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Inosine 5′-monophosphate dehydrogenase inhibition by mycophenolic acid impairs maturation and function of dendritic cells☆

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Abstract

Background: The mechanism of action of mycophenolic acid (MPA) has been described as a blockade of inosine 5′-monophosphate dehydrogenase (IMPDH) and is thought to selectively influence T- and B-lymphocytes due to their strong dependency on guanine nucleotides synthesized via the de novo purine synthesis pathway. Recent evidence suggests MPA to affect antigen-presenting cells.
Methods: Using $CD14^+$ derived human dendritic cells (DC) we have investigated the effects of MPA on differentiation, maturation and function and studied intracellular nucleotide content and IMPDH activity.
Results: GTP content and IMPDH activities of DC were strongly and dose-dependently decreased when MPA was present during the entire culture period or was added after the fifth (immature DC) or the seventh (mature DC) day of culture. Concurrent to low GTP levels, a dose-dependent reduction in the expression of CD80, CD86, CD40, CD54 and CD83 was seen which was accompanied by a decreased capacity of DC to stimulate T-cells. Our data for the first time shows a direct effect of MPA on the maturation and function of human $CD14^+$ derived DC, indicates a role of IMPDH and a dependency on the de novo purine synthesis pathway.

Keywords: Dendritic cells; Mycophenolic acid; Immunosuppression; IMPDH; GTP

1. Introduction

Dendritic cells (DC) are considered professional antigen presenting cells (APC) controlling immunity via their interaction with lymphocytes. In vivo, DC are widely distributed in tissues; capture and process antigen; and, upon stimulation seen in, for example, inflammation or acute infection, undergo a process of maturation. During this maturation phase DC migrate to lymphoid tissues where a spectrum of primary immune responses may be induced [1]. More recent evidence has focused on the potential of DC to induce tolerance. In this scenario, DC are thought to create peripheral tolerance via the induction of regulatory T-cells and deletion of reactive lymphocytes [2]. Thus, DC enable a wide spectrum of immune responses ranging from the induction of tolerance and prevention of autoimmunity to the initiation of lymphocyte response leading to the protection against infection, cancer or allograft rejection [3,4].

Considering their pivotal role in immune responses, it is not surprising that DC have moved into the focus of many investigators' attention. In the field of transplantation, immunosuppressive drugs have for the most part been developed to target lymphocyte function. Yet, the initiation of primary immune responses has been shown to result from the interaction of APC and T-lymphocytes. The discussion of immunosuppressive effects based on T-cells alone may be incomplete. In this context, Mehling et al. [5] have reported mycophenolate mofetil (MMF; CellCept®), the morpholinoethylester of mycophenolic acid (MPA) to directly affect DC function in a murine model. This finding prompted our

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studies on an effect of MPA on human DC and on a possible mechanism of action.

MMF is a well established agent and is included in a number of clinically relevant immunosuppressive regimens to prevent rejection in transplant recipients. Currently, in vitro and in vivo studies are investigating the value of MMF in chronic allograft rejection [6]. Furthermore, the substance has shown efficacy in the treatment of autoimmune diseases [7–19]. MMF is usually administered at a fixed dosage of 1 g twice daily, leading to plasma concentrations between 5 and 10 μM of the active metabolite MPA [20].

The agent's mechanism of action is best understood as a selective, non-competitive and reversible inhibition of inosine 5′-monophosphate dehydrogenase (IMPDH) [21]. The function of this enzyme represents the first committed step in the biosynthesis of guanosine 5′-monophosphate (GMP) from inosine 5′-monophosphate (IMP). A schematic overview of GTP metabolism is depicted in Fig. 1. IMP in turn may originate either from the de novo purine pathway [first committed step catalysed by 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase] or from the salvage of hypoxanthine by hypoxanthine-phosphoribosyltransferase. The salvage of guanine is catalysed by guanine-phosphoribosyltransferase into GMP and is not dependent on IMPDH. Thus, in case of IMPDH inhibition by MPA a dependency on the de novo purine synthesis pathway typical for lymphocytes predisposes T- and B-cells to GTP depletion and failure to synthesize RNA/DNA which is a prerequisite to a proliferative response upon antigenic or mitogenic stimulation. Even further, the dependency on the de novo synthesis is all the more severe since guanine nucleotide depletion (as well as adenine nucleotide accumulation) will lead to a decrease in the function of 5-PRPP synthetase and thus create a general decrease in de novo purine synthesis [22]. The selectivity of the immunoregulatory effects of MPA is further enhanced by a five times increased sensitivity of the type II isoform of IMPDH which is typically expressed in antigen or mitogen activated T- and B-lymphocytes [23,24].

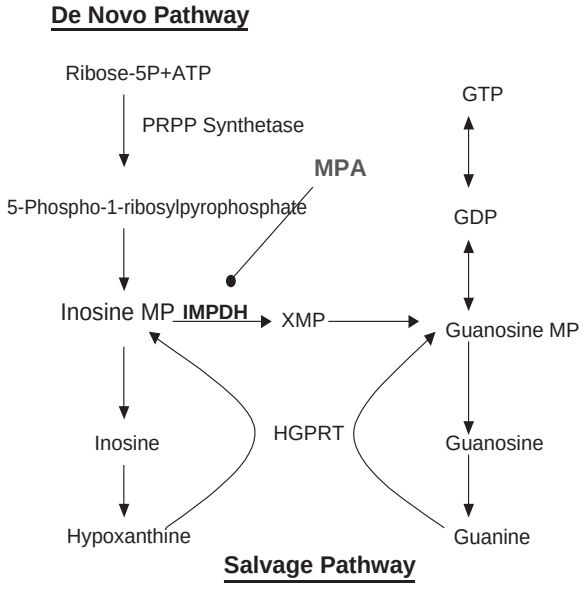

Fig. 1. Inosine monophosphate dehydrogenase (IMPDH) and the substrate inosine monophosphate take a central position in purine metabolism. The simplified scheme focuses on the synthesis of guanine nucleotides from metabolites of the de novo and the salvage pathway (HGPRT=hypoxanthine guanosine phosphoribosyl transferase; XMP=xanthosine monophosphate; PRPP=phosphoribosyl pyrophosphate).

In the present study, we have investigated the effect of MPA on the differentiation of monocytes to immature DC and on the maturation of immature to mature DC. As previously described, we have found MPA to induce an inhibition of the expression of CD83 and costimulatory molecules (CD80, CD86 and CD40) at the cell surface of mature DC [5,25]. On a functional level, mature DC cultured in the presence of MPA exhibited a reduced lymphocyte stimulatory capacity. MPA decreases the activity of IMPDH in immature and mature DC leading to a dose-dependent decrease in intracellular GTP pools. Thus, we have been able to show a direct effect of MPA on human DC. The depletion of intracellular GTP pools and IMPDH inhibition during MPA influence point to a similar mechanism of action as described in lymphocytes and point to a role of IMPDH in DC maturation. Furthermore, DC exhibits a strong dependency on the de novo pathway of purine synthesis.

2. Materials and methods

2.1. Isolation of $CD14^+$ PBMC and differentiation into mature DC

Healthy donors were recruited by the University Clinic of Surgery, Vienna. Venipuncture and apheresis were performed after written informed consent. PBMC were isolated from fresh buffy coats using a Ficoll-Hypaque (Amersham Pharmacia Biotech AB, Upsala, Sweden) density gradient centrifugation. $CD14^+$ cells were isolated using an immunomagnetic method. PBMC were labelled with a magnetic bead-conjugated anti CD14 monoclonal antibody (CD14 Microbeads, Miltenyi Biotec, Bergisch Gladbach, Germany) and passed over a separation column according to the manufacturer's instructions (BS/CS separation column, Miltenyi Biotec, Bergisch Gladbach, Germany). Cell purity was assessed by flow cytometry and was >97%. $CD14^+$ cells were cultured in growth medium (GM; RPMI-1640 medium, 10% heat-inactivated FCS, and 50 μg/ml gentamycin; all from Life Technologies, Grand Island, NY, USA) at 1×10^6 $CD14^+$ cells/ml. GM was supplemented with 1000 U/ml GM-CSF (Novartis Pharmaceuticals, Basel, Switzerland) and 1000 U/ml IL-4 (Strathmann PBH, Hamburg, Germany). Incubation was carried out in a 5% CO_2 humidified atmosphere at 37 °C. On day 2, cultures were replenished with half the volume of fresh growth medium and cytokines. On day 5, cells

were washed and resuspended in growth medium supplemented with 1000 U/ml GM-CSF and 100 ng/ml TNFα (kindly provided by Dr. HR Alexander Jr., National Cancer Institute, Bethesda, MD, USA). This protocol has previously been published by our group in the context of a clinical trial in solid organ cancer [26] and in the context of an autologous human system [27]. Cells were cultured in the presence of MPA (Sigma, St. Louis, MO, USA) at graded concentrations between 1 μM and 7.5 μM or vehicle (methanol), both of which were added at every change of medium. In order to test the specificity of MPA effects guanosine (Sigma, St. Louis, USA) at 100 μM was added to cultures when indicated.

2.2. Isolation of T-cells

T-cells were isolated by an immunomagnetic method (Pan T cell Isolation Kit, Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Using a cocktail of hapten-conjugated CD11b, CD16, CD19, CD36 and CD56 antibodies (Ab) in combination with a magnetic bead-conjugated anti-hapten antibody, all cells apart from T-cells were depleted. Flow cytometric analyses of T-cells showed a purity above 95%. Cryopreservation was carried out at −70 °C in a mixture of RPMI 1640 medium containing 10% DMSO (Sigma, St. Louis, USA) and 20% FCS.

2.3. Analysis of DC phenotype by flow cytometry

Aliquots of 2×10^5 cells were incubated with the following FITC or PE conjugated mouse anti-human monoclonal Ab (mAb): CD83 (IgG2b, Immunotech, Marseille, France), CD1a (IgG1, Immunotech, Marseille, France), CD80 (IgG1, Immunotech, Marseille, France), CD86 (IgG2b, Immunotech, Marseille, France), CD40 (IgG1, Immunotech, Marseille, France), HLA-ABC (IgG1, BD PharMingen, San Jose, CA, USA), HLA-DR (IgG1, BD PharMingen, San Jose, CA), CD11c (IgG1, Immunotech, Marseille, France), CD14 (IgG1, BD PharMingen, San Jose, CA) and ICAM-1 (IgG1, R&D, UK) and appropriate isotype controls. At the end of a 30-min incubation period cells were washed, and counter-stained with propidium iodide (Sigma, St. Louis, USA). After another washing step, cells were analysed using an Epics XL flow cytometer (Coulter, Krefeld, Germany) equipped with Expo 32 ADC software. A minimum of 5000 propidium iodide-negative cells per sample was analysed.

2.4. Determination of intracellular nucleotide pools

Five million cells (monocytes at day 0, DCs at days 2, 5, 7 and 10 of culture) were washed and resuspended in 130 μl of ice-cold Dulbecco's phosphate-buffered saline (Gibco BRL, USA) without calcium and magnesium. After removing 30 μl for the measurement of protein content 100 μl of ice-cold 0.2 M perchloric acid was added to lyse the cells. Lysed cells were neutralized with 0.5 M K_2HPO_4 and centrifuged. 100 μl of the supernatant was analysed by HPLC according to Weigel et al. [28]. The intraassay coefficient of variation was 7.6%, the interassay coefficient of variation was 10.3%.

2.5. Determination of IMPDH activity

Five million DC were washed extensively and mixed with 50 μl of human AB-serum (Bio Whittaker, Walkersville, MD, USA) alone or 50 μl of human AB-serum containing MPA at final concentrations of 5 or 7.5 μM. 5.7 μl of ^{14}C-hypoxanthine (55 mCi/mmol, Amersham Pharmacia, Buckinghamshire, UK) was added prior to incubation at 37 °C for 150 min. Cells were washed and resuspended in 50 μl of cold 0.9% sodium chloride after which cell lysis was induced by the addition of 20 μl of 0.2 M perchloric acid. Centrifugation at $400 \times g$ for 5 min yielded the supernatant, 50 μl of which was neutralized by the addition of 0.5 M triethanolamine and 2.0 M potassium carbonate. The neutral lysate was again centrifuged, the supernatant spotted onto a PEI cellulose chromatography plate (Merck, Germany) with carrier xanthosine 5′-monophosphate, guanosine 5′-monophosphate, inosine 5′-monophosphate, and hypoxanthine and allowed to dry. The plate was then developed in an ascending system in 0.86 M potassium phosphate. The spots were detected under UV-light and cut out. Radioactivity was measured in a liquid scintillation counter (Wallac 1410, Turku, Finland). Results are expressed as pmol of ^{14}C-xanthosine 5′-monophosphate and ^{14}C-guanosine 5′-monophosphate formed per 10^6 DC/h. All measurements were done in triplicate. The intraassay coefficient of variation was 12% while the interassay coefficient of variation was 16.2%.

2.6. Proliferation assays

DC were cultured until day 7 in the presence of MPA at concentrations ranging from 0 to 7.5 μM and, after thorough washing, were applied in graded numbers to 1×10^5 freshly thawed PBMC or T-cells adding up to a final volume of 200 μl. After a 5-day culture period in RPMI 1640 supplemented with 10% FCS and 50 μg/ml gentamycin in 96-well round bottom plates, 1 μCi of [^{3}H] thymidine was added for an 18 h incubation period. Cells were harvested and [^{3}H] thymidine incorporation was measured in a liquid scintillation counter (Wallac 1450 Microbeta, Turku, Finland) and values are given as mean counts per minute for triplicate wells. Experiments were carried out in both autologous and allogeneic settings.

MPA may be released into fresh media by DC that have been cultured in the presence of the substance and may cause a direct effect on lymphocytes used in the proliferation assay. In order to rule out this possibility, we have established controls containing DC (cultured in the presence

of 7.5 μM MPA) seeded into the media used for the proliferation assays. A HPLC method to detect MPA [29] was used to verify that no significant amounts of MPA were present during the co-incubation at 2 h and 48 h.

2.7. Statistical analyses

To detect statistical significance of differences in MFI, intracellular GTP pools, IMPDH activities and proliferation rates of T-cells, a two-tailed Student's *t* test for unpaired data was employed. A *p*-value of <0.05 was considered significant.

3. Results

3.1. MPA leads to a decreased expression of T-cell stimulatory molecules and maturation markers on the DC surface

APC function relies on the expression of immunomodulatory molecules crucial for the activation of T-cells. To investigate the influence of MPA on DC phenotype we analyzed surface expression of T-cell stimulatory molecules and maturation markers of DC by flow cytometry on day 7 of culture in the graded presence or absence of MPA. Control cultures yielded phenotypically mature DC in terms of their expression of HLA-DR, CD1a, CD80, CD86, CD40, CD83 and CD54. Further phenotypical and functional analyses of DC generated in this protocol have previously been published by our group [26,27]. Table 1 shows the mean fluorescence intensity (MFI) and the surface molecule expression in terms of % positive cells. CD83, CD40, CD80, CD86 and CD54 showed a markedly reduced, dose-dependent expression when MPA at concentrations of 1 μM, 2.5 μM, 5 μM, or 7.5 μM was present during the culture period from monocytes to mature DC. This down-regulation was also seen as a trend in HLA-DR expression and was missing for the markers CD11c and CD1a. When Guanosine (Gua) was added together with MPA from the beginning of the culture period, the inhibitory effects were neutralized and the MFIs seen were comparable to those cultures where no MPA had been added. Furthermore, when MPA was washed out of DC culture medium before day 5 the expression of T-cell stimulatory molecules was similar to those treated with vehicle. When, on the other hand, MPA was added on day 5 and cells were analysed 48 h later, the above described down-regulation of surface markers was very similar to cells cultured from the beginning with MPA as specified in Table 1 (data not shown).

3.2. DC cultured in the presence of MPA show dose-dependent functional defects concerning their T-cell stimulatory capacity

In order to obtain information on DC function under the influence of MPA, a proliferation assay was used to assess

Table 1
DC propagated in the presence of increasing concentrations of MPA until day 7 of the culture period show a dose-dependent reduction in the expression of T-cell stimulatory molecules

CD	0 μM MPA	1 μM MPA	2.5 μM MPA	5 μM MPA	7.5 μM MPA
	Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.
CD86 (MFI)	147.4±10.0	134.4±8.9	99.9*±13.0	88.7*±10.6	54.1*±9.5
CD86 (%+cells)	99.0±2.1	97.0±2.0	98.0±1.0	99.0±1.0	98.3±5.2
CD80 (MFI)	222.3±18.4	201.3±15.0	182.3±10.9	149.0*±10.7	98.0*±8.6
CD80 (%+cells)	99.2±3.2	98.3±2.1	97.3±2.1	99.0±1.0	94.8±2.0
CD40 (MFI)	64.8±7.2	63.3±5.9	54.3*±7.7	44.4**±8.6	39.9**±5.2
CD40 (%+cells)	99.4±5.2	97.1±1.2	96.0±4.0	97.2±4.0	98.0±6.0
CD54 (MFI)	110.4±10.1	92.3±4.5	73.7*±6.2	57.8*±5.8	42.9**±3.7
CD54 (%+cells)	99.0±4.0	99.5±1.0	97.1±3.0	98.2±3.2	94.3±4.2
CD83 (MFI)	79.7±5.2	67.3*±3.3	55.4*±8.1	43.0*±3.8	36.1*±5.8
CD83 (%+cells)	86.5±2.0	87.2±4.0	78.3±5.0	81.2±4.2	76.3±9.0
MHCII (MFI)	543.0±15.0	522.0±12.0	487.0±18.0	510.0±9.0	440.0±16.0
MHCII (%+cells)	99.4±7.0	99.2±1.0	97.0±2.0	96.0±3.0	98.7±2.0
MHCI (MFI)	302.0±12.0	289.0±13.0	320.0±17.0	289.0±6.0	270.0±7.0
MHCI (%+cells)	99.5±4.0	99.1±0.4	97.2±4.1	98.2±2.0	98.2±2.0
CD11c (MFI)	52.2±7.0	44.4±5.0	49.2±15.0	51.4±9.0	45.9±12.0
CD11c (%+cells)	99.2±1.0	98.5±2.0	99.8±0.2	96.2±1.0	99.8±2.0
CD1a (MFI)	52.9±3.0	53.2±8.0	55.6±14.0	45.2±4.0	48.5±8.0
Cd1a (%+cells)	97.7±1.0	99.5±0.3	98.4±0.2	99.9±0.4	98.5±2.0

Results are expressed as the mean fluorescence intensity (MFI) of the entire population.
Three experiments from individual donors have been compiled and the MFI and the standard deviation (S.D.) are depicted. Furthermore, the percentage of positive cells for each investigated marker (%+cells) is shown in the same fashion. Triplicates were tested for statistical significance comparing each MPA dose to the control (0 μM MPA).
* $p<0.05$.
** $p<0.01$.

T-cell stimulatory capacity. Fig. 2 shows the decreased T-lymphocyte reaction upon stimulation with allogeneic DC cultured in the presence of MPA. MPA given at the highest dosage of 7.5 μM led to an approximately 50% decrease in observed CPM rates as compared to vehicle treated controls. The functional defect was seen as dose-dependent (Fig. 2). As an additional control group DC cultured in the presence of 100 μM Gua and 7.5 μM MPA were used. The addition of Guanosine (Gua) led to an abrogation of the functional defect (Fig. 2). Data shown is one representative experiment of five.

3.3. MPA induces reduced GTP levels during monocyte differentiation and DC maturation

Having established both phenotypical and functional consequences after MPA incubation, further studies focused on possible mechanisms of action. In B- and T-lymphocytes MPA is well known to induce an anergy in response to mitogenic stimulation due to the inability to synthesize GTP. We surmised that the phenotypical and functional inhibition seen in the previous experiments could be explained by similar changes in intracellular nucleotide pools.

Upon incubation with MPA intracellular GTP levels dropped dramatically and in a dose-dependent manner on day 2 of monocyte culture. At a maximum dose of 7.5 μM MPA cells showed only 7.2% (±4.7%) of GTP content as compared to controls. At days 5 and 7, 32.7% (±8.4%) and 48.9% (±15%), respectively, of control GTP levels were found at the maximum dosage; the decrease was dose-dependent (Fig. 3a). The mean intracellular concentration of GTP per protein at 0 μM MPA (100%) was 8.4 nmol/mg at day 2, 9.4 nmol/mg at day 4, and 8.1 nmol/mg at day 7.

Concurrent to GTP decline we could observe a parallel decline in GDP levels, while levels of ATP (Fig. 3b), UDP and CTP did not show any significant changes. The mean intracellular concentration of ATP per protein at 0 μM MPA (100%) was 36.6 nmol/mg at day 2; 51.2 nmol/mg at day 4 and 40.9 nmol/mg at day 7. Further experiments included discontinuation of MPA on days 2 and 5. In these experiments, GTP levels returned to control levels until day 7 of culture (data not shown).

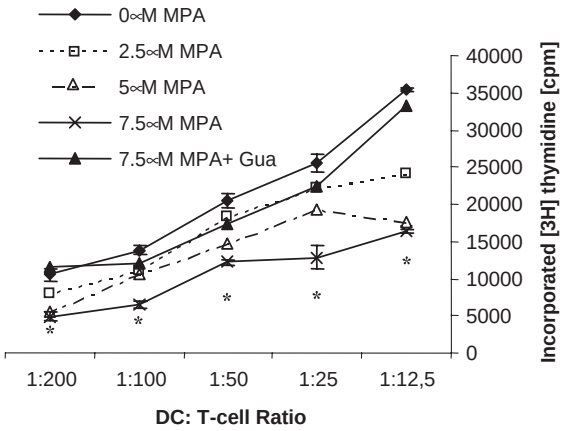

Fig. 2. DC cultured in the presence of MPA show an inhibition in T-cell stimulatory capacity. DC propagated in the presence of graded doses of MPA or vehicle for 7 days were used to stimulate allogeneic T-cells during a subsequent 5 day co-culture period. *$p<0.01$ for DC cultured in the presence of 7.5 μM MPA vs. control (0 μM MPA). As an additional control, DC cultured in 7.5 μM MPA in the presence of Gua were used. One representative experiment of five conducted is shown.

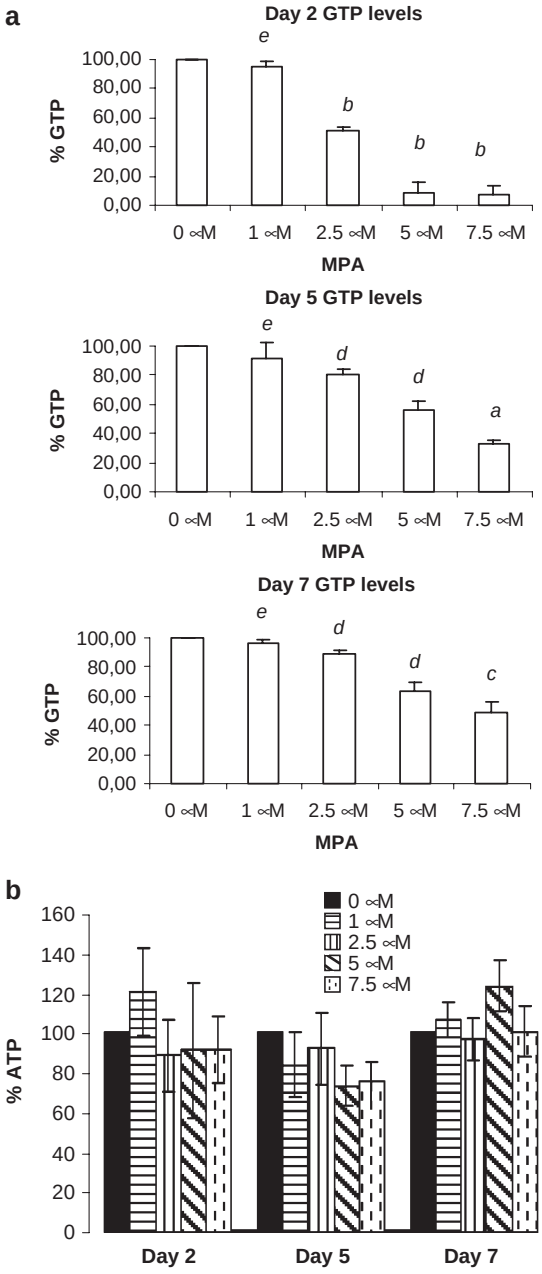

Fig. 3. (a) MPA leads to a strong decline in intracellular GTP pools throughout the culture period. MPA was administered in concentrations ranging from 0 μM to 7.5 μM from the beginning of the DC culture period and HPLC analysis was carried out on days 2, 5 and 7. The mean relative decrease of GTP of three independent experiments is depicted. [a]$p<0.001$, [b]$p<0.005$, [c]$p<0.01$, [d]$p<0.05$ vs. control; [e]not significant. (b) Percentual changes of intracellular ATP pools of the same 3 experiments depicted in Fig. 3a are shown. ATP was measured in parallel to GTP and the mean value of the control (0 μM MPA) is defined as 100%. In contrast to GTP pools, there were no significant increases or decreases found.

In a next step we cultivated DC until day 5 when cells showed an immature $CD83^{negative}$, $CD80/CD86^{low}$ phenotype and added MPA during the maturation phase until day 7. GTP levels dropped in a dose-dependent manner to 18.2% (±6.6%) of controls at the maximum of 7.5 μM of MPA (Fig. 4a). Furthermore, DC were matured until day 7 ($CD83^{high}$ and $CD80/CD86^{high}$; Table 1) washed and resuspended in growth medium supplemented with GM-CSF and MPA in graded concentrations and cultivated until day 10. In this setting, the effect of MPA was somewhat mitigated with 63.3% (±1.9%) of control levels at the maximum concentration of 7.5 μM; again we observed a dose-dependent decline (Fig. 4b). The mean intracellular concentration of GTP per protein at 0 μM MPA (100%) was 14.5 nmol/mg at day 7 and 8.7 nmol/mg at day 10.

Interestingly, we found a complete abrogation of the MPA effect when guanosine (Gua) was added exogenously at a dose of 100 μM concomitantly to MPA in both experimental settings (Fig. 4a, b).

3.4. IMPDH activity

In order to investigate an involvement of IMPDH a radionuclide assay measuring the enzyme products xanthosine 5′-monophosphate and guanosine 5′-monophosphate was performed, to determine the enzyme activity during the incubation with MPA or vehicle. IMPDH activity responded as a function of MPA concentration during the 150-min incubation period of the assay. One representative experiment performed in triplicate of four conducted for immature DC (day 5, Fig. 5a) and mature DC (day 7, Fig. 5b) is shown.

IMPDH showed a strong and dose-dependent inhibition by MPA. In mature DC the inhibition was at a maximum at a concentration of 7.5 μM with a 61% drop in activity. In comparison to mature DC, cells incubated and analyzed on day 5 of the culture period showed the same trend of enzyme inhibition. We found a 25% lower rate of enzyme activity at 7.5 μM MPA.

4. Discussion

CellCept® (MMF) has evolved not only as a major component of many immunosuppressive regimens in organ transplantation but has gained a considerable reputation in so-called “non-transplant uses” [12]. A growing number of reports investigating the effect of

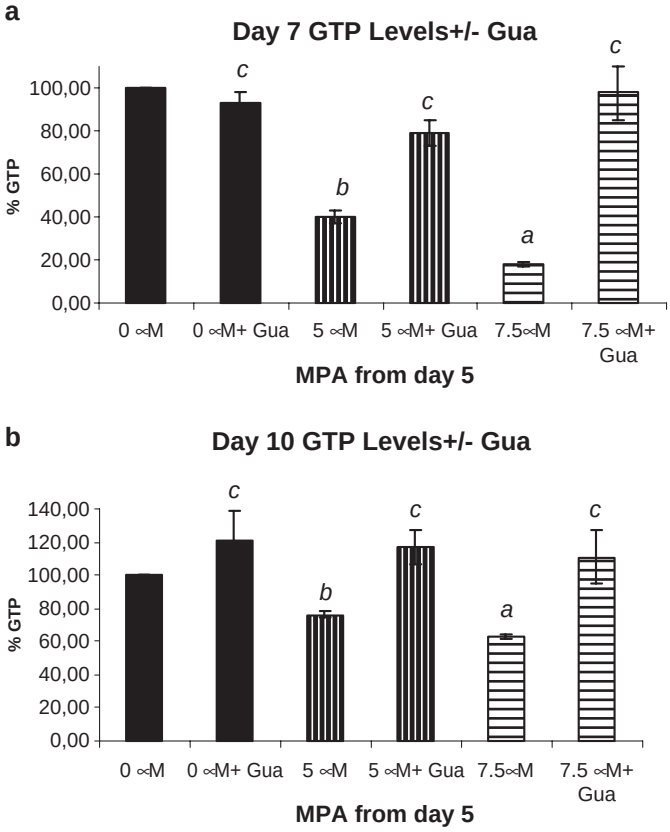

Fig. 4. MPA leads to a strong decline in intracellular GTP pools when (a) MPA was added on day 5 of culture and cells were analysed 2 days later (full maturation) for GTP content. Guanosine (Gua) was added at the same time and caused an abrogation of the MPA effect. (b) MPA was added on day 7 of culture and cells were analysed on day 10 for GTP content. The decline in GTP levels could be suspended by adding exogenous Gua together with MPA. The mean relative decrease of GTP in % of three independent experiments is depicted. [a] $p<0.01$, [b] $p<0.05$, [c] not significant vs. control.

MMF in autoimmune diseases such as glomerulonephritis and systemic lupus nephritis have been reviewed [19] and have received favourable commentary. Furthermore, the drug has been reported to be useful in the treatment of autoimmune neuromuscular disease [7,9,11,13,16–18], a number of autoimmune skin disorders [14], inflammatory bowel disease [10] and autoimmune hepatitis [8,15]. IMPDH inhibition, intracellular GTP depletion by MMF and the consequences in lymphocytes have been thoroughly described [6]. This paradigmatic view of MPA pharmacodynamics was supplemented by findings in a mouse model [5]: Mehling et al. showed an impairment of DC maturation and function by MMF. A more recent report by Colic et al. reported both functional and phenotypical responses of human DC to MPA [25]. In the present study, we have been able to confirm a direct effect of MPA on human DC. In addition, we have investigated the involvement of IMPDH, an enzyme yet undescribed in DC.

We show a direct in vitro effect of MPA on differentiating monocytes, immature and mature DC in several experimental settings: 1) MPA led to a very strong decline in intracellular GTP levels when present throughout the culture period. The decrease in GTP was also observed when monocyte-derived immature or mature DC were challenged. The specificity of this effect was demonstrated when GTP levels were restored to control levels by exogenously added Gua. 2) The reduction of intracellular GTP was corroborated by a decrease in IMPDH activity. The radionuclide assay demonstrated a reduced IMPDH activity both in immature and mature DC in the presence of MPA. 3) T-cell stimulatory molecules and maturation markers of mature DC were down-regulated concurrent with decreased GTP levels and IMPDH activity which presumably led to 4) a reduced capacity to stimulate the proliferation of T-cells.

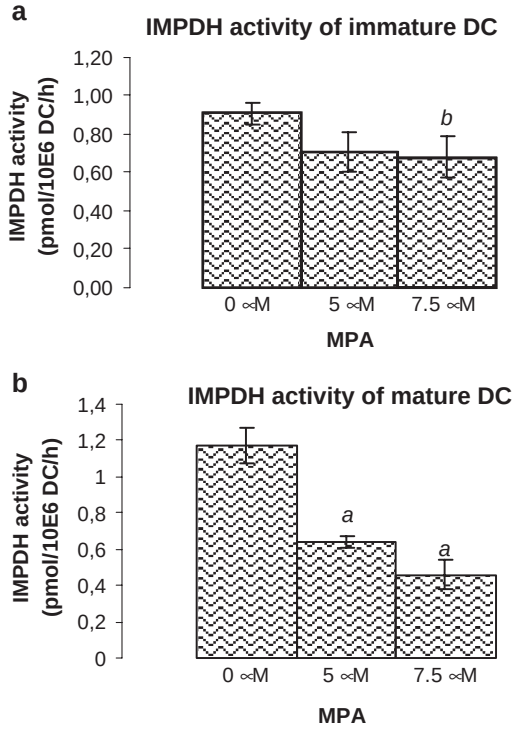

Fig. 5. (a) Inhibition of IMPDH activity in immature DC by MPA. DC were cultured until day 5 and incubated with MPA for 150 min after which cells were lysed and measurement of IMPDH products was performed. One representative experiment (performed in triplicate) of four conducted is shown. $^{b}p=0.04$ vs. control. (b) Inhibition of IMPDH activity in mature DC by MPA. DC were cultured until day 7 and incubated with MPA for 150 min after which cells were lysed and measurement of IMPDH products was performed. One representative experiment (performed in triplicate) of four conducted is shown. $^{a}p<0.001$ vs. control.

$CD14^{+}$-derived DC share many of the presently known molecular and functional aspects of in vivo developing DC [30,31]. Moreover, peripheral blood monocytes as non-dividing DC precursors are presently often used for clinical protocols of DC immunotherapy due to their easy accessibility [26]. Thus, monocyte-derived DC stand out as an important and practical model for our investigations.

The decrease in IMPDH activity and a strong intracellular GTP drop during MPA incubation stand at the core of our findings. Monocytes have been described as sensitive to MPA treatment [21]. The effects of MPA present from the beginning of the culture period may, in this experimental setting, be explained by affecting differentiating monocytes and leading to an impairment of DC differentiation and maturation as a late consequence. Thus, it was most interesting to find dropping GTP levels and IMPDH inhibition in immature and mature DC upon treatment with MPA after the fifth day of culture and previously unchallenged by MPA. When compared to GTP levels and IMPDH inhibition of lymphocytes in similar experimental settings the measure of guanine nucleotide decline and IMPDH inhibition was similar [32]. Importantly, this study demonstrates that indeed immature and mature DC are affected by MPA. In contrast to the previous report in the human system [25], we have been able to rule out the possibility that an initial defect in monocytes is responsible for all further effects seen during the culture to DC.

The up-regulation of costimulatory molecules and the maturation marker CD83 was inhibited under MPA influence. The activation state of APC has been described to differentiate between T-cell activation and tolerance [33]. In vivo, GTP depletion in lymphocytes has been shown to inhibit lymphocyte response in terms of proliferation of reactive T-cell clones [34]. GTP depletion in DC may result in changes of their antigen presenting characteristics due to reduced expression of immunomodulatory molecules. In line with previously published findings both in the murine [5] and the human [25] system the in vitro data in this report points to a functional consequence of the observed phenomena: MLR assays have been used to prognosticate graft versus host disease and allograft rejection in vivo [35]. The inhibition of T-cell stimulatory capacity via defects

inflicted by MPA thus adds to the importance of our findings.

Another mechanism for the reduced co-stimulation by monocyte-derived DC of lymphocytes may be a reduced glycosylation of surface molecules. Although this idea is still speculative in view of DC, a reduced glycosylation of surface molecules on lymphocytes has been well documented by Allison and Eugui [6]. Changes in the glycosylation of surface molecules under MPA have also been shown in prior work of the laboratory of Bertalanffy et al. on human umbilical vein endothelial cells [36]. More specific for DC it has been demonstrated that CD83 is glycosylated and that glycosylation changes the binding of antibodies [37]. It is in view of these findings that DC phenotype and function under MPA influence require further investigations that go beyond the scope of this study.

In conclusion, we report a direct effect of MPA on human monocyte-derived dendritic cells. IMPDH inhibition and intracellular GTP depletion by MPA reveal the enzyme IMPDH as an important factor in the maturation and function of monocyte-derived DC. We have thus not only shown dendritic cell purine metabolism to be dependent on the de novo synthesis of guanine nucleotides but also describe a potentially novel tool in the immunomodulation of dendritic cells.

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References

[1] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392(6673):245–52.
[2] Steinman RM, Nussenzweig MC. Inaugural article: avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. Proc Natl Acad Sci U S A 2002;99(1):351–8.
[3] Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767–811.
[4] Fong L, Engleman EG. Dendritic cells in cancer immunotherapy. Annu Rev Immunol 2000;18:245–73.
[5] Mehling A, Grabbe S, Voskort M, Schwarz T, Luger TA, Beissert S. Mycophenolate mofetil impairs the maturation and function of murine dendritic cells. J Immunol 2000;165(5):2374–81.
[6] Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. Immunopharmacology 2000;47(2–3):85–118.
[7] Schneider C, Gold R, Reiners K, Toyka KV. Mycophenolate mofetil in the therapy of severe myasthenia gravis. Eur Neurol 2001;46(2):79–82.
[8] Richardson PD, James PD, Ryder SD. Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. J Hepatol 2000;33(3):371–5.
[9] Mowzoon N, Sussman A, Bradley WG. Mycophenolate (CellCept) treatment of myasthenia gravis, chronic inflammatory polyneuropathy and inclusion body myositis. J Neurol Sci 2001;185(2):119–22.
[10] Miehsler W, Reinisch W, Moser G, Gangl A, Vogelsang H. Is mycophenolate mofetil an effective alternative in azathioprine-intolerant patients with chronic active Crohn's disease? Am J Gastroenterol 2001;96(3):782–7.
[11] Meriggioli MN, Rowin J. Treatment of myasthenia gravis with mycophenolate mofetil: a case report. Muscle Nerve 2000;23(8):1287–9.
[12] Jayne D. Non-transplant uses of mycophenolate mofetil. Curr Opin Nephrol Hypertens 1999;8(5):563–7.
[13] Hauser RA, Malek AR, Rosen R. Successful treatment of a patient with severe refractory myasthenia gravis using mycophenolate mofetil. Neurology 1998;51(3):912–3.
[14] Geilen CC, Orfanos-Boeckel H, Offermann G, Orfanos CE. Mycophenolate mofetil: a new immunosuppressive drug in dermatology and its possible uses. Hautarzt 2000;51(2):63–9.
[15] Dhawan A, Mieli-Vergani G. Mycophenolate mofetil—a new treatment for autoimmune hepatitis? J Hepatol 2000;33(3):480–1.
[16] Ciafaloni E, Massey JM, Tucker-Lipscomb B, Sanders DB. Mycophenolate mofetil for myasthenia gravis: an open-label pilot study. Neurology 2001;56(1):97–9.
[17] Chaudhry V, Cornblath DR, Griffin JW, O'Brien R, Drachman DB. Mycophenolate mofetil: a safe and promising immunosuppressant in neuromuscular diseases. Neurology 2001;56(1):94–6.
[18] Caponnetto C, Rossi E, Primavera A. Mycophenolate mofetil: a new immunosuppressive approach. Successful treatment in a case of myasthenia gravis associated with incomplete lupus erythematosus syndrome and hepatitis C virus infection. Eur Neurol 2001;46(1):53–4.
[19] Adu D, Cross J, Jayne DR. Treatment of systemic lupus erythematosus with mycophenolate mofetil. Lupus 2001;10(3):203–8.
[20] Weigel G, Griesmacher A, Zuckermann AO, Laufer G, Mueller MM. Effect of mycophenolate mofetil therapy on inosine monophosphate dehydrogenase induction in red blood cells of heart transplant recipients. Clin Pharmacol Ther 2001;69(3):137–44.
[21] Allison AC, Eugui EM. Purine metabolism and immunosuppressive effects of mycophenolate mofetil (MMF). Clin Transplant 1996;10:(1 Pt 2):77–84.
[22] Garcia RC, Leoni P, Allison AC. Control of phosphoribosylpyrophosphate synthesis in human lymphocytes. Biochem Biophys Res Commun 1977;77(3):1067–73.
[23] Nagai M, Natsumeda Y, Weber G. Proliferation-linked regulation of type II IMP dehydrogenase gene in human normal lymphocytes and HL-60 leukemic cells. Cancer Res 1992;52(2):258–61.
[24] Konno Y, Natsumeda Y, Nagai M, et al. Expression of human IMP dehydrogenase types I and II in *Escherichia coli* and distribution in human normal lymphocytes and leukemic cell lines. J Biol Chem 1991;266(1):506–9.
[25] Colic M, Stojic-Vukanic Z, Pavlovic B, Jandric D, Stefanoska I. Mycophenolate mofetil inhibits differentiation, maturation and allostimulatory function of human monocyte-derived dendritic cells. Clin Exp Immunol 2003;134(1):63–9.
[26] Stift A, Friedl J, Dubsky P, et al. Dendritic cell-based vaccination in solid cancer. J Clin Oncol 2003;21(1):135–42.
[27] Bachleitner-Hofmann T, Stift A, Friedl J, et al. Stimulation of autologous antitumor T-cell responses against medullary thyroid carcinoma using tumor lysate-pulsed dendritic cells. J Clin Endocrinol Metab 2002;87(3):1098–104.
[28] Weigel G, Griesmacher A, DeAbreu RA, Wolner E, Mueller MM. Azathioprine and 6-mercaptopurine alter the nucleotide balance in endothelial cells. Thromb Res 1999;94(2):87–94.
[29] Seebacher G, Weigel G, Wolner E, et al. A simple HPLC method for monitoring mycophenolic acid and its glucuronidated metabolite in transplant recipients. Clin Chem Lab Med 1999;37(4):409–15.
[30] Zhou LJ, Tedder TF. $CD14^+$ blood monocytes can differentiate into functionally mature $CD83^+$ dendritic cells. Proc Natl Acad Sci U S A 1996;93(6):2588–92.
[31] Kiertscher SM, Roth MD. Human $CD14^+$ leukocytes acquire the phenotype and function of antigen-presenting dendritic cells when cultured in GM-CSF and IL-4. J Leukoc Biol 1996;59(2):208–18.

[32] Griesmacher A, Weigel G, Seebacher G, Muller MM. IMP-dehydrogenase inhibition in human lymphocytes and lymphoblasts by mycophenolic acid and mycophenolic acid glucuronide. Clin Chem 1997;43(12):2312–7.
[33] Steinman RM, Turley S, Mellman I, Inaba K. The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 2000;191(3):411–6.
[34] Eugui EM, Mirkovich A, Allison AC. Lymphocyte-selective antiproliferative and immunosuppressive activity of mycophenolic acid and its morpholinoethyl ester (RS-61443) in rodents. Transplant Proc 1991;23(2 Suppl 2):15–8.
[35] Bishara A, Brautbar C, Nagler A, et al. Prediction by a modified mixed leukocyte reaction assay of graft-versus-host disease and graft rejection after allogeneic bone marrow transplantation. Transplantation 1994;57(10):1474–9.
[36] Bertalanffy P, Dubsky P, Wolner E, Weigel G. Alterations of endothelial nucleotide levels by mycophenolic acid result in changes of membrane glycosylation and E-selectin expression. Clin Chem Lab Med 1999;37(3):259–64.
[37] Lechmann M, Kremmer E, Sticht H, Steinkasserer A. Overexpression, purification, and biochemical characterization of the extracellular human CD83 domain and generation of monoclonal antibodies. Protein Expr Purif 2002;24(3):445–52.