The kinase inhibitor imatinib mesylate inhibits TNF-α production in vitro and prevents TNF-dependent acute hepatic inflammation

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Imatinib exerts potent antileukemic effects in vitro and in vivo. Despite its well known antitumor activity, the potential of imatinib for the treatment of inflammatory diseases remains elusive so far. Our current report provides strong evidence that imatinib has potent antiinflammatory effects. It potently inhibits LPS- and Con A-induced TNF-α production by human myeloid cells in vitro (peripheral blood mononuclear cells, CD14-selected monocytes, and monocyte-derived macrophages). Of note, the production of the antiinflammatory cytokine IL-10 was not significantly regulated by imatinib. In line with this observation, phosphorylation of IκB and subsequent DNA binding of NF-κB, which is critically involved in TNF-α, but not IL-10 expression, was reduced by imatinib. Using several murine models of acute hepatitis, we could corroborate our in vitro findings, as imatinib prevented macrophage- and TNF-α-dependent inflammatory damage of the liver induced by injection of either Con A or d-galactosamine/LPS by inhibition of hepatic TNF-α production. Of note, d-galactosamine/TNF-induced hepatitis was not affected, showing that imatinib does not directly inhibit TNF-α-induced hepatocellular cell death. These findings suggest a potent antiinflammatory role of imatinib by modulation of TNF-α production in monocytes/macrophages. This observation might be of therapeutic value for the treatment of TNF-mediated diseases.

cytokines | monocytes/macrophages | immunosuppression

Imatinib mesylate, also known as STI571 or Gleevec (Novartis, Basel, Switzerland), has been approved for the treatment of chronic myeloid leukemia, gastrointestinal stromal tumors, systemic mastocytosis, and hypereosinophilic syndromes. Its clinical benefit is also being tested in various other malignant diseases. Imatinib reversible binds to several target kinases, among which c-kit, c-abl, and platelet-derived growth factor receptor-α are most sensitive to drug-induced inhibition of kinase activity (1). These kinases are known to be causally involved in the pathogenesis of the above-mentioned diseases. However, the same kinases similarly regulate key functions of immune cells, particularly macrophages, T cells, and dendritic cells (DCs) (2). This observation led to the hypothesis that, because of its broad spectrum of target kinases, imatinib might also modulate nonmalignant cell types, such as cells of the immune system.

Appel and coworkers (3) demonstrated that imatinib impaired the function and maturation of DCs from CD34+ progenitors. Other groups very recently emphasized the inhibitory role of imatinib on T cell activation with reduced phosphorylation of Lck (4) and ERK1/2 and subsequent inhibition of a delayed-type hypersensitivity reaction in a murine model of inflammation (5). Borg and coworkers (6) provided evidence that the antitumor effect in patients with gastrointestinal stromal tumors without detectable c-kit mutation might at least in part be caused by the natural killer (NK) cell-activating properties of imatinib. Those reports demonstrated that therapeutic concentrations of imatinib exert profound effects on DCs, NK cells, and T cells. Therefore, it is of critical relevance for both the understanding of drug-induced side effects and the evaluation of possible new indications (i.e., treatment of inflammatory or autoimmune diseases) to gain deeper insight into the immunomodulatory effects of imatinib in vitro and in vivo.

Autoimmune hepatitis in humans has been demonstrated to be initiated by activation of T cells and macrophages (7). These cells either directly attack liver parenchymal cells or induce tissue damage by the release of several proinflammatory cytokines, such as TNF-α and IFN-γ. Several well described murine models of acute hepatitis have been published so far. Injection of Con A induces fulminant hepatitis within 8 h after application (8). In this model, liver damage critically depends on macrophage-derived TNF-α (9) and T cell-derived IFN-γ (10). Alternatively, sensitizing mice with d-galactosamine (GalN) before application of recombinant TNF-α serves as a model of acute hepatic failure induced by the direct cytoxic effects of TNF-α, whereas injection of mice with GalN followed by LPS administration induces TNF-α production by macrophages, which in turn kills sensitized hepatic parenchymal cells (11). Our current report provides evidence that imatinib inhibits the TNF-α production of human myeloid cells in vitro. In vivo imatinib prevented the development of TNF-α-dependent acute hepatic injury induced by either Con A or GalN/LPS via inhibition of hepatic TNF-α expression. These data might define a unique role of imatinib in immunologically mediated diseases well beyond malignant disorders and might even set the stage for future clinical testing of this substance in inflammatory diseases whose pathogenesis is driven by TNF-α.

Materials and Methods

Preparation of Peripheral Blood Mononuclear Cells (PBMCs), Monocytes, and Macrophages. Peripheral blood was drawn from healthy volunteers after obtaining written informed consent. CD14+ monocytes were selected from PBMCs by magnetic bead separation (Miltenyi Biotec, Bergisch-Gladbach, Germany). For preparation of macrophages, CD14+ selected monocytes were grown with 1,000 units/ml granulocyte–macrophage colony-stimulating factor for 10 days. Cells were exposed to either saline or imatinib at the indicated concentrations of imatinib (ranging from 0.1 to 10 μM) for 1 h followed by stimulation with 100 ng/ml LPS (Sigma) for an additional 3–16 h.

Detection of Cytokines by ELISA. Detection of human or murine TNF-α, IFN-γ, IL-2, IL-6, IL-8, and IL-10 was performed with

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Abbreviations: DC, dendritic cell; GalN, d-galactosamine; NK, natural killer; PBMC, peripheral blood mononuclear cell; INOS, inducible NO synthase; ALT, alanine aminotransferase; MAPK, mitogen-activated protein kinase.

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ELISA kits (BD Pharmingen) strictly according to the manufacturer’s instructions.

**Real-Time RT-PCR.** One microgram of total RNA isolated with Trizol Reagent was reverse-transcribed into cDNA and amplified by using the following primers: sense TNF-α, ATCTTCTCG-GACACCCCGAGTGA; antisense TNF-α, CCGTTCAGCCCATCTGGAGCT; probe TNF-α, CCCCATGTGAGACCAACCTCTCAAAGTGTA; sense IL-10, GGGAGAAGGTAAGAGG-3’; antisense IL-10, TGCTCCTTTTCTACAGGGAAG; and probe IL-10, CTGAGGCCTAGGGCGCTGTCATCG. The GAPDH expression level in each sample was assayed by using primers specific for human GAPDH (Applied Biosystems) as control. Primers and TaqMan probes specific for murine TNF-α and IL-10 were obtained from TaqMan Predeveloped Assay Reagents (Applied Biosystems). The primer set and probe for murine inducible nitric oxide synthase (iNOS) were: sense iNOS, 5’-CAGCCTCTCTGGAAGACTGTTG-3’; antisense iNOS, 5’-CACTGGGTCATCAAACCTT-3’; and iNOS probe, 5’-FAM-CG GGCTCTGGAACCTGTTGAT-3’. For endogenous control, β-glucuronidase (GUS) expression in each sample was assayed with mouse GUS Predeveloped TaqMan control reagents (Applied Biosystems). Quantitative real-time RT-PCR was performed with the Applied Biosystems PRISM 7700 Sequence Detection System.

**Flow Cytometry.** PMBCs or monocytes were cultured in serum-free overnight, then incubated with imatinib (0.1–10 μM) or saline for 30 min followed by the addition of 100 ng/ml LPS or solvent for 1–30 min. Cells were fixed, permeabilized, and incubated with a FITC-labeled monoclonal antiphosphotyrosine antibody (Cell Signaling Technology, Beverly, MA) or the corresponding isotype control. Finally, the cells were acquired with a FACSCalibur and then analyzed by CELLQUESTPRO software. Apoptosis/necrosis was analyzed by propidium iodide staining.

**Protein Extraction.** PMBCs or monocytes were incubated with 10 μM imatinib or solvent for 30 min and subsequently stimulated with 100 ng/ml LPS for varying time points (0–3 h). Protein was extracted by lysing cells in lysis buffer.

**NF-κB Activity.** NF-κB activation was measured with the EZ-Detect NF-κB p65 Transcription Factor Kit (Pierce) according to the manufacturer’s instructions.

**EMSA.** Nuclear proteins were extracted from the macrophage cell line RAW-37 as described by Schreiber et al. (12). The following oligonucleotides were used: NF-κB sense, 5’-AGCTTCCA-GAGGGGACCTTCCGAGAGG-3’ and NF-κB, antisense 5’-TCGACCTCTCGGAAGTCTTTCTGTA-3’. Gel mobility-shift assay and supershift assays were performed as described (13). For supershift assays, 1 μg of the appropriate anti-NF-κB subunit antibody (p50, p65; Santa Cruz Biotechnology) was added. For competition studies, a 30-fold excess of unlabeled oligonucleotide probe was used.

**Western Blot.** Twenty micrograms of protein from each sample was used. Membranes were incubated with an anti-IκB antibody, an antibody specific for phosphorylated IκB (Cell Signaling Technology), or an anti-GAPDH antibody. The total IκB and p-IκB protein signal was quantified by scanning densitometry using an image analysis system (Bio-Rad).

**Animals.** Six- to 8-week-old female BALB/c mice were obtained from Harlan (Borchen, Germany). They were maintained under controlled conditions and had free access to standard Chow and water. All animals received humane care according to the legal requirements in Austria.

**Animal Treatment.** All reagents were injected in a total volume of 200 μl per mouse for i.v. and 100 μl per mouse for i.p. injections and dissolved in pyrogen-free saline. Either 75 mg/kg imatinib or saline was i.p.-injected 24, 12, and 0.5 h before treatment with the corresponding hepatotoxin. Imatinib pretreatment was followed by injection of: (i) 15 mg/kg Con A (Sigma); i.v. (ii) 700 mg/kg GalN (Roth, Karlsruhe, Germany) and 250 ng/kg LPS from Salmonella abortus equi (Sigma) i.p.; and (iii) 700 mg/kg GalN with 4 μg/kg recombinant mouse TNF-α (Peprotech, London) i.v. For testing the therapeutic potential of imatinib, a single dose of imatinib (75 mg/kg) was injected i.p. 30 min after Con A administration.

**Sampling of Material.** For determination of circulating TNF-α, IL-2, and IL-6 levels, blood samples were taken from the tail vein 2 h after challenge. For detection of IL-10 and IFN-γ, blood was taken by cardiac puncture after 8 h. Livers were removed, and one part was frozen in liquid nitrogen (quantification of TNF-α, IL-10, and iNOS mRNA level). Another part was embedded in Tissue-TEK and stored at −20°C until in situ detection of DNA fragments (TUNEL staining) or hematoxylin/eosin staining.

**Analysis of Liver Enzymes.** Liver injury was quantified by measurement of plasma enzyme activities of alanine aminotransferase (ALT) and aspartate aminotransferase by using an automated procedure.

**Pharmacologic Kinase Inhibition.** Human PBMCs or CD14-selected monocytes were stimulated with LPS at a concentration of 100 ng/ml for 12 h in the presence of either solvent or the indicated kinase inhibitor (LY294002, SB203580, PD98059, and JNK-Inhibitor II, all used in a final concentration of 35 μM and purchased from Calbiochem).

**DNA Fragmentation.** In situ detection of DNA fragments was performed by using a FITC-conjugated TUNEL test (Roche, Mannheim, Germany).

**Hematoxylin/Eosin Staining of Liver Sections.** After fixation of the livers with 4% formalin/PBS, liver tissue was sliced and stained with hematoxylin/eosin.

**Statistical Analysis.** Results are expressed as the mean ± SEM. Statistical analyses were performed by using Student’s t test. A value of P ≤ 0.05 was considered significant. Survival curves were compared by using the log rank test. All analyses were performed with PRISM software (GraphPad, San Diego).

**Results**

**Imatinib Inhibits the Production of the Proinflammatory Cytokine TNF-α.** Preincubation of human PBMCs, CD14-selected monocytes, or monocyte-derived macrophages revealed a significant reduction of LPS-induced TNF-α production (Fig. 1A; *, P ≤ 0.05). Fig. 1B demonstrates the dose-dependent inhibitory effect of imatinib on LPS-induced TNF-α production in PBMCs. The inhibitory effect of imatinib reached statistical significance at a concentration of 0.5 μM when compared with controls (Fig. 1B; *, P ≤ 0.01). A similar effect was seen in primary monocytes and monocyte-derived macrophages (data not shown). Quantitative PCR data confirmed the protein data, as imatinib dose-dependently decreased LPS-induced TNF-α mRNA expression in PBMCs (Fig. 1C; *, P ≤ 0.01) and monocytes (data not shown). The reduction of TNF-α was not caused by induction of cell death as determined by propidium iodide staining of imatinib-treated myeloid cells (data not shown). Additionally, imatinib dose-dependently inhibited the production of LPS-induced IL-6 and IL-8 (Fig. 7, which is published as supporting information on the PNAS web site), whereas the production of
the antiinflammatory cytokine IL-10 was not significantly regulated by imatinib (Fig. 8, which is published as supporting information on the PNAS web site).

**LPS-Induced Tyrosine Phosphorylation Is Dose-Dependently Inhibited by Imatinib.** It is well known that imatinib specifically inhibits the activity of several tyrosine kinases. Flow cytometry using a phosphotyrosine-specific antibody revealed that imatinib dose-dependently reduced LPS-induced tyrosine phosphorylation in monocytes (Fig. 2A).

**LPS-Mediated Activation of NF-κB Is Down-Regulated by Imatinib.** NF-κB represents a key transcription factor for the regulation of LPS-induced cytokine gene expression (14). Therefore, we analyzed the activation of NF-κB upon LPS stimulation in cells pretreated with either imatinib or solvent. Indeed, LPS-mediated activation of NF-κB was significantly inhibited by imatinib preexposure (Fig. 2B; *P < 0.05). Accordingly, imatinib-induced inhibition of LPS-induced NF-κB DNA binding was most pronounced 30 min after the addition of LPS (Fig. 2C). The specificity of the NF-κB/probe interaction was confirmed by addition of an excess of unlabeled “cold” probe.

**Fig. 1.** Imatinib inhibits TNF-α production in human myeloid cells. Human PBMCs, monocytes, or macrophages were exposed to either saline or imatinib (1 h, 1 μM) followed by 3-h stimulation with 100 ng/ml LPS. (A) LPS-induced TNF-α levels in supernatants of all three cell types as determined by ELISA (*P < 0.01). (B and C) Imatinib dose-dependently inhibits LPS-induced TNF-α protein (*P < 0.01) and TNF-α mRNA expression (*P < 0.01) in human PBMCs.

**Fig. 2.** Imatinib reduces phosphorylation of IκBα and subsequent NF-κB activation. (A) Monocytes were treated with increasing imatinib concentrations (0.1–10 μM) for 30 min followed by LPS (100 ng/ml) for 15 min, stained for intracellular phosphotyrosine content, and analyzed by flow cytometry. A representative example from three independent experiments is shown. The percentage of phosphotyrosine-positive cells in the monocyte gate and the mean fluorescence intensity (MFI) is given in each diagram. (B) Monocytes were exposed to either saline or imatinib followed by LPS stimulation. Whole-cell lysates were prepared at the indicated time points, and the LPS-induced NF-κB p65 activation was determined by using a chemiluminescent assay (*P < 0.05). H&E, hematoxylin/eosin. (C) LPS-induced NF-κB DNA binding was detected by EMSA. Nuclear extracts were prepared from solvent- or imatinib-treated macrophages stimulated with LPS (50 ng/ml) for 0–60 min. One of three representative experiments is shown. For supershift assays, p50 and p65 anti-NF-κB subunit antibodies were used. (D) The phosphorylation state of IκBα was determined by Western blot using antibodies specific for phosphorylated IκBα and total IκB. A representative result from three independent experiments is shown. Densitometry confirms the decrease in IκBα phosphorylation in response to imatinib (*, *P < 0.01). Results are expressed as percentage of control sample.
Supershift analyses using p50 and p65 anti-NF-κB subunit antibodies revealed that imatinib preferentially inhibited LPS-induced DNA binding of the p65 subunit.

**Imatinib Inhibits LPS-Induced IκBα Phosphorylation.** Phosphorylation of the inhibitory protein IκBα is a prerequisite for subsequent NF-κB activation, as it enables nuclear translocation and DNA binding of NF-κB. Imatinib preexposure of CD14-selected monocytes and PBMCs (data not shown) inhibited LPS-induced phosphorylation of IκBα (Fig. 2D), thereby retaining the NF-κB complex in an inactive state in the cytoplasm. Of note, significant inhibition was seen only at early time points, i.e., 10 min after LPS stimulation.

**p38 Mitogen-Activated Protein Kinase (MAPK) and Mitogen-Activated Protein Kinase Kinase (MEK1) Regulate LPS-Induced TNF-α, but Not IL-10 Production.** We next concentrated on possible upstream signaling events that might be modulated by imatinib by using several kinase inhibitors. Direct pharmacologic inhibition of the p38 MAPK and MEK1 significantly inhibited TNF-α but not IL-10 production, whereas a phosphatidylinositol 3-kinase inhibitor and the c-Jun N-terminal kinase inhibitor prevented the production of both cytokines upon LPS exposure of human PBMCs (Fig. 9, which is published as supporting information on the PNAS web site) and primary monocytes (data not shown).

**Imatinib Prevents Con A-Induced Liver Injury by Inhibition of TNF-α Production.** To translate our *in vitro* findings into an *in vivo* model of TNF-α-dependent inflammatory disease, we tested the effect of imatinib in well characterized murine models of TNF-dependent hepatic failure. Imatinib pretreatment protected animals from Con A-induced liver damage as shown by a significant inhibition of liver enzyme release (Fig. 3A; *, *P* < 0.01). In addition, imatinib prevented histological changes (Fig. 3B) and the induction of hepatic apoptosis shown by a marked reduction of TUNEL-positive cells within the liver (Fig. 10, which is published as supporting information on the PNAS web site). The protective effect was paralleled by a significant down-regulation of hepatic TNF-α mRNA expression (Fig. 3C; *, *P* < 0.05) and a significant reduction of TNF-α plasma levels (Fig. 3D; *, *P* < 0.05).

**Fig. 3.** Inhibition of TNF-α- and macrophage-dependent acute liver injury in mice. Either imatinib- or saline-pretreated BALB/c mice were challenged with Con A (15 mg/kg, *n* = 8 per group). (A) Plasma ALT-levels were determined 8 h after Con A injection (*, *P* = 0.0001). (B) Imatinib prevents histomorphologic liver changes [hematoxylin/eosin (H&E) staining]. A representative example from each group is shown (magnification: ×150). (C and D) Hepatic mRNA levels of TNF-α (C) and the corresponding TNF-α plasma levels (D) were determined 2 h after Con A injection by real-time PCR and ELISA, respectively (*, *P* < 0.05).

**Fig. 4.** Imatinib prevents activation of macrophages *in vivo*. Con A-induced induction of hepatic IL-10 mRNA (A) and circulating plasma levels of IL-10 (B) were not modulated by imatinib (*P* > 0.05).

**Fig. 5.** Therapeutic administration of imatinib prevents acute liver failure. Mice received either imatinib or saline 0.5 h after Con A administration (*n* = 6 per group). (A) Plasma ALT-levels were determined after 8 h (*, *P* < 0.05). (B) TNF-α plasma levels were determined by ELISA 2 h after Con A injection (*, *P* < 0.05).
circulating TNF-α levels (Fig. 3D; *, P ≤ 0.05). Of note, hepatic expression of IL-10 mRNA (Fig. 4A) and circulating IL-10 levels (Fig. 4B) were not significantly modulated by imatinib pretreatment. Moreover, macrophage-derived mediators such as hepatic iNOS mRNA and IL-6 plasma levels were significantly reduced by imatinib pretreatment (Fig. 11 A and B, which is published as supporting information on the PNAS website). To rule out an inhibitory effect of imatinib on Con A-induced T cell activation, we determined plasma levels of the T cell-derived cytokines IL-2 and IFN-γ. Both cytokines were only marginally affected by imatinib pretreatment (Fig. 11 C and D).

**Therapeutic Administration of Imatinib Prevents Acute Liver Failure.** To test the therapeutic potential of imatinib in TNF-mediated acute liver injury we administered imatinib 0.5 h after Con A injection. Imatinib-injected animals showed a significant reduction of liver enzyme release (Fig. 5A; *, P ≤ 0.05), which was paralleled by a significant inhibition of TNF-α plasma levels (Fig. 5B; *, P ≤ 0.05).

**Imatinib Protects from GalN/LPS-induced but Not from GalN/TNF-induced Liver Injury.** Imatinib pretreatment protected mice also from macrophage-dependent GalN/LPS hepatitis, as shown by a significant decrease of liver enzyme release (Fig. 6A; *, P ≤ 0.01). Prevention from hepatic damage appeared to be mediated by inhibition of LPS-induced production of TNF-α (Fig. 6B; *, P ≤ 0.01). In contrast, imatinib rather increased liver damage in GalN-sensitized animals subsequently injected with TNF-α (Fig. 6C). This observation further strengthens the idea that imatinib potently inhibits myeloid cells, but does not prevent the induction of TNF-α-mediated cell death in hepatocytes.

**Imatinib Significantly Improved Survival upon GalN/LPS Administration.** Imatinib pretreatment showed a highly significant improvement of survival rates of mice receiving a lethal dose of GalN/LPS (Fig. 6D; *, P ≤ 0.001). Ninety percent of the GalN/LPS-treated animals died within 26 h (n = 10), whereas only 10% of the imatinib-pretreated mice died within the follow-up (96 h, n = 10), with 9 of 10 animals being long-term survivors (8 weeks).

**Discussion**

Imatinib mesylate has been developed for targeted inhibition of the Abelson kinase (c-ABL) (15), which is activated when translocated to the genetic locus of the breakpoint cluster region (leading to the BCR/ABL fusion gene) and which represents the causative pathogenetic event for chronic myeloid leukemia. Of note, because of its physicochemical properties, kinase specificity of imatinib is limited. Despite its well documented clinical efficacy mediated by inhibition of constitutively activated tyrosine kinases, such as BCR/ABL in chronic myeloid leukemia (16, 17), platelet-derived growth factor receptor-α in hypertensive syndrome (18), and mutated c-kit in patients with gastrointestinal stromal tumors (19), other tyrosine kinases such as Flt-3, Lck, and MAPK are affected as well (20). The relevance of the inhibition of other kinases for therapeutic purpose and a deeper understanding of imatinib-induced side effects remain elusive so far.

Our current report shows that therapeutic doses of imatinib dramatically reduced LPS-induced production of the proinflammatory cytokine TNF-α in human myeloid cells. Of note, the production of IL-10 was not significantly regulated, thereby excluding an IL-10-mediated suppression of TNF-α (21). LPS-induced transcriptional activation of TNF-α is critically governed by the transcription factor NF-κB (22). Activation of NF-κB is regulated by phosphorylation of the inhibitor of κBα, which is induced by IκBα kinases and subsequently degraded via the ubiquitin/proteasome pathway. We show that LPS-induced whole tyrosine phosphorylation was dose-dependently inhibited by imatinib. Of note, phosphorylation of IκBα and subsequent NF-κB DNA binding was also inhibited by imatinib preexposure, showing that imatinib impaired LPS-induced activation of NF-κB. Imatinib reduced only nuclear translocation of the p65, while the p50 subunit remained unaffected, which is in line with previous data showing that imatinib modulates distinct NF-κB subunits depending on the cell type studied (3, 23). It is noteworthy that imatinib targets only tyrosine kinases, which excludes it as an direct inhibitor of the serine/threonine kinase family member IκB kinase (24). Therefore, it is conceivable that modulation of upstream signaling events, such as the activation of MAPK (i.e., p38) or c-Jun, led to the observed inhibition of NF-κB (25). Indeed, direct inhibition of p38 MAPK and mitogen-activated protein kinase kinase (MEK1), but not inhibition of phosphatidylinositols 3-kinase or c-Jun N-terminal kinase potentially abrogated the secretion of TNF-α, without affecting IL-10 production. Hence, inhibition of p38 MAPK and/or MEK1 might contribute to the observed effects on myeloid cells via inhibition of NF-κB. Activation of the latter has recently been shown to be inhibited by imatinib in CD34+ progenitor cells and monocytes, thereby inhibiting their reg-
ular differentiation into mature DCs (3, 23). In addition, our data are in line with very recent reports showing a T cell-suppressive effect of imatinib in vitro and in vivo (4, 5, 26). Of note, the immunomodulatory effects of imatinib seem to be cell type-specific, as Borg and coworkers (6) provided evidence that it enhances NK cell function by affecting host DCs in patients with gastrointestinal stromal tumors responding to imatinib carrying the nonmutated WT c-kit. In addition, treatment of murine antigen-presenting cells with imatinib in vitro restored the responsiveness of tolerant T cells from tumor-bearing hosts (27).

TNF-α is known to be a pleiotropic cytokine, which is critically involved in a wide variety of pathologies, such as acute lung and liver injury, graft-versus-host disease, and septic shock (reviewed in ref. 28). TNF-α is primarily produced by macrophages. Inhibition of TNF-α by means of antibodies or soluble decoy receptors has proven to have a clinical benefit in patients suffering from immune-mediated diseases, such as inflammatory bowel disease (29) or rheumatoid arthritis (30). To translate our in vitro findings into murine models of TNF-α-dependent inflammatory disease, we tested the therapeutic efficacy of imatinib in several models of acute inflammation. In Con A-induced Con A hepatitis has recently been described to critically depend on Kupffer cell-derived TNF-α (31). Indeed, we could demonstrate a protective effect of imatinib in Con A-induced hepatitis with a dramatic decrease in circulating TNF-α levels. The hypothesis that imatinib primarily inhibits macrophages in vivo is further supported by the observation that imatinib also inhibited Con A-induced synthesis of IL-6, which is predominantly produced by Kupffer cells (32). Moreover, the expression of the iNOS, which regulates TNF-α production upon Con A application, was down-regulated by imatinib as well (33). The protective effect of imatinib is most likely not caused by T cell inhibition, as the levels of circulating T cell-derived cytokines IL-2 and IFN-γ remained unaffected by imatinib. Moreover, application of imatinib inhibited the inflammatory cascade induced by systemic Con A application in a therapeutic setting. Its application 30 min after Con A challenge significantly down-regulated the release of liver transaminases and the production of TNF-α. To provide further evidence that T cells are not the primary target cell population of imatinib, we tested its efficacy in macrophage-dependent GalN/LPS-induced hepatitis. Again, imatinib protected mice from hepatic failure induced by GalN/LPS, which was paralleled by a significant decrease of TNF-α. In keeping with this observation, imatinib rescued almost all animals from a lethal dose of GalN/LPS. In contrast, liver failure after direct administration of TNF-α with the GalN/TNF-induced model was even aggravated by imatinib. This observation revealed that imatinib most likely protects from acute liver failure via inhibition of TNF-α production by hepatic macrophages (i.e., Kupffer cells) and probably does not directly interfere with TNF-α-induced cell death pathways within hepatic parenchymal cells.

Our findings provide evidence that imatinib potently inhibits TNF-α production in myeloid cells in vitro and in vivo. This finding might be of clinical benefit under conditions of TNF-α-mediated organ damage, as seen in immune-mediated diseases.

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