianne Haimerl for the excellent technical assistance, and Dr G. Oremek, Department of Internal Medicine, J.W. Goethe-University, Frankfurt am Main, is thanked for the measurement of LDH. This work was supported by a grant of the Fresenius Stiftung.

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Received for publication: 12.5.99
Accepted in revised form: 15.9.99