Imatinib impairs the proliferation and function of CD4+CD25+ regulatory T cells in a dose-dependent manner

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Abstract. The tyrosine kinase inhibitor imatinib has been reported to inhibit CD8+ T lymphocytes. Little is known about its effects on CD4+CD25+ regulatory T cells (T_{reg} cells) which might regulate the graft-vs.-leukemia (GVL) reaction after allogeneic stem cell transplantation (allo-SCT) and donor lymphocyte infusion (DLI). This is of particular interest in patients with relapse of chronic myeloid leukemia (CML) after allo-SCT, as the two therapeutical options DLI and imatinib might interact reversely. Here, we demonstrate that the proliferation of CD4+CD25+ T_{reg} cells and their production of IL-10, TGF-ß1 and granzyme B as markers of activation were significantly down-regulated by imatinib in a dose-dependent manner. In addition, the expression of surface CD69, both surface and intracellular GITR, FoxP3, CD152 (CTLA) of activated CD4+CD25+ T_{reg} cells were inhibited by imatinib in a dose-dependent manner. In light of these findings, clinical administration of imatinib might not result in a reduction of the GVL effect on CML patients receiving imatinib after allo-SCT and/or DLI or other CD8+ T lymphocyte based immunotherapies as the function of CD8+ cytotoxic T lymphocytes and CD4+CD25hi T_{reg} cells is hampered in a similar way by imatinib.

Introduction

Imatinib mesylate (STI571, Gleevec®, Glivec®; Novartis, Basel, Switzerland) is a highly effective oral drug which has been well established as front-line therapy for patients with Philadelphia chromosome positive leukemia (CML) (1-3). Earlier reports described an immunosuppressive effect of imatinib on CD8+ T cells (4-6) and CD4+ T helper cells (7). However, little is known about its potential effects on CD4+CD25+ T_{reg} cells which are considered to control T cell mediated immune responses toward tumor-associated antigens and viral antigens (8-10). T_{reg} cells have also been described to be critical in the reduction of the graft-vs.-leukemia (GVL) effect observed after allo-SCT (9,11-13). Therefore, the impact of different immunosuppressive drugs on T_{reg} cell function *in vivo* may be of relevance for the transplantation biology and constitute an option to enhance CD8+ T lymphocyte based anti-tumor immunotherapies. In patients with relapse of CML after allo-SCT, two therapeutical options are in current clinical practice: donor lymphocyte infusions (DLI) and imatinib (14-16). The combination of both has been synergistic, but also contradictory reports have been published (16).

Here, we investigated the effect of imatinib on the proliferation and activation of CD4+CD25+ T_{reg} cells *in vitro*. The results might be of interest in terms of long-term toxicity of the drug as well as of novel yet only partly explored applications of the drug. Clinical administration of imatinib might not result in a reduced efficacy of the GVL effect or other CD8+ T lymphocyte based immunotherapies as the function of both CD8+ cytotoxic T lymphocytes and CD4+CD25hi T_{reg} cells is hampered in a similar way.

Materials and methods

Samples from healthy volunteers. All samples were taken from healthy blood donors after their informed consent was obtained. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Biocoll Separation Solution (Biochrom, Berlin, Germany) density gradient centrifugation from EDTA (Delta-Pharma, Pfullingen, Germany) anticoagulated blood buffy coat preparations from healthy blood donors. The viability of PBMCs obtained was always >95%, as determined by trypan blue staining (Trypan Blue Solution 0.4%, Sigma-Aldrich, Munich, Germany). The viable cells were quantified in a Neubauer chamber (Zeiss, Oberkochen, Germany). For cellular assays, Ficoll separated PBMCs were tested freshly or cryopreserved in RPMI medium 1640 (Biochrom AG,

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Berlin, Germany) containing 20% human AB serum (Red Cross Blood Bank, Ulm, Germany) and 10% dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany), and stored in liquid nitrogen.

Imatinib. Imatinib powder was generously provided by Novartis Pharmaceuticals (Basel, Switzerland) and was dissolved in DMSO. The 10 mM stock solution was stored at -20˚C and protected from light until use, diluted to the final concentration in X-VIVO 10 medium (CellGenics, Freiburg, Germany), added to T cell cultures at a serial concentration (0, 1, 5, 10 μM) immediately on the first day. The final concentration of DMSO in the media was <0.01% to 0.1%, and mere DMSO had no effect on the cell growth inhibition in the present study (data not shown).

Purification of T cell subsets by magnetic cell sorting (MACS). $CD4^+$ T cells were purified from PBMCs through positive selection by using magnetic beads and then sorted into $CD4^+CD25^+$ or $CD4^+CD25^-$ populations with according to the manufacturer's instructions ($CD4^+CD25^+$ Regulatory T cell Isolation Kit, human, Miltenyi Biotec, Gladbach, Germany). Briefly, $CD4^+$ T cells were first isolated through positive selection by removing all other cell types. Pre-isolated $CD4^+$ T cells were incubated with 10 μl of magnetic beads coupled with anti-CD25 antibodies (for 10^7 cells) to separate the $CD4^+CD25^+$ from the $CD4^+CD25^-$ T cell population. The purity of the resulting T cell population was confirmed to be >95%, as determined by fluorescence activated cell sorting (FACS) analysis (data not shown).

$CD4^+CD25^+$ and $CD4^+CD25^-$ T cell cultures. $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated human AB serum/2 mM L-glutamine/100 units/ml penicillin/100 μg/ml streptomycin. Cultures were incubated at 37˚C with 5% CO_2. To propagate $CD4^+CD25^+$ T cells for better evaluation and to circumvent anergy and apoptosis, 10^5 cells per 100 μl-well were activated by plate-bound anti-CD3 antibody (5 μg/ml, BD Biosciences, Heidelberg, Germany, clone UCHT1) and stimulated with soluble anti-CD28 antibody (10 μg/ml, BD Biosciences, clone 28.2) in a round-bottom 96-well microplate (Costar, Cambridge, MA) in the presence of recombinant human IL-2 at a final concentration of 10 ng/ml (Sigma, St. Louis, USA). For plate-bound anti-CD3 stimulation, 50 μl of the anti-CD3 Ab diluted in PBS (Life Technologies, NY, USA) at the concentration of 5 μg/ml was added to each well, placed at 37˚C for 4 h, and then washed twice with PBS. Imatinib was added at different concentrations as indicated above.

Antibodies and FACS analysis. Six-color flow cytometry analysis was performed on the cells harvested after 72 or 96 h cultures using the following antibodies (Ab): phycoerythrin (PE)-Cy7 conjugated anti-CD4 (BD Biosciences), allophycocyanin (APC)-Cy7-conjugated anti-CD25 (BD Biosciences), peridinin chlorophyll-a protein (PerCP)-conjugated anti-CD69 (BD Biosciences), intracellular or extracellular APC-conjugated anti-CD152 (CTLA-4) (BD Biosciences), intracellular or extracellular PE-conjugated anti-GITR (R&D System, Wiesbaden, Germany), and intracellular fluorescein isothiocyanate (FITC)-conjugated anti-FoxP3 (eBioscience, Kranenburg, Germany) with the appropriate normal isotype-matched control IgG. For extracellular staining, cells were incubated for 30 min at 4˚C with optimal dilution of each antibody. For intracellular staining, the cells were fixed with Reagent A and permeabilized with Reagent B (IntraStain, DakoCytomation, Germany). The cells were analyzed on a FACSAria™ flow cytometer (Becton-Dickinson) using the CellQuest software (Becton-Dickinson). The area of positivity was determined using an isotype-matched control IgG, setting gates at the 1% level of the respective isotype-matched control. In all cases, at least 10,000 events were collected to evaluate analysis. Dead cells and debris were excluded by forward and side ward scatter gating.

Proliferation assays (5-bromo-2-deoxyuridine labeling of cells). To assess the inhibition of proliferation of $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells by imatinib, a total of $1x10^5$ sorted $CD4^+CD25^+$ T cells or $CD4^+CD25^-$ T cells were cultured for 72 h as described above. BrdU incorporation for the last hour of culture was measured as an indicator of cell proliferation. Briefly, the cells were labelled with BrdU (BD Biosciences) *in vitro* with 10 μM for 1 h. The cells were fixed and permeabilized by BD Cytofix/Cytoperm buffer (BD Biosciences) for 30 min on ice, treated with 30 μg/ml DNase for 1 h at 37˚C, and stained with FITC-conjugated anti-BrdU (BD Biosciences), and analyzed by FACS.

Analysis of the inhibitory function of $CD4^+CD25^+$ T cells. Transwell experiments were performed in 24-well transwell plates (3 μm pore size; Costar, NY) for suppression assays. $CD4^+CD25^+$ and $CD4^+CD25^-$ cell populations were isolated as described above and cultured in transwell plates. Both chambers of transwell received soluble anti-CD3 plus soluble anti-CD28 in the presence of IL-2 as described above. After three days of culture, the proliferation of $CD4^+CD25^-$ cells ($1x10^5$) plated in the lower chamber of each transwell was measured in the presence or absence of $1x10^5$ $CD4^+CD25^+$ T cells by BrdU incorporation.

Assessment of IL-10 and TGF-ß1 production by enzyme-linked immuno-sorbent assay (ELISA). To assess the IL-10 and TGF-ß1 levels in the culture medium, ELISAs using commercially available kits (BD Biosciences-PharMingen, San Jose, USA) were performed on cell-free supernatants after 96 h of culture of $CD4^+CD25^+$ and $CD4^+CD25^-$ cells as indicated above according to the manufacturer's instructions. Briefly, supernatants were collected from 96-well round plates after 72-96-h culture indicated above. The samples were diluted 1:2 to 1:5, and IL-10 as well as TGF-ß1 were captured by specific primary monoclonal antibodies (mAb) pre-coated on the microplate and detected by horseradish peroxidase-labeled secondary mAbs. Plates were read at a wavelength of 450 nm using a microplate reader (Model Spectra Max 190; Bio-Rad Labs, Hercules, CA). Samples were quantified by comparison with standard curves obtained with purified recombinant human IL-10 and TGF-ß1 (BD PharMingen). Samples and standards were run in duplicate.

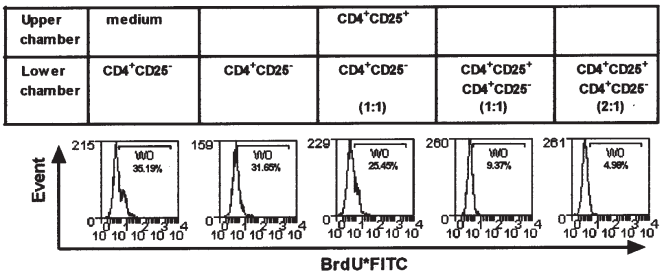

Figure 1. $CD4^+CD25^+$ T cells suppress the proliferation of $CD4^+CD25^-$ T cells when cocultured. $CD4^+CD25^-$ T cells ($1x10^5$/well) were stimulated in the lower chamber of a transwell plate in the absence of additional T cells or in the presence of $CD4^+CD25^+$ T cells that were stimulated with anti-CD3/CD28 antibodies either in the same chamber at different ratios of $CD4^+CD25^+$ T cells to $CD4^+CD25^-$ T cells, or in the separate upper chamber of the transwell plate. After 3 days of culture, BrdU incorporation was detected by FACS analysis to evaluate the proliferation of $CD4^+CD25^-$ T cells. The figure displays a representative result from three independent experiments with similar results.

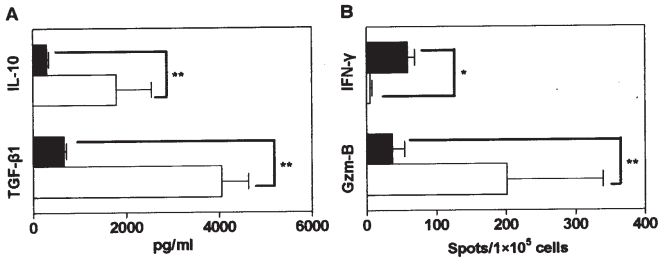

Figure 2. Cytokine profile of $CD4^+CD25^+$ (white bars) and $CD4^+CD25^-$ T cells (black bars). $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells were stimulated with anti-CD3/CD28 antibodies described in Materials and methods. On day 4, supernatants were collected and assessed for IL-10 and TGF-ß1 levels by ELISA (A), while cells releasing granzyme-B and IFN-γ were detected by ELISPOT (B). The data are representative of four independent experiments with similar results and are presented as mean ± SD. Error bars indicate SD. $^*p<0.05$ and $^{**}p<0.01$.

Detection of IFN-γ and granzyme B (Gzm-B) by enzyme-linked immuno-spot (ELISPOT) assays. IFN-γ and Gzm-B ELISPOT assays were performed according to the manufacturer's instructions as previously described (17,18). Briefly, 96-well nitrocellulose plates (Millipore, Schwalbach, Germany) were coated with IFN-γ mAbs (Mabtech, Hamburg, Germany) and incubated overnight at 4˚C. Thereafter, they were blocked with 10% human AB serum (Red Cross Blood Bank) for 2 h at 37˚C. Anti-CD3/anti-CD28 Abs ($1x10^5$) stimulated $CD4^+CD25^+$ or $CD4^+CD25^-$ T cells were added to each well. Following overnight incubation in RMPI-1640 medium, plates were washed with 1X PBS (supplemented with 0.05% Tween-20), IFN-γ mAbs (0.2 μg/ml) were added to each well, and then incubated at room temperature for 2 h. After washing with streptavidine alkaline phosphatase (1 μg/ml, Mabtech, Hamburg, Germany) for 2 h, BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium; Sigma-Aldrich, Munich) was used for colorization according to the manufacturer's instructions, and thereafter evaluated by the use of an ELISPOT reader (CTL, Reutlingen, Germany). Similarly, the Gzm-B ELISPOT assay was performed according to the manufacturer's instructions as previously described (17,18).

The responses were considered significant if: i) the mean spot-forming cells (SFCs) in duplicate of anti-CD3/CD28 Abs stimulated wells was significantly greater ($p<0.05$) than in wells without anti-CD3/CD28 Abs stimulation; ii) the net SFCs of duplicate wells (the mean of the duplicate SFCs/$1x10^5$ cells in anti-CD3/CD28 Abs stimulated wells-mean of the duplicate SFCs/$1x10^5$ cells in wells without anti-CD3/CD28 stimulation) was greater than 10 SFCs/$1x10^5$ cells; and iii) the index (the ratio of mean SFCs/$1x10^5$ cells in anti-CD3/CD28 Abs stimulated wells to mean SFCs/$1x10^5$ cells in wells without anti-CD3/CD28 Abs stimulation) was greater than 2.0.

Statistical analysis. All data are representative of at least three independent experiments using the peripheral blood from different donors with similar results. The data are presented as the mean ± standard deviation (SD). The significance of the difference between means ± SD of different study groups was determined by the 2-tailed Student's t-test or one-way variance (ANOVA), and the differences were considered statistically significant at $p<0.05$. The statistical analyses were performed using the statistical software package SPSS Version 11.5 for Windows (SPSS, Chicago, IL, USA).

Results

Definition of regulatory T cells as $CD4^+CD25^+$ cells. $CD4^+CD25^+$ T cells were separated from PBMNs of healthy volunteers. A reduction of proliferation of $CD4^+CD25^-$ T cells was observed in BrdU incorporation assays when either co-cultured or separated in transwell plates with $CD4^+CD25^+$ T cells as shown in Fig. 1. These $CD4^+CD25^+$ T cells were defined as T_{reg} cells in all consecutive assays.

Cytokine profile of $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells. The cytokine expression profile of both $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells was analyzed and compared. Freshly isolated $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells from healthy volunteers were stimulated with plate-bound anti-CD3 Ab, soluble anti-CD28 Ab and IL-2 for 96 h. The culture supernatants were then measured for IL-10 and TGF-ß1 production by ELISAs, while production of both Gzm-B and IFN-γ was detected by ELISPOT assays. As shown in Fig. 2, $CD4^+CD25^+$ T cells predominantly secreted IL-10, TGF-ß1 and Gzm-B in contrast to little or no secretion of IFN-γ, whereas $CD4^+CD25^-$ T cells mainly produced IFN-γ and very low levels of IL-10, TGF-ß1 and Gzm-B.

Imatinib inhibits the proliferation of human $CD4^+CD25^+$ T_{reg} cells. To determine the $CD4^+CD25^+$ T_{reg} cell proliferation in cultured cells by FACS analysis, $CD4^+CD25^+$ T_{reg} cells cultured for 96 h were labeled with BrdU. The T_{reg} cell subpopupation constitutes a small T cell subset. To propagate T_{reg} cells for better evaluation and to avoid anergy or even apoptosis, T_{reg} cells were stimulated unspecifically by IL-2 and antibodies against CD3/CD28 (19,20). Imatinib reduced the proliferation of these cells stimulated with anti-CD3/

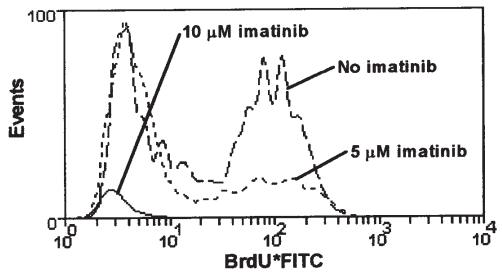

Figure 3. Imatinib inhibits the proliferation of CD4+CD25+ T_{reg} cells in a dose-dependent manner. Increasing concentrations of imatinib (0-10 μM) were added to cultures in which purified CD4+CD25+ T_{reg} cells were stimulated with anti-CD3/CD28 antibodies in the presence of IL-2. Three days after the initiation of the cultures, CD4+CD25+ T_{reg} cells were labelled with BrdU. Data presented are overlaid histograms representing the fluorescence intensity of BrdU-labelled CD4+CD25+ T_{reg} cells. The y-axis displays the percentage of BrdU-labelled CD4+CD25+ T_{reg} cells depending on different concentrations (0-10 μM) of imatinib. The data show the results of one representative experiment.

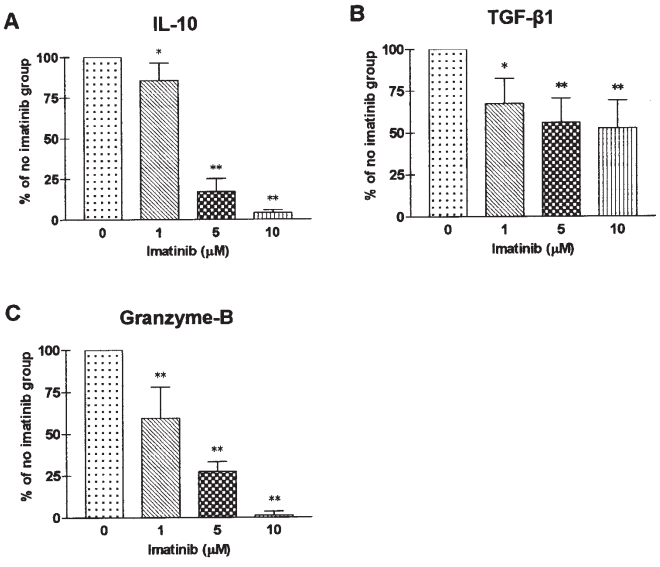

Figure 4. Imatinib inhibits the secretion of IL-10, TGF-ß1 and granzyme-B of CD4+CD25+ T_{reg} cells in a dose-dependent manner. Increasing concentrations of imatinib (0-10 μM) were added to cultures in which purified CD4+CD25+ T_{reg} cells were stimulated with anti-CD3/CD28 antibodies in the presence of IL-2. Four days after the initiation of the cultures, supernatants were collected, and IL-10 (A) and TGF-ß1 (B) were measured by ELISA. (C) Presents the results of ELISPOT assays for the release of granzyme-B by CD4+CD25+ T_{reg} cells stimulated with anti-CD3/CD28 antibodies in the absence or the presence of increasing concentrations of imatinib (0-10 μM) 4 days after the initiation of cultures. All data of panels A-C are from three independent experiments and are expressed as mean values in % of the 'no imatinib group' ± SD at each imatinib concentration. Error bars indicate SD. $^{*}p<0.05$ and $^{**}p<0.01$ relative to the group of CD4+CD25+ T_{reg} cells without imatinib treatment.

CD28 Abs in the presence of IL-2 in a dose-dependent manner, and the T_{reg} cell proliferation was almost completely inhibited at a concentration of 10 μM (Fig. 3).

Reduction of cytokine level produced by stimulated CD4+CD25+ T_{reg} cells after exposure to imatinib at different concentrations. To evaluate the inhibitory effect of imatinib on the production of cytokines, freshly isolated CD4+CD25+ T cells were stimulated with plate-bound anti-CD3 and soluble anti-CD28 Abs in the presence of IL-2 without or with different concentrations of imatinib. ELISAs were performed to assess the production of IL-10, TGF-ß1, while ELISPOT assay was used to assess the secretion of Gzm-B by CD4+CD25+ T_{reg} cells. As shown in Fig. 4, imatinib reduced the secretion of detectable IL-10, TGF-ß1 and Gzm-B gradually in a dose-dependent manner. The maximal amount of IL-10 produced by anti-CD3/CD28 Abs stimulated CD4+CD25+ T_{reg} cells was 1.485±682 pg/ml without imatinib (Fig. 4A); the maximal amount of TGF-ß1 was 4,229±561 pg/ml without imatinib (Fig. 4B). The maximal number of Gzm-B spots of stimulated CD4+CD25+ T_{reg} cells was 209±153 per $1x10^5$ cells without imatinib (Fig. 4C).

Imatinib down-regulates the expression of surface CD69 and GITR, as well as intracellular GITR, FoxP3, and CD152 of CD4+CD25+ T_{reg} cells. We then investigated the surface expression of CD69, GITR, and the intracellular expression of GITR, FoxP3, CD152 (CTLA) which are considered to be CD4+CD25+ T_{reg} cell activation markers. We analyzed the expression of these markers by flow cytometry on purified human CD4+CD25+ T_{reg} cells after 3 days following stimulation by anti-CD3/CD28 Abs without imatinib or in the presence of imatinib at different concentrations. Expression of these markers in resting CD4+CD25+ T_{reg} cells was lower than 5% (data not shown). Upon stimulation with anti-CD3/CD28 Abs, CD4+CD25+ T_{reg} cells up-regulated the expression of these markers. Without imatinib, the maximum expression of surface CD69 and GITR as well as intracellular GITR, FoxP3 and CD152 (CTLA-4) of anti-CD3/CD28 Abs-stimulated CD4+CD25+ T_{reg} cells was 52.62±20.02%, 73.47±11.29%, 43.13±5.35%, 72.80±7.04% and 31.50±5.31%. However, imatinib significantly inhibited the up-regulation of these CD4+CD25+ T_{reg} cell activation markers in a dose-dependent fashion. Fig. 5A displays the mean ± standard deviation (SD) of three independent experiments showing all these markers of CD4+CD25+ T_{reg} cells. For all markers, a significant (Fig. 5A, $^{*}p<0.05$) or highly significant (Fig. 5A, $^{**}p<0.01$) down-regulation was observed dependent on the concentration of imatinib. Fig. 5B shows the results of one representative experiment.

Discussion

Imatinib is a potent tyrosine kinase inhibitor that is increasingly used for CML patients even at relapse after allogeneic transplantation. We reported here that imatinib inhibits CD4+CD25+ T_{reg} cell proliferation and activation after anti-CD3/CD28 Abs stimulation *in vitro*. To propagate T_{reg} cells for better evaluation and to avoid anergy or even apoptosis, T_{reg} cells were stimulated unspecifically by IL-2 and antibodies against CD3/CD28 (19,20). The inhibitory effects on T_{reg} cells were dose-dependent with some inhibition detectable at 1 μM and severe T_{reg} cell impairment at an imatinib concentration of 5 μM, which corresponds to the mean steady-state level of 2.5-5μM achieved after daily

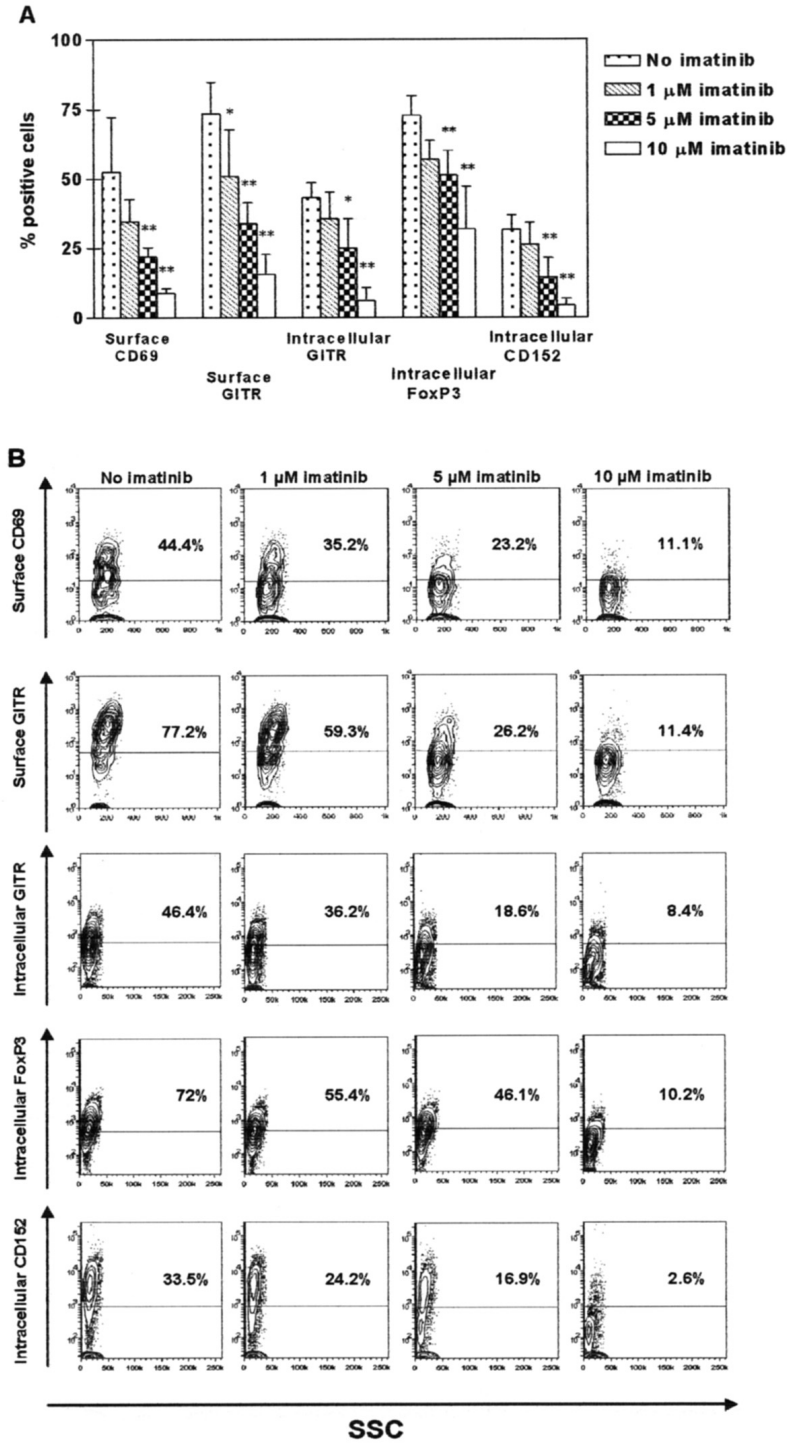

Figure 5. Imatinib treatment results in an efficient down-regulation of surface expression of CD69 and GITR as well as intracellular expression of GITR, FoxP3 and CD152 of $CD4^+CD25^+$ T_{reg} cells in a dose-related fashion. $CD4^+CD25^+$ T_{reg} cells were stimulated with anti-CD3/CD28 anti-bodies in the presence of IL-2 for 4 days. Cell surface expression of CD69 and GITR as well as intracellular expression of GITR, FoxP3 and CD152 were assessed by FACS analysis. (A) The percentage of $CD4^+CD25^+$ T_{reg} cells expressing the respective markers from three independent experiments with similar results. The columns give mean values, the error bars indicate SD. $^*p<0.05$ and $^{**}p<0.01$ relative to the group of $CD4^+CD25^+$ T_{reg} cells without imatinib treatment. (B) The results of one representative experiment.

administration of 400 mg imatinib. The proliferation of T_{reg} cells was almost completely suppressed at an imatinib concentration of 10 μM.

The definition of T_{reg} cells in humans has been controversial, and several studies have shown that the mechanism of action of $CD4^+CD25^+$ T_{reg} cells is mainly cell contact dependent. In our study, we defined T_{reg} cells as $CD4^+CD25^+$ T cells since this population consistently suppressed the *in vitro* proliferation of $CD4^+CD25^-$ T cells and also expressed characteristic markers such as FoxP3, GITR and CD152 (CTLA).

$CD4^+CD25^+$ T_{reg} cells produce IL-10 which is a pleiotropic cytokine with well-known immunosuppressive properties mediated through the inhibition of type 1 cytokine production

(21,22). *In vivo*, a suppression of CD8+ T cell mediated tumor rejection by CD4+CD25+ T_{reg} cells was demonstrated to require an intact TGF-ß receptor II on CD8+ T cells (8). Furthermore, a recent study showed that TGF-ß1 signaling in T_{reg} cells may promote FoxP3 expression, subsequently an immunosuppressive function of T_{reg} cells (23). Both IL-10 and TGF-ß1 are therefore considered to be the functional cytokines of CD4+CD25+ T_{regs} especially *in vivo*. Grossman *et al* have reported that Gzm-B is highly up-regulated in activated human T cells bearing a T regulatory cell phenotype (25). Gzm-B is one of the key components of T_{reg} cell-mediated suppression. Induction of T_{reg} cell activity is correlated with the up-regulation of Gzm-B expression.

In this study, addition of imatinib resulted in a reduced production of IL-10 (Fig. 4A) and TGF-ß1 (Fig. 4B) by CD4+CD25+ T_{reg} cells after 96 h of stimulation with anti-CD3/CD28 Abs in a dose-dependent manner. As shown in Fig. 4C, also the release of Gzm-B by CD4+CD25+ T_{reg} cells was inhibited by imatinib in a dose-dependent manner.

Activated CD4+CD25+ T_{reg} cells up-regulate CD69 and CD152 (CTLA-4); GITR was described as an important marker for T_{reg} cells, and triggering of GITR has been shown to eradicate their contact-dependent suppression activity (26). FoxP3, a nuclear protein considered to act as a transcription repressor, is a more specific marker for T_{reg} cells, which plays a central role in the development and function of these cells (27-31).

Consistent with the inhibitory effect of imatinib on the proliferation and secretion of IL-10, TGF-ß1 and Gzm-B in this report, we also demonstrated that the expression of surface CD69 and GITR as well as intracellular GITR, FoxP3 and CD152 (CTLA-4) was down-regulated by imatinib in a dose-dependent manner. The expression of surface GITR was significantly inhibited by imatinib at concentrations as low as 1 μM, while the inhibition of surface CD69, intracellular GITR, intracellular FoxP3 and intracellular CD152 required imatinib at a concentration of 5 μM. The mean peak plasma concentration after single administration of 400 mg is 4-5 μM, but can reach 7.5 μM at a steady state with 400 mg imatinib administered twice a day (32).

Given the potent immunosuppressive properties of CD4+CD25+ T_{reg} cells, their presence may also be detrimental during an immune response to tumors, pathogens, and in patients after cancer vaccine administrations. Recently, our group and others have demonstrated that imatinib impairs the function of anti-viral and anti-leukemic CD8+ T cell (6,33). According to the present study, imatinib also inhibits the immunosuppressive function of T_{reg} cells in a dose-dependent fashion suggesting that the clinical impact of imatinib does not necessarily result in a reduced efficacy of the GVL effect observed after allo-SCT and/or DLIs or vaccination therapy, but might depend rather on the balance of anti-leukemic CD8+ cytotoxic T cells and immunosuppressive CD4+CD25+ T_{reg} cells.

Acknowledgements

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