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ORIGINAL ARTICLE

Effects of sotrastaurin, mycophenolic acid and everolimus on human B-lymphocyte function and activation

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Summary

Humoral rejection processes may lead to allograft injury and subsequent dysfunction. Today, only one B-cell-specific agent is in clinical use and the effects of standard and new immunosuppressant substances on B-cell activation and function are not fully clarified. The impact of sotrastaurin, mycophenolic acid and everolimus on human B-lymphocyte function was assessed by analysing proliferation, apoptosis, CD80/CD86 expression and immunoglobulin and IL-10 production in primary stimulated B cells. In addition, B-cell co-cultures with pre-activated T cells were performed to evaluate the effect of the different immunosuppressive agents on T-cell-dependent immunoglobulin production. Sotrastaurin did not inhibit B-cell proliferation, CD80/CD86 expression, and IgG production and had only minor effects on IgM levels at the highest concentration administered. In contrast, mycophenolic acid and everolimus had strong effects on all B-cell functions in a dose-dependent manner. All immunosuppressive agents caused decreased immunoglobulin levels in T-cell-dependent B-cell cultures. The data provided here suggest that mycophenolic acid and everolimus, but not sotrastaurin, are potent inhibitors of human B-lymphocyte function and activation.

Introduction

During the last two decades, the threat of T-cell-mediated kidney allograft rejection has been largely overcome due to the development of novel potent immunosuppressive drugs for both induction and maintenance therapies that primarily block T-cell activity. The incidence of acute cellular rejection decreased below 15% with current protocols [1,2]. Today B-lymphocytes as antigen-presenting cells and producers of antibodies against donor human leukocyte antigen are considered an important barrier to successful renal transplantation and long-term graft survival. B cells play important but not yet fully understood roles in different clinical situations as a pathogenic factor [3,4]. Donor-specific antibodies (DSA) are involved in early subacute humoral rejection processes, which cause early morbidity and graft loss. At later time points after kidney transplantation, antibody-mediated rejection (AMR) may occur alone or in combination with cellular reactions. Furthermore, the development of transplant glomerulopathy is associated with DSA and chronic AMR, and today there is no effective treatment for this condition leading to decreased long-term graft survival.

B cells as mediators of acute and chronic antibody-mediated injury are now recognized as therapeutic targets for standard and also novel immunosuppressive substances with different mechanisms of action. Current protocols for the treatment of AMR [1,2] include plasmapheresis, intraveneous immunoglobulin (IVIG) and rituximab, which is a chimeric monoclonal antibody against CD20 and the only B-cell-specific agent used in solid organ transplantation [5]. Nevertheless, the outcome of IVIG and plasmapheresis therapy is not satisfactory and the effectiveness of rituximab is critically discussed [6–8].

Newer approaches to the treatment of AMR involve terminal complement inhibition with eculizumab [9] and plasma cell depletion via proteasome inhibition with bortezomib [10,11]. Clinical experience with bortezomib in the context of AMR after renal transplantation is limited, but the results are promising [7,12,13]. As immunosuppressive drugs with specific mechanisms blocking B-lymphocyte activation and proven efficacy in transplantation are presently not available, AMR therapy remains a challenge and is being treated with agents that do not aim specifically at B cells. Recent studies imply that standard immunosuppressive agents have differential influence on humoral reactions [14,15]. It would therefore be of interest to elucidate the direct effects currently used agents have on B-cell function. As humoral immune reactions like immunoglobulin production fundamentally depend on T-cell help, it is also important to address the question how different immunosuppressants affect T-cell-dependent B-cell responses. We therefore present here a detailed evaluation regarding the *in vitro* effects of the new protein kinase C inhibitor sotrastaurin and standard substances mycophenolic acid (MPA) and everolimus on human B-lymphocyte function parameters including proliferation, apoptosis, cytokine production, immunoglobulin production independent and dependent on T-cell help and costimulatory surface molecule expression.

Materials and methods

Cells, culture conditions and immunosuppressive substances

Isolation of peripheral blood mononuclear cells (PBMC) from at least six healthy volunteers was performed by density gradient centrifugation over Ficoll-Paque (GE Healthcare, Uppsala, Sweden). Human B-lymphocytes were isolated by positive selection of cells expressing CD19 antigen using MACS® separation (Miltenyi Biotec, Bergisch Gladbach, Germany). Purities of typically more than 95% were obtained. The cells were cultured in complete medium [RPMI 1640 with 2 mM l-glutamine (Biochrome AG, Berlin, Germany) and 1% penicillin/streptomycin (Biochrome AG)], supplemented with 10% FCS (Biochrome AG) and incubated at 37 °C in 5% CO_2. The standardized and validated stimulation of human B cells was performed with CpG oligonucleotide type B-Human TLR9 ligand ODN2006 (2,5 μM; InvivoGen, San Diego, CA, USA), anti-human CD40 (1 μg/ml; R&D Systems, Abington, UK) and recombinant human IL-4 (10 ng/ml; Cellgenix, Freiburg, Germany) [16–18].

Sotrastaurin (gift from Novartis Pharma; diluted in DMSO) was used at final concentrations of 5, 50 and 500 nM. The MPA (Sigma-Aldrich, Hamburg, Germany; diluted in Ethanol) concentrations administered to the cells were 1, 10, 100, 1 and 10 μg/ml; everolimus (Sigma-Aldrich; diluted in DMSO) was used at final concentrations of 0.01, 0.1, 1, 10 and 100 ng/ml. The concentration range was chosen based on known trough levels of sotrastaurin (400–2000 ng/ml), MPA (1.0–3.5 μg/ml) and everolimus (3–8 ng/ml) and also on maximal effects on cells that were already observed using lower concentrations.

Human B-lymphocyte proliferation

Human B cells were washed and labelled with 2 μM carboxyfluorescein diacetate succinimidylester (CFSE) (Invitrogen, Karlsruhe, Germany) in PBS for 3.5 min at room temperature (RT). CFSE-labelled cells were washed three times, stimulated in complete medium and either treated with immunosuppressants or left untreated with solvent controls in round-bottom 96-well plates at 1.5×10^5 per well. After 6d, cells were harvested, and cell division of CD19+ cells was analysed by measuring CFSE intensity using flow cytometry.

Apoptosis of human B-lymphocytes

1.5×10^5 human B cells per well were seeded in flat-bottom 96 well-plates, stimulated and treated with sotrastaurin, MPA, everolimus or the adequate solvent as control. After 72 h, the cells were harvested, washed and analysed using the Annexin V-FITC apoptosis Detection kit I (BD Biosciences, Heidelberg, Germany). According to the manufacturer's instructions, cells were resuspended in binding buffer and incubated with Annexin V-FITC and propidium iodide for 15 min at RT in the dark. Following the addition of binding buffer, flow cytometric analysis was performed immediately.

Expression of surface markers CD80 and CD86 on human B-lymphocytes

Human B-lymphocytes were seeded at 1.5×10^5 per well in 96-well flat-bottom plates and either treated with immunosuppressants in graded concentrations or remained untreated with solvent controls for 72 h. After incubation, the cells were harvested, washed three times and stained with labelled a-CD80 and a-CD-86 monoclonal antibody (mAb) (BD Biosciences) according to the manufacturer's protocol. Samples were analysed by flow cytometry, and the geometric mean of CD80 and CD86 on CD19+ cells was determined.

Immunglobulin G and M and cytokine production of human B-lymphocytes

The supernatants from proliferation experiments described above were analysed for IgM and IgG concentrations with

ELISA quantification sets (Bethyl Laboratories, Inc., Montgomery, AL, USA). Plates were coated with coating antibody for 1 h at RT. After washing, blocking solution was added to the wells and incubated for 30 min at RT. Following a washing step, standard or sample was applied, incubated for 1 h at RT and washed again. Horseradish peroxidase conjugated detection antibody was added to the wells and incubated for 1 h at RT. After washing, 3,3′,5,5′-tetramethylbenzidine substrate solution was applied to the wells and the enzymatic colour reaction was allowed to develop at RT in the dark. The reaction was stopped by adding 0.18 M H_2SO_4 and measured on a plate reader at 450 nm. Supernatants from sotrastaurin experiments were also analysed for cytokine concentrations. IL-10 levels were measured with the Human IL-10 ELISA Kit II (BD OptEIA™; San Jose, CA, USA) according to the manufacturer's instructions.

T-cell-dependent human B-lymphocyte activation

Human T-lymphocytes were obtained after PBMC isolation by positive selection of cells expressing CD4 antigen using MACS® separation (Miltenyi Biotec, Bergisch Gladbach, Germany). 5×10^5 human T cells were cultured in complete medium and pre-stimulated for 48 h with 5 μg/ml a-CD28 mAb (BD Biosciences) and 100 U/ml recombinant human IL-2 (ebioscience, Frankfurt, Germany) in 24-well plates that had been coated with a-CD3 mAb (5 μg/ml; BD Biosciences). After harvesting and washing, 5×10^4 T-lymphocytes were cocultured as previously described [19] with 5×10^4 autologous human B-lymphocytes for 8d. 2.5 μg/ml ODN 2006 was administered to increase immunoglobulin production and immunosuppressive substances or solvent as control was added to the cultures. Additionally, human T cells alone and human B cells alone were incubated as controls under the same culture conditions with stimulus and immunosuppressants or solvent controls. The supernatants were examined for IgG and IgM levels as described above.

Statistical analysis

The statistical significance of the results ($^*P < 0.05$) was calculated with the non-parametric Wilcoxon matched pairs test applying PASW Statistics 18.

Results

Effects of immunosuppressants on human B-lymphocyte proliferation

The influence of immunosuppressive substances on human B-lymphocyte proliferation was analysed by measuring the proliferation rates of CFSE-labelled CD19+ B cells that were stimulated in the absence or presence of sotrastaurin, MPA or everolimus, resp. (exemplified in Fig. 1a). The protein kinase C (PKC) inhibitor sotrastaurin was not effective in inhibiting B-lymphocyte proliferation (Fig. 1b), instead caused even a mild increase in B-cell proliferation. Low concentrations of MPA did not have influence, but concentrations of 100 ng/ml and higher strongly and significantly inhibited B-lymphocyte proliferation (Fig. 1c). All everolimus concentrations administered to the B-lymphocyte cultures had a profound inhibitory effect on B-cell proliferation (Fig. 1d).

Effects of immunosuppressants on apoptosis of human B-lymphocytes

The effect of immunosuppressive regimens on B-cell proliferation might also reflect their ability to induce apoptosis of activated B-lymphocytes. Hence, stimulated human B-lymphocytes were treated with immunosuppressive agents or with solvent controls. The effects of graded concentrations of immunosuppressants were analysed via Annexin-V and PI staining. Percentage of living cells and the sum of cells in the early apoptotic, late apoptotic state, as well as dead cells, were analysed. Cells treated with sotrastaurin at 50 and 500 nM displayed a tendency towards decreased percentages of living cells and also increased percentages of early apoptotic/late apoptotic/dead cells compared with controls, but did not reach statistical significance. MPA and everolimus at the concentrations used did not induce apoptosis (Fig. 2a–c).

Effects of immunosuppressants on CD80 and CD86 expression on human B-lymphocytes

B cells are potent antigen-presenting cells and express various costimulatory molecules upon activation including the CD80/CD86 surface molecules. To explore the impact of immunosuppressants on the antigen-presenting capability of activated primary human B cells, we examined CD80 and CD86 induction on CD19+B cells by flow cytometry after stimulation and treatment with or without immunosuppressants. Sotrastaurin – in the tested concentrations – had no effect on the surface expression of CD80, but caused increased CD86 expression on B-lymphocytes (Fig. 3a and d). In contrast to the PKC inhibitor, MPA inhibited the surface marker expression significantly at concentrations starting with 100 ng/ml for CD80 (Fig. 3b) and 1 μg/ml for CD86 (Fig. 3e). Again, everolimus affected the CD80 and CD86 expression at all concentrations (Fig. 3c and f).

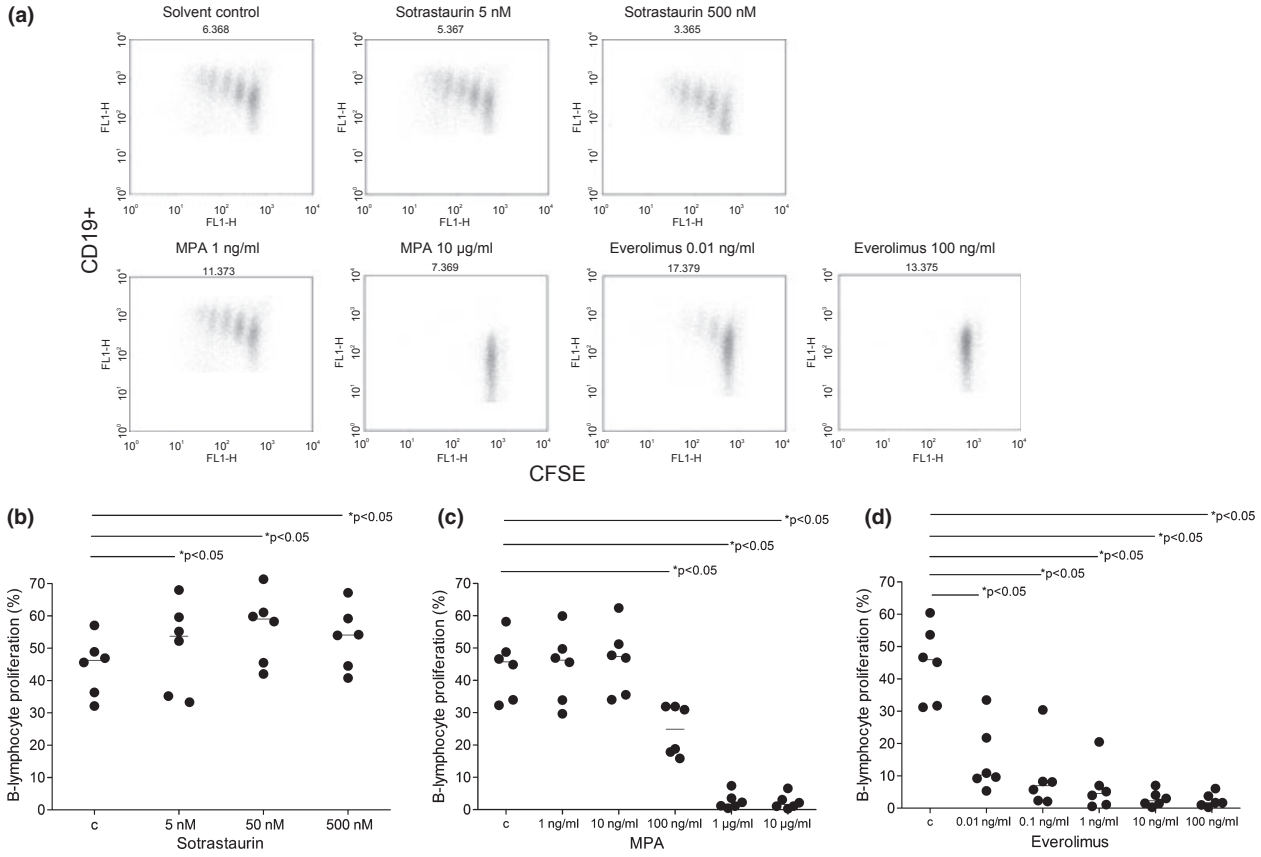

Figure 1 B-lymphocyte proliferation rates are reduced after mycophenolic acid (MPA) and everolimus, but not sotrastaurin administration. Proliferation assays with stimulated B cells that were either treated or untreated with immunosuppressive substances were performed. Proliferation rates of CFSE-labelled CD19+ B cells were analysed by flow cytometry after 6 days (n = 6) (a) Depicted are dot plots of B-lymphocytes treated with the lowest and highest concentration of immunosuppressants. Percentage of CD19+ B cells that had entered cell division is slightly heightened after sotrastaurin (b) administration. MPA (c) and everolimus (d) inhibited B-cell proliferation significantly in a dose-dependent manner. Horizontal bars indicate median values.

Effects of immunosuppressants on the immunoglobulin production of human B-lymphocytes

IgG and IgM levels in supernatants from stimulated human B-lymphocyte cultures were analysed after treatment with graded concentrations of immunosuppressive substances or solvent controls. Sotrastaurin did not inhibit IgG production at the concentrations used and IgM production only at the highest concentration (Fig. 4a and d). Both MPA and everolimus effectively suppressed immunoglobulin production at high or all concentrations used (Fig. 4b, c, e and f).

Effects of immunosuppressants on T-cell-dependent immunoglobulin production

A previously described culture system [19] was applied to analyse the influence of sotrastaurin, MPA and everolimus on T-cell-dependent B-cell activation defined as IgG and IgM production. B cells were activated with prestimulated autologous T cells and also ODN2006 and treated with immunosuppressants or solvent controls. Prestimulated T cells were required in this culture system since ODN2006-stimulated B cells alone produced significantly lower levels of immunoglobulines (Fig. 5a and b). IgG production was significantly suppressed by 500 nM sotrastaurin and all MPA and everolimus concentrations used. The administration of 50 nM sotrastaurin led to slightly increased IgG levels (Fig. 5c). Sotrastaurin and everolimus inhibited IgM production significantly in the highest concentration administered compared with solvent controls. MPA decreased IgM levels significantly in both concentrations (Fig. 5d).

Effects of sotrastaurin on B-lymphocyte cytokine production

IL-10 concentrations in supernatants from stimulated B-lymphocyte cultures were examined after treatment with sotrastaurin, MPA, everolimus or solvent as control.

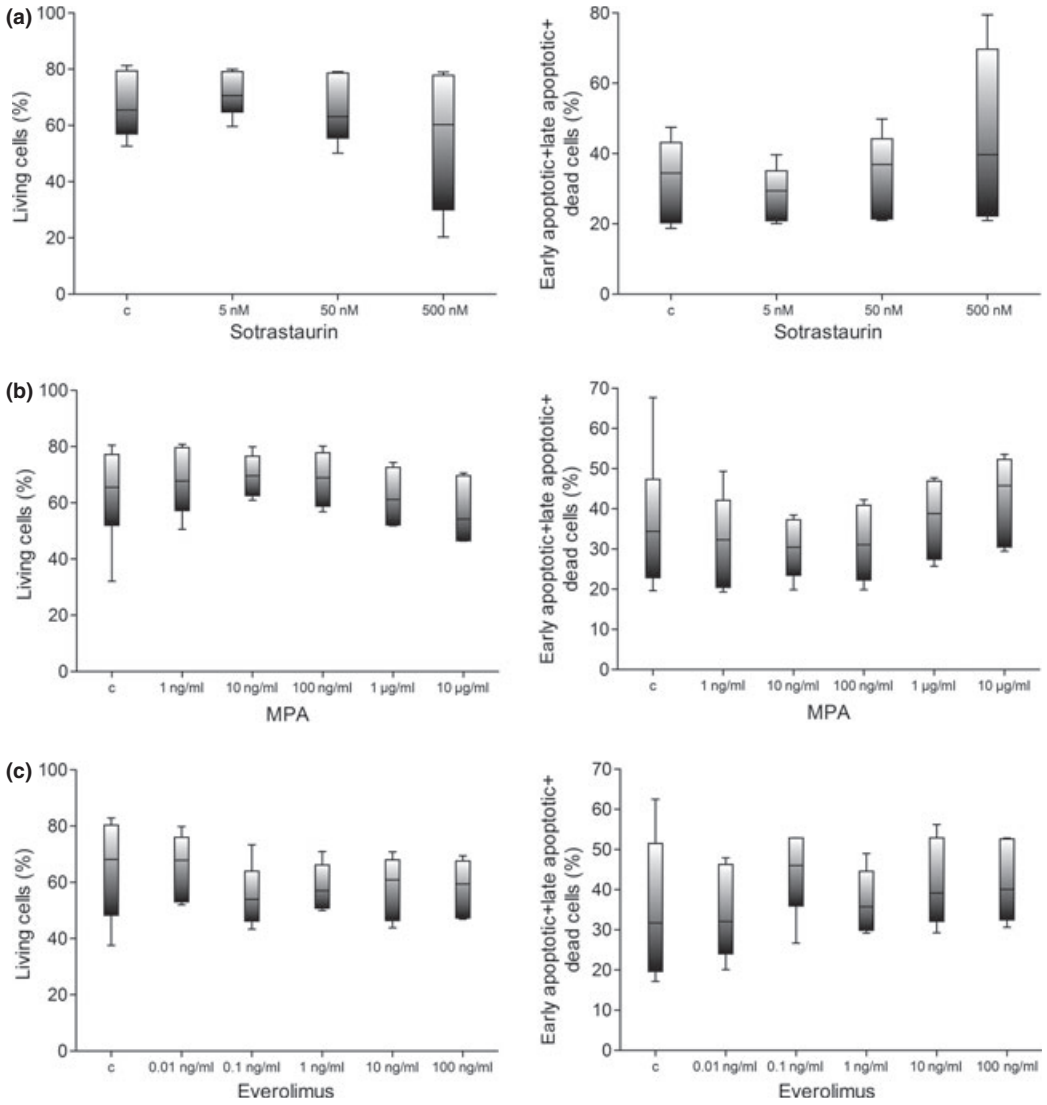

Figure 2 Apoptosis in B-lymphocytes is not induced by sotrastaurin, mycophenolic acid (MPA) and everolimus. After 3 days of stimulation and treatment with immunosuppressants, B cells were stained with Annexin-V and PI and analysed by flow cytometry (n = 6). Unstained cells are alive, Annexin-V stained cells are in the early apoptotic and PI/Annexin-V stained cells in the late apoptotic state. Cells that are only PI stained are dead. The administration of high sotrastaurin concentrations tended to result in increased percentages of early apoptotic/late apoptotic/ dead cells and decreased percentages of living cells, but this effect did not reach statistical significance (a). MPA and Everolimus administration did not induce B-lymphocyte apoptosis (b and c).

The administration of both sotrastaurin concentrations induced IL-10 production significantly, whereas MPA and everolimus inhibited cytokine expression in a dose-dependent manner (Fig. 6a–c).

Discussion

B-lymphocytes have several mechanisms by which they might affect kidney allograft survival, including antigen presentation, cytokine production, immune regulation and differentiation into memory cells and alloantibody-producing plasma cells. Plasma cells are generated by T-lymphocyte-dependent activation, which is defined as clonal expansion and differentiation after B-cell-receptor cross-linking and costimulation. Alloantibody primarily targets the donor organ endothelium and causes damage via complement-dependent or independent pathways. After endothelial damage, pathological features like platelet activation and thrombosis, endothelial and smooth muscle cell proliferation and direct organ damage medi-

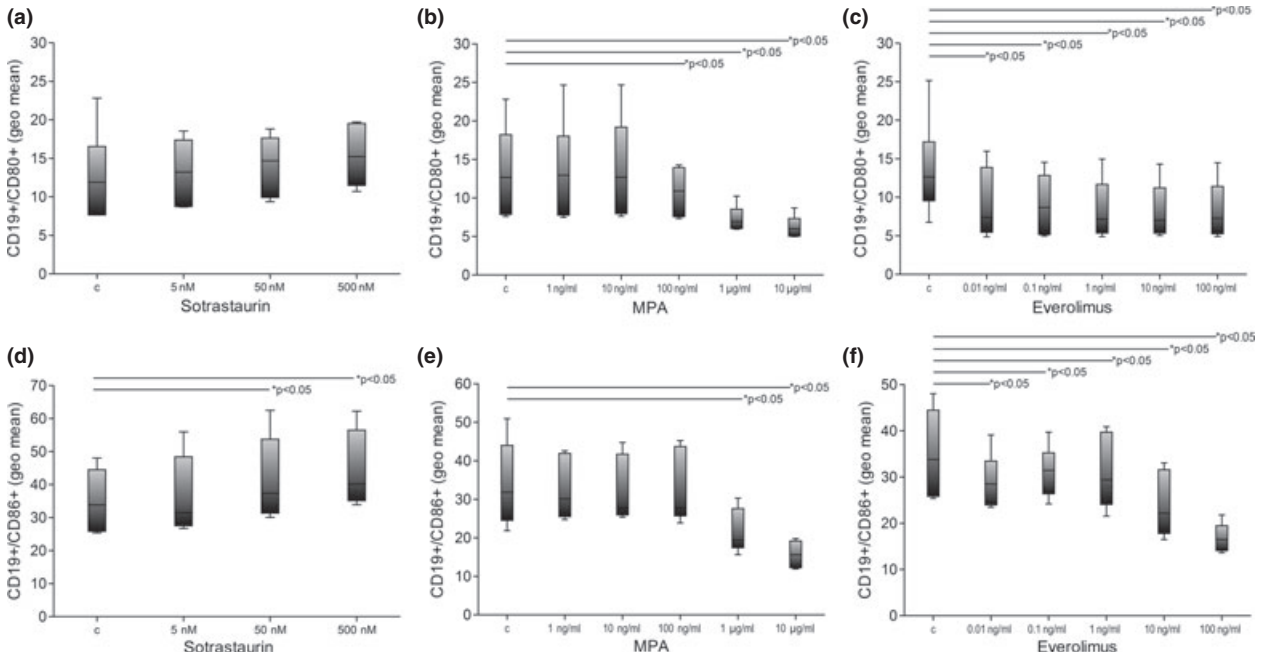

Figure 3 Mycophenolic acid (MPA) and everolimus, but not sotrastaurin treatment inhibits B-lymphocyte expression of the costimulatory markers CD80 and CD86. A phenotypic characterization of CD19+ B-cells after stimulation in the absence or presence of immunosuppressive substances was performed by flow cytometry (n = 6). The expression levels of surface markers CD80 and CD86 on CD19+ B cells were investigated. The administration of MPA (b and e) and everolimus (c and f), but not sotrastaurin (a and d), led to a significant downregulation of the expression of CD80 and CD86 compared with solvent controls.

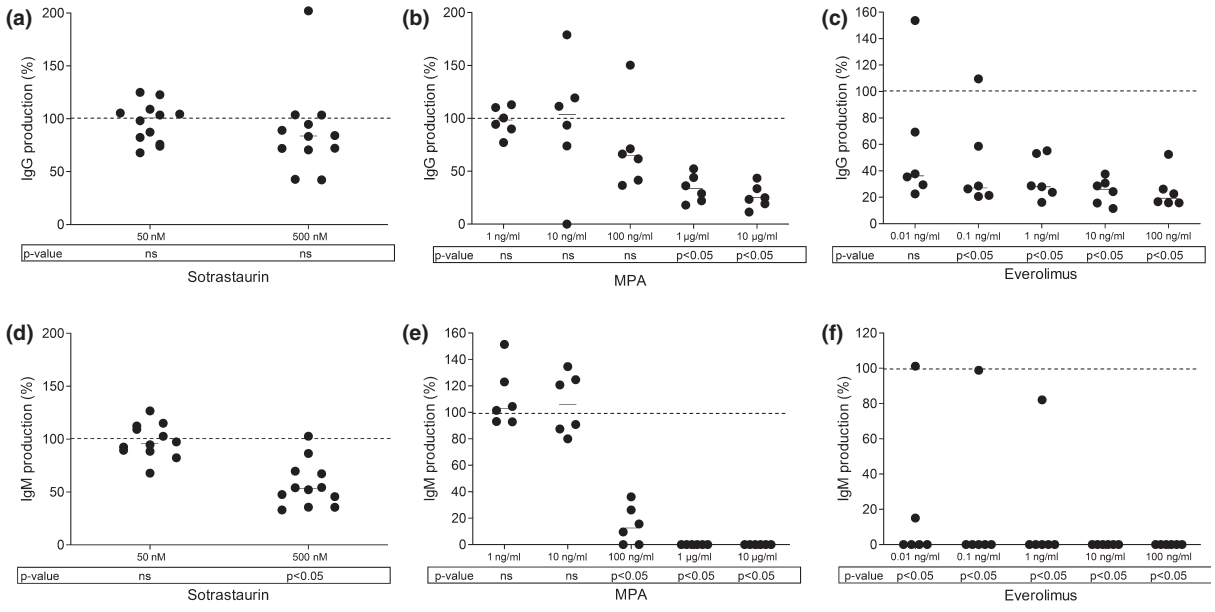

Figure 4 Sotrastaurin, mycophenolic acid (MPA) and everolimus have diverse effects on B-lymphocyte immunoglobulin production. Supernatants from B-lymphocyte cultures that were stimulated and either treated or untreated with immunosuppressive substances were analysed for IgG and IgM levels with ELISA. Sotrastaurin (n = 12) in the concentrations administered had no effect on IgG production and inhibited IgM production only at the highest concentration of 500 nM (a and d). MPA (n = 6) administration of 1 and 10 µg/ml caused significantly decreased IgG levels (b), IgM production was inhibited by the same concentrations and also by 100 ng/ml (e). Everolimus treatment (n = 6) with concentrations starting at 0.1 ng/ml led to diminished IgG production (c), IgM levels were significantly decreased at all administered concentrations compared with solvent controls (f) (- - - solvent controls; — median values).

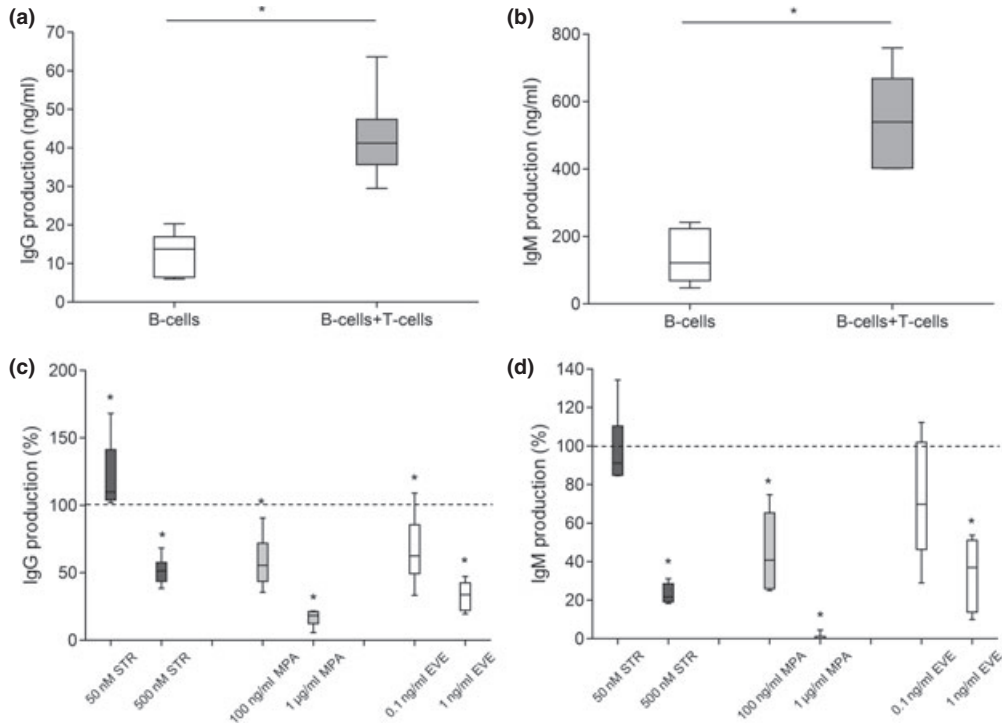

Figure 5 Sotrastaurin, mycophenolic acid (MPA) and Everolimus affect T-cell-dependent B-lymphocyte immunoglobulin production. Cocultures of B cells with ODN-CPG as stimulus and prestimulated T cells in the presence or absence of immunosuppressive substances were performed (n = 6). Supernatants were harvested after 8 days and analysed for IgG and IgM levels. B cells alone produced significantly less IgM and IgG compared with B cells cocultured with prestimulated T cells (a and b). T-cell-dependent IgG production was slightly induced by 50 nM sotrastaurin (STR) administration and suppressed by 500 nM sotrastaurin, 100 ng/ml and 1 µg/ml MPA and also 0.1 and 1 ng/ml everolimus (EVE) compared with solvent controls (—) (c). IgM levels were inhibited by 500 nM sotrastaurin, 100 ng/ml and 1 µg/ml MPA and 1 ng/ml everolimus compared with solvent controls (—) (d).

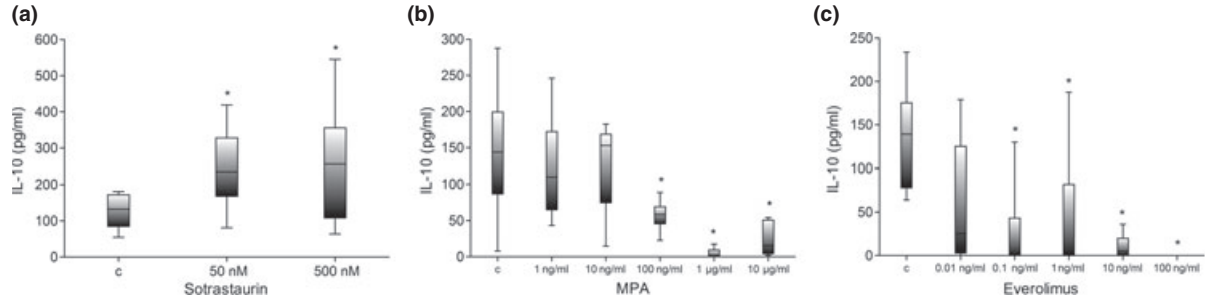

Figure 6 Sotrastaurin, mycophenolic acid (MPA) and everolimus affect B-lymphocyte cytokine production. Supernatants from B-lymphocyte cultures that were stimulated and either treated or untreated with immunosuppressive agents were analysed for IL-10 levels with ELISA. Sotrastaurin administration caused elevated levels of IL-10 (a) (n = 8), whereas MPA and everolimus inhibited IL-10 production significantly at high concentrations (b and c) (n = 6). *P < 0.05.

ated by humoral or cellular infiltrates occur [20,21]. It is generally known that the development of especially DSA is associated with acute and chronic AMR leading to poor prognosis [22,23]. Usually DSA persist and may lead to transplant glomerulopathy, which is a major cause for graft loss beyond the first year. Even though plasmapheresis, IVIG and rituximab are recommended by current guidelines [2], the efficacy of this regimen is limited [5]. Although the chimeric monoclonal antibody rituximab depletes immature and mature B-lymphocytes effectively, it does not affect antibody-producing plasma cells. As a consequence, novel treatment strategies have to be devel-

oped, which should be based on a better understanding of the impact current and novel immunosuppressants have on B-cell biology. In this context, it is important to point out that T- and B-lymphocytes always depend on each other in the alloimmune responses and therefore the production and appearance of DSA are also related to the functionality of T-lymphocytes. The data presented here elucidate the impact of novel and standard maintenance immunosuppressive agents with different mechanisms of action on B cells directly and indirectly through interference with T-cell help.

Sotrastaurin is a new low molecular mass synthetic compound that potently and reversibly inhibits PKC-α, PKC-β and PKC-θ with lesser activity on PKC-δ, but not atypical PKC isoforms [24]. It affects early T-cell activation signals 1 and 2 through PKC [25]. Sotrastaurins strong impact on T-cell activation and absent influence on DC-maturation *in vitro* has been previously described [26]. After early termination of two phase II trials [27,28], the PKC inhibitor is currently under investigation in phase II studies for efficacy and safety in combination with everolimus in a calcineurin inhibitor (CNI)-free regimen and also in combination with tacrolimus. Despite several reports on the effective inhibitory effect of sotrastaurin on T-cell activation [29], to date, there are no data available concerning sotrastaurins effect on B-lymphocyte function. The analyses of our experiments presented here suggest that sotrastaurin has no inhibitory effect on human B-cell proliferation; in contrast, sotrastaurin elicited a weak proliferative response of B cells. Sotrastaurin exerted only minimal effects on apoptosis and had no direct inhibitory effect on B-cell activation defined as the combination of costimulatory molecule CD80 expression on the cell surface, IgG/IgM and cytokine production. There was even a tendency towards increased CD86 expression and IL-10 cytokine production. These results were unexpected since B cells express several PKC family members, including PKCα, β, δ, ε, η, ζ and λ [30,31], and isoforms have been identified as crucial participants in B-cell survival, development and tolerance [32–35]. Nevertheless, the highly complex expression profile and function of individual PKC isoforms –dependent on the specific B-cell subset – are not completely understood. The finding that activation of the two highly important transcription factors NFκB (nuclear factor kappa B) and NFAT (nuclear factor of activated T cells) occurs via distinct mechanisms in B cells [36] might explain some results of our experiments. Whereas activation of NFκB depends on the conventional PKC isoform β, that of NFAT might involve non-conventional PKCδ. As mentioned above, sotrastaurin has profound effects only on classical and novel PKC isoforms but not on nonconventional ones [25]. In our experiments, we stimulated B cells with ODN 2006 in combination with a-CD40 and IL-4. It has been previously described that ligation of CD40 receptors in conjunction with IL-4 induces NFAT in normal murine B cells [37] and that signals from TLR9 and CD40 in the presence of IL-4 synergize to promote B-cell activation [38]. Thus, the induction of NFAT might – in our experimental setting – have been sufficient to cause nonconventional PKCδ-dependent B-cell activation, which was not adequately suppressed by sotrastaurin. Similarly, the increased IL-10 production under sotrastaurin could be caused by the higher proliferation rates and NFAT-dependent pathways. This approach could be supported by a very recent study suggesting NFAT as a crucial transcription factor responsible for IL-10 production during B-cell receptor signalling [39]. Interestingly, Naylor *et al.* recently described a differential IL-10 production after sotrastaurin administration in activated B-cell-like subtypes of diffuse large B-cell-lymphoma cell lines that was CD79A/B mutation-dependent. Future studies should elucidate the mechanisms behind PKC inhibition through sotrastaurin and the effects on cytokine production in the context of B-cell activation [40]. Even though sotrastaurin was not efficient in directly inhibiting B-lymphocytes, it strongly affected – at a high concentration – immunoglobulin production by the effective inhibition of T-cell help in a co-culture system of autologous pre-activated T cells and B cells.

MPA preferentially inhibits *de novo* pathway of guanosine nucleotide synthesis in T and B-lymphocytes. In the presented study, it potently suppressed all tested B-cell functions including CD80 and CD86 expression, proliferation and IgG/IgM and cytokine production in a dose-dependent manner. Surprisingly, apoptosis as a relevant effector mechanism for immunosuppressive agents was not induced. A possible explanation for this observation might be the addition of IL-4 to the cultures, which could protect B-lymphocytes from apoptosis induction by MPA (and also everolimus) [41–45]. A combination of CNI and MPA has successfully been administered as rescue therapy for acute or chronic rejection [46–49]. As previous reports describe that CNI do not affect B cells directly [50], it could be concluded that MPA might have a greater potential in reverting humoral rejection episodes after kidney transplantation than CNIs alone. Furthermore, T-cell-dependent IgG and IgM production was strongly suppressed by MPA, which is consistent with the findings described above that MPA affects both B cells and T cells directly [51].

Inhibitors of mammalian target of rapamycin (mTOR) are potent immunosuppressants with antiproliferative and antimigratory capacities. Sirolimus as a representative of this group displayed potency in controlling an already established humoral alloimmune response as indicated by

decreased circulating alloantibodies as well as peritubular C4d in the kidney in a renal allotransplantation rat model [52]. Its administration also led to a reduction in anti-equine antibody formation after renal transplantation in man treated with equine antithymocyte globulin [53] and strong inhibition of B-cell responses *in vitro* [50]. Also, patients receiving mTOR inhibitors after solid organ transplantation had lower seroprotection rates and lower geometric mean titres following pandemic influenza H1N1–2009 vaccination [54]. In our study, the mTOR inhibitor everolimus displayed strong effects on the B-cell functions, CD80 and CD86 expression, proliferation, IgG/IgM and cytokine production in a dose-dependent manner. Strong suppressive influence was already observed at very low concentrations. Nevertheless, a recent study shows that everolimus allowed primary humoral immune response and left secondary T-cell-dependent and independent immune responses intact after vaccination of stable renal transplant recipients [14]. Also, everolimus does not completely control humoral processes of chronic allograft nephropathy in a sensitized rat model [55]. These controversial findings need to be examined further in the context of large randomized prospective trials to investigate the incidence of AMR and DSA under mTOR inhibitor-based regimens. Apart from that, it has been described previously that everolimus is a potent suppressor of T-lymphocyte function [56], which is in line with our findings that T-cell-dependent immunoglobulin production was suppressed by that agent.

In summary, sotrastaurin seems to be able to act on B-lymphocyte function only indirectly by suppressing T-lymphocyte help. In contrast, both MPA and everolimus are capable of inhibiting humoral responses directly and indirectly via inhibition of T-cell help. The treatment of humoral rejection processes after renal transplantation is and will remain a major challenge in transplant medicine due to currently unavailable B-cell-specific agents. Neither the dose nor the best combination of immunosuppressive agents for the treatment of an established humoral rejection is based on solid evidence.

It is consequently essential to investigate the ability of standard and new immunosuppressive agents to intervene with B-cell function and T-cell help further and in the context of clinical trials.

Authorship

MM: participated in research design, writing of the manuscript, in the performance of the research, in data analysis. ML, CL, KF and SD: participated in the performance of the research. NU, UAW and HHN: participated in research design. MFM: participated in research design and data analysis. KB: participated in research design and writing of the manuscript.

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