Proteasome inhibitor bortezomib modulates TLR4-induced dendritic cell activation

Alessio Nencioni, Karin Schwarzenberg, Katharina M. Brauer, Susanne M. Schmidt, Alberto Ballestrero, Frank Grünebach, and Peter Brossart

Evidence from the animal model suggests that proteasome inhibitors may have immunosuppressive properties; however, their effects on the human immune system remain poorly investigated. Here, we show that bortezomib, a proteasome inhibitor with anticaner activity, impairs several immune properties of human monocyte-derived dendritic cells (DCs). Namely, exposure of DCs to bortezomib reduces their phagocytic capacity, as shown by FITC-labeled dextran internalization and mannose-receptor CD206 down-regulation. DCs treated with bortezomib show skewed phenotypic maturation in response to stimuli of bacterial lipopolysaccharide (LPS) and endogenous sources (including TNF-α and CD40L), as well as reduced cytokine production and immunostimulatory capacity. LPS-induced CCL-2/MCP-1 and CCL5/RANTES secretions by DCs were prevented by DC treatment with bortezomib. Finally, CCR7 up-regulation in DCs exposed to LPS as well as migration toward CCL19/MIP-3β were strongly impaired. As a suitable mechanism for these effects, bortezomib was found to down-regulate MyD88, an essential adaptor for TLR signaling, and to relieve LPS-induced activation of NF-κB, IRF-3, and IRF-8 and of the MAP kinase pathway. In summary, inhibition of DC function may represent a novel mechanism by which proteasome inhibitors exert immunomodulatory effects. These compounds could prove useful for tuning TLR signaling and for the treatment of inflammatory and immune-mediated disorders. (Blood. 2006;108:551-558) © 2006 by The American Society of Hematology

Introduction

Pharmacologic inhibitors of the proteasome have recently received much attention in the light of their potent antitumor activity.1,2 In this setting, bortezomib (Velcade) was demonstrated to be effective in the treatment of multiple myeloma, when taken alone or in combination with traditional anticancer drugs.3,4 Besides, preliminary evidence indicates that also patients with non-Hodgkin lymphoma, acute leukemia, or some type of solid tumors may take advantage of treatment with this kind of drugs.5-9 The mechanism through which proteasome inhibitors prevent tumor growth is not completely understood even though inhibition of NF-κB was suggested to play a relevant role, especially in those malignancies in which this transcription factor is constitutively activated, such as multiple myeloma.1,2

Cumulating evidence indicating the importance of the ubiquitin-proteasome pathway in different aspects of the immune response, including antigen processing, apoptosis, cell cycle, costimulation, adhesion, and chemotaxis, has fostered the evaluation of proteasome inhibitors as immunosuppressive agents.10 Evidence from the animal model indicates a potential role for proteasome inhibitors in the treatment of graft-versus-host disease.11-14 Similarly, these drugs proved useful in the animal model for the prevention of allograft rejection, arthritis, experimental autoimmune encephalomyelitis, and psoriasis.10,15-17 Whether proteasome inhibitors affect the immune function in humans remains controversial, since this kind of evaluation is hampered by the pre-existing immunodepression or by heavy pretreatment of the patients in the clinical studies carried out to date. However, lymphopenia was frequently detected in patients treated with bortezomib,14,18 which appears to be due to a direct inhibitory and proapoptotic effect of this compound in human lymphocytes11,14,19 (A.N., unpublished observations, October 2005).

A central role in the initiation of adaptive immune responses is played by dendritic cells (DCs), a trace leukocyte population with antigen-presenting cell (APC) function that patrols the peripheral tissues for the presence of unwanted antigenic material of infectious (and possibly tumoral) source.17,20 Alert signals such as pathogen-derived products are sensed by DCs through Toll-like receptors (TLRs) expressed at the cell surface and thereby trigger phenotypic and functional changes in DCs that lead to their migration to the afferent lymphoid organs.22,23 Here, DCs carry out their ultimate function by presenting the antigens captured in the periphery to T lymphocytes for the initiation of an antigen-specific response.23 TLR signaling in DCs on antigen encounter is believed to be critical for the subsequent immune outcome.23 Therefore, this is presently the subject of intense investigations which will possibly lead researchers to identify novel targeted immunomodulatory therapeutics.22

Given the potential of proteasome inhibitors to interfere with signaling pathways involved in APC function such as NF-κB,1,2 we have evaluated in the present study the effects of the proteasome
inhibitor bortezomib on human monocyte-derived DCs. We show herein that this drug affects DC function at multiple levels by impeding antigen uptake through phagocytosis and by down-modulating DC response to the pathogen-derived product lipopolysaccharide (LPS), as well as to endogenous inflammatory cytokines and prostaglandins.\(^{20,22,24,25}\) LPS-induced signaling via NF-κB, interferon regulatory factors (IRFs), and the mitogen-activated protein (MAP) kinase pathway were all found to be impaired by bortezomib in DCs, thus suggesting novel molecular targets of proteasome inhibitors in immune cells.

---

**Materials and methods**

**Reagents**

The medium used for cell cultures was RPMI 1640 supplemented with 10% inactivated FCS, 50 nM 2-mercaptoethanol, and antibiotics, all purchased from Gibco-BRL (Grand Island, NY). Bortezomib (Velcade) was obtained from the pharmacy of the University Clinics in Tübingen (Tübingen, Germany). Human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) (molgramostim, Leucomax) was from Novartis (Basel, Switzerland). Human recombinant interleukin-4 (IL-4), IL-1, IL-6, and TNF-α were purchased from R&D Systems (Wiesbaden, Germany). CD40-ligand (CD40L) was from Bender Medsystems (Vienna, Austria). PGE\(_2\); was from Biomol (Plymouth Meeting, PA). LPS was obtained from Sigma (Steinheim, Germany).

**DC generation from adherent monocytes**

DCs were generated from adherent monocytes as described elsewhere.\(^{26,29}\) Buffy coat preparations from healthy volunteers were obtained from the blood bank of the University of Tübingen (Tübingen, Germany). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll/Paque (Biochrom, Berlin, Germany) density gradient centrifugation. Cells were resuspended in serum-free X-VIVO 20 medium (Cambrex, Verviers, Belgium) and allowed to adhere (1 × 10\(^7\) cells/well) in 6-well plates in a final volume of 2 mL. After 2 hours of incubation at 37°C, nonadherent cells were removed by extensive washing. DCs were generated by culturing the adherent monocytes in RPMI-based medium supplemented with 100 ng/mL GM-CSF and 20 ng/mL IL-4. The medium was replenished with cytokines every other day. Cells were harvested for further experiments at day 6 of culture.

**Immunostaining and cell viability assay**

Cells were stained using fluorescein isothiocyanate (FITC)– or phycoerythrin-conjugated mouse monoclonal antibodies against CD14, CD25, CD69, CD80, HLA-DR, CD54, CD206 (Becton Dickinson, Heidelberg, Germany); CD40, CD86 (PharMingen, Hamburg, Germany); CD83 (Immunotech, Marseille, France); DC-SIGN and CCR7 (R&D Systems, Minneapolis, MN), and mouse IgG isotype control (Becton Dickinson). Cells were analyzed on a FACSCalibur cytometer (Becton Dickinson). To calculate the percentage of positive cells, a proportion of 1% false-positive events was accepted in the negative control samples throughout.

For the determination of cell viability, cells were stained with 5 μg/mL propidium iodide (PI) and analyzed by flow cytometry.

**Mixed leukocyte reaction (MLR) assay**

A total of 10\(^5\) responding cells from allogeneic PBMCs were cultured in 96-well flat-bottom microplates (Nunc, Roskilde, Denmark) with 10\(^4\) stimulator cells. Thymidine incorporation was measured on day 5 by a 16-hour pulse with [\(^3\)H]thymidine (0.5 μCi [0.0185 MBq]/well; Amersham Life Science, Little Chalfont, United Kingdom). For the evaluation of the lymphocyte phenotype following culture with allogeneic DCs, CD3\(^+\) cells were isolated from total PBMCs, making use of CD3 MicroBeads from Miltenyi (Bergisch Gladbach, Germany) according to the manufacturer’s instructions. CD3\(^+\) PBMCs (1 × 10\(^6\)) per well were seeded in 24-well plates in the presence of 10\(^5\) DCs of allogeneic source/well. Cells were harvested 48 hours later, washed with PBS, stained, and analyzed by flow cytometry by gating on the CD3\(^+\) lymphocyte population.

**Migration assay**

Cells (2 × 10\(^5\)) were seeded into a transwell chamber (8 μm; BD Falcon, Heidelberg, Germany) in a 24-well plate, and migration to chemokine-CC motif-ligand 19 (CCL19/MIP-3β; 100 μg/mL; R&D Systems, Wiesbaden, Germany) was analyzed after 16 hours by counting gated DCs for 60 seconds on a FACSCalibur cytometer.\(^{29}\)

**Analysis of endocytic capacity**

For the analysis of endocytic activity, 1 × 10\(^5\) cells were incubated with FITC-dextran (40 000 MW, molecular probes; Invitrogen, Karlsruhe, Germany) for 1 hour at 37°C. As a control, 1 × 10\(^5\) cells were precooled to 4°C prior to the incubation with dextran at 4°C for 1 hour. The cells were washed 4 times and immediately analyzed on a FACSCalibur cytometer.\(^{27}\)

**Cytokine determination**

DCs were incubated at 1 × 10\(^6\)/well in 2 mL medium and treated with different stimuli as indicated. Supernatants were collected and stored at −70°C until use for cytokine determination. Cytokine concentrations were measured with commercially available 2-site sandwich enzyme-linked immunosorbent assays (ELISAs) from Beckman Coulter (Hamburg, Germany; IL-12 and TNF-α) and R&D Systems (Wiesbaden, Germany; CCL5/RANTES, CCL2/MCP-1), according to the manufacturers’ instructions.

**Polyacrylamide gel electrophoresis (PAGE) and Western blotting**

Nuclear extracts were prepared from DCs as described previously.\(^{24,27}\) For the preparation of whole-cell lysates, cells were lysed in a buffer containing 1% Igepal, 0.5% sodium-deoxycholate, 0.1% SDS, 2 mM EDTA, 1 mM PMSF, 2 μg/mL aprotinin, and 1 mM sodium-orthovanadate. Protein concentrations of protein lysates were determined using a bicinchoninic acid (BCA) assay (Pierce, Perbio Science, Bonn, Germany). For the detection of nuclear localized NF-κB family members, approximately 20 μg nuclear extracts was separated on a 10% SDS–polyacrylamide gel and transferred onto nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). Ponceau S staining of the membrane was performed to confirm that equal amounts of protein were present in every lane. The blot was probed with antibodies for RelB (C-19, rat polyclonal), IRF-3 (C-20, goat polyclonal), or IRF-8 (goat polyclonal), all from Santa Cruz Biotechnology (Santa Cruz, CA). The activation state of the MAP kinases p38 and ERK, as well as MyD88 levels, was determined by separating 20 to 30 μg whole-cell lysates on a 12% SDS–polyacrylamide gel and subsequent transfer to nitrocellulose membranes. These were probed with antibodies specific for Phospho-p38 (Thr180/Tyr182, rabbit polyclonal), Phospho-p44/p42 (Thr202/Tyr204, mouse monoclonal), p38 MAP kinase (rabbit polyclonal; all from Cell Signaling Technology, New England Biolabs, Frankfurt, Germany), ERK1 (C-16, rabbit polyclonal; Santa Cruz Biotechnology) and MyD88 (N-19, goat polyclonal; Santa Cruz Biotechnology).\(^{29}\)

**Reverse transcriptase–polymerase chain reaction (RT-PCR) for TLR4 expression**

Total RNA was isolated from cell lysates using QIAGEN RNeasy Mini anion-exchange spin columns (QIAGEN, Hilden, Germany) according to the instructions of the manufacturer. Total RNA (1 μg) was subjected to a 20-μL cDNA synthesis reaction using SuperScript RTII (Invitrogen, Karlsruhe, Germany). Oligo(dT) was used as primer. PCR amplification was performed using 2 μL cDNA. Primers and PCR conditions for β2m were described previously.\(^{25}\) Primer sequences for TLR4 were
5′-CTCAATGGATCAAGGACCA-3′ and 5′-TCCCACTCCAGGTAAGTGTT-3′. PCR temperature profiles were as follows: 5 minutes of pretreatment at 94°C and 22 cycles at 94°C for 30 seconds, annealing at 60°C for 30 seconds and 72°C for 30 seconds. RT-PCR reactions (10 μL) were electrophoresed on a 3% agarose gel and stained with ethidium bromide for visualization under ultraviolet light.

Statistical analysis
Each experiment was performed at least 3 times with similar results. Representative experiments are shown. Unpaired t tests were performed to evaluate the significance of the results.

Results
Bortezomib prevents DC maturation in response to inflammatory signals

In an attempt to evaluate whether the proteasome inhibitor bortezomib would affect DC immune properties, we found that exposure to pharmacologic concentrations of this compound did not modify the phenotype of DCs that were generated from peripheral blood monocytes by incubation with GM-CSF and IL-4 (data not shown). However, treatment with bortezomib inhibited the phenotypic changes that were induced by stimulation of DCs with LPS, a pathogen-derived signal that induces DC maturation and increases DC capacity to prime lymphocyte-mediated reponses. Namely, DC pretreatment with the proteasome inhibitor prevented the up-regulation of the costimulatory molecules CD80, CD86, and CD40 (Figure 1). The levels of CD83, a surface marker expressed by mature DCs, were markedly reduced. Similarly, DCs exposed to bortezomib exhibited a down-regulation of the integrin CD54 and of DC-SIGN, a C-type lectin that mediates adhesion with T cells by stabilizing the DC/T-cell contact zone. The normal up-regulation of HLA class 2 molecules in response to LPS was slightly reduced by the proteasome inhibitor while CD14 remained undetectable, thus ruling out de-differentiation to monocytes. DC maturation in response to the endogenous signals TNF-α, CD40L, or a monocyte-conditioned medium mimics that includes IL-1, IL-6, PGE2, and TNF-α was also profoundly affected, as shown by the levels of CD1a and CD83 expression (Figure 2), indicating that bortezomib acts as a powerful inhibitor of DC function in response to a multiplicity of inflammatory stimuli (Figure 2).

Bortezomib blunts DC allostimulatory capacity and cytokine secretion

The effect of bortezomib on DC immunostimulatory capacity was subsequently evaluated in MLRs. Here, we found that treatment of immature DCs with bortezomib does not substantially affect their capacity to prime allogeneic lymphocyte proliferation (Figure 4A). However, consistent with the effects of the proteasome inhibitor on the phenotypic response to LPS, we found that pretreatment with bortezomib reduced the capacity of LPS-primed DCs to induce proliferation (Figure 4B) and expression of activation markers (CD25, CD69, and HLA-DR) (Figure 4C) by allogeneic T cells.
Besides the effect of bortezomib on the expression of costimulatory molecules by DCs, it is likely that a reduced capacity to produce immunostimulatory cytokines is also responsible for this effect, because TNF-\(\alpha\) and IL-12 secretion by DCs on stimulation with LPS were found to be profoundly reduced (Figure 5A-B).

**Figure 5. IL-12 and TNF-\(\alpha\) secretion by DCs are modulated by bortezomib.** (A-B) Adherent monocytes cultured in the presence of GM-CSF and IL-4 for 6 days were stimulated with or without bortezomib at the indicated concentrations (in ng/mL) for 24 hours. LPS (100 ng/mL) was added during the last 16 hours of incubation. Supernatants were subsequently collected, and IL-12 (A) and TNF-\(\alpha\) (B) were determined by commercially available ELISAs.

**Impaired migratory capacity in DCs exposed to bortezomib**

Mature DCs typically acquire the capacity to migrate in response to CCL19/MIP-3\(\beta\) because of the expression of the cognate receptor CCR7. This effect is believed to be crucial to ensure that DCs reach the afferent lymphoid tissues.\(^\text{20,21}\) Here, we found that DCs exposed to bortezomib and thereafter stimulated with LPS lack capacity to migrate toward CCL19 (Figure 6A). In parallel, we observed that bortezomib blunted the expression of CCR7, the corresponding receptor for CCL19, on LPS-stimulated DCs, which likely accounts for the impaired migration ability (Figure 6B). Note that LPS-induced secretion of chemokine-CC motif-ligand 2 (CCL2/MCP-1), a cytokine involved in monocyte recruitment to the sites of inflammation,\(^\text{31}\) and of chemokine-CC motif-ligand 5 (CCL5/RANTES), a powerful chemoattractant and leukocyte activator,\(^\text{32}\) are also reduced when DCs are exposed to bortezomib (Table 1).

**Effects of bortezomib on DC intracellular signaling cascades**

The effect of the proteasome inhibitor bortezomib on intracellular signaling in DCs was subsequently evaluated, making use of LPS as a stimulus. It has become clear that LPS binding to TLR4...
induces a large number of genes through activation of NF-κB, MAP kinases, and IRFs. Proteasome inhibition affects NF-κB signaling by preventing degradation of the NF-κB inhibitor IκBα, an effect that appears to account, at least partially, for the antitumor activity of proteasome inhibitors. We monitored herein LPS-induced nuclear relocalization of RelB, a NF-κB family member known to be relevant in DC differentiation and response to inflammatory stimuli. In fact, treatment with bortezomib led to reduced levels of this transcription factor in nuclear extracts after stimulation with LPS (Figure 7A). The MAP kinase pathway, which is involved in the regulation of DC maturation and survival, was also affected by bortezomib. In particular, we found here that DCs treated with bortezomib had reduced levels of phosphorylated p42/ERK2 protein on stimulation with LPS. Conversely, p38 phosphorylation was unaffected (Figure 7B). Finally, IRF-3 as well as IRF-8/ICSBP nuclear relocalizations were also impeded by bortezomib (Figure 7A), indicating that proteasome inhibition affects TLR4 signaling at multiple levels.

TLR intracellular signaling is mediated by adaptor proteins that are recruited to the TLR4-MD2 complex on ligand binding. Myeloid differentiation primary response gene 88 (MyD88) plays a key role because it specifically interacts with the IL-1 receptor-associated kinases (IRAKs), IRAK1 and IRAK4, which in turn activate the MAP kinase cascade and NF-κB. Monitoring MyD88 levels in DCs that had been exposed to bortezomib demonstrated that the proteasome inhibitor blunts MyD88 expression (Figure 7C), an effect that may account for TLR signaling inhibition by bortezomib, at least with respect to NF-κB and MAP kinase relieving. Conversely, TLR4 levels in DCs were only found to be minimally affected by exposure to high concentrations of proteasome inhibitor (Figure 7D) as detected by semiquantitative RT-PCR.

### Reduced endocytic capacity in DCs treated with bortezomib

To the completion of our observations on the effects of the proteasome inhibitor bortezomib on DCs, we monitored DC capacity to internalize FITC-labeled dextran as an assay of DC endocytic capacity. To this end, we decided to use immature DCs because these normally show pronounced internalization capacity as compared with mature DCs. Interestingly, pretreatment of DCs with bortezomib reduced their endocytosis ability (Figure 8), indicating that proteasome inhibition may affect DC function also by impeding antigen uptake for further processing and presentation. Subsequent analysis demonstrated how DC exposure to bortezomib prevents the expression of the mannose receptor CD206, which is likely to contribute to impair endocytosis in DCs (Figure 9A). Finally, bortezomib-induced CD206 down-regulation was also observed in DCs that were stimulated to undergo maturation in response to TNF-α, LPS, CD40L, and a monocytic conditioned medium mimics (Figure 9B).

### Discussion

The present study indicates the potential of proteasome inhibitors to interfere with DC function by down-modulating DC responsiveness to inflammatory signals and by reducing DC endocytic activity.
capacity, DC encounters with proinflammatory stimuli of exogenous (pathogen-derived products such as LPS) and endogenous (ie, TNF-α, IL-1, prostaglandins) sources induce phenotypic and functional changes that enhance DC immunogenicity and regulate their life span.20,21,41,42 Here, we found that, although not significantly affecting phenotype and immunostimulatory capacity of immature DCs, pharmacologic concentrations of bortezomib rather prevent phenotypic changes and activation that are induced in DCs by proinflammatory signals.8 Namely, treatment with the proteasome inhibitor affected the up-regulation of costimulation (CD40, CD80, CD86) and adhesion (CD54, DC-SIGN) molecules and DC markers (CD1a, CD83) in response to LPS, TNF-α, CD40L, and a monocyte-conditioned medium mimics. Bortezomib inhibitory properties extended to DC capacity to secrete activatory cytokines such as IL-12 and TNF-α and to induce lymphocyte proliferation in MLRs. DCs that were exposed to bortezomib showed impaired migratory capacity toward CCL19/MIP-3β and reduced secretion of chemokine ligands involved in inflammation and lymphocyte recruitment such as CCL2/MCP-1 and CCL5/RANTES.43 The latter results are consistent with those of a previous study that evaluated the effect of MG132 on retinal epithelial cells44 while diverging from those of another report in which no effect of proteasome inhibition on RANTES secretion was detected.45 Finally, we found that bortezomib also interferes with antigen capture by DCs because it reduced FITC-labeled dextran uptake and down-regulated the scavenger mannose receptor CD206.46

It should be observed that, although these effects appear to be merely functional in response to low bortezomib concentrations (up to 1 ng/mL), high concentrations of proteasome inhibitor also affect DC viability, as detected in our experiments and consistent with recent data obtained with whole PBMCs.19 This effect appears to correlate with the up-regulation of proapoptotic Bcl-2 family members such as Bax in DCs exposed to bortezomib (A.N., unpublished data, March 2005; data not shown). Therefore, increased susceptibility to undergo apoptosis is likely to contribute to further down-regulate DC function in response to bortezomib, particularly when this compound is taken at high concentrations.

Following the observation that bortezomib affects DC maturation and viability, we evaluated potential effects of this proteasome inhibitor on intracellular signaling pathways that are known to regulate DC immune properties and survival.20,21,28,29 To this end, we used LPS as a stimulus. Two major signaling pathways are normally triggered on TLR4 binding by LPS:22,33 one is dependent on Toll-IL-1 receptor (TIR) domain-containing adaptor protein (TIRAP) and MyD88. MyD88 recruits members of the IRAKs and tumor necrosis factor (TNF) receptor–associated factor 6 (TRAF6) to finally activate NF-κB and the MAP kinase pathways. The other signaling pathway uses adaptor proteins such as TRIF-related adaptor molecule (TRAM) and TIR domain-containing adaptor inducing IFN-γ (TRIF, also known as TICAM-1) to activate the IRFs (as well as NF-kB), thereby leading to production of type I IFN. NF-κB family members control DC development and different aspects of DC function, RelB being particularly relevant in this context.20,21,34,35 However, the IFN pathways are also being assessed growing importance in DC biology. IRF-3 is a well-known mediator of IFN production by inflammatory cells and of endotoxic shock in response to LPS.46,47 IRF-8 is expressed in hematopoietic progenitors and is required for plasmacytoid DC development in mice.48-50 Interestingly, this molecule has also been related to the normal development and trafficking of Langerhans cells and dermal DCs, because it favors CCR6 and CCR7 expression.51 IRFs participate with NF-kBs in the control of IL-12 production.47,52-54 Thus, the current view suggests that IRFs and NF-κB signaling cooperate at multiple levels and are both necessary for inducing an optimal DC response to pathogens. Finally, MAPK kinases were also reported to be involved in modulating DC response to LPS.36-39

Here, we found that bortezomib blocks RelB activation on stimulation with LPS. NF-κB inhibition is a well-known effect of proteasome inhibitors and is attributed to the impeded degradation of the inhibitor IκBα.1,2 Thus, this observation is consistent with previous studies. The MAPK pathway in DCs stimulated with LPS was also observed to be affected. Namely, although not preventing p38 activation, bortezomib reduced ERK phosphorylation. Such an effect was recently detected in studies with hematologic malignancies and suggests a selective effect of proteasome inhibition on the ERK pathway.55-57 Finally, we found that bortezomib interferes with IRF-3 and IRF-8 activation in response to LPS in DCs. Given the reported role for these IRFs in DC migratory capacity and IL-12 secretion,51-54 it is appealing to speculate a particular relevance for the obstruction of this signaling pathway in the inhibitory effects carried out by bortezomib.

We describe here for the first time that a proteasome inhibitor affects the expression of MyD88, an adaptor protein that plays a crucial role in upstream TLR signaling.22,33 The current model of LPS-induced signal cascades suggests that this effect may contribute to affect NF-κB and MAP kinase activation by IRAKs. On the contrary, the mechanism by which proteasome inhibition affects the IFN pathway, which is believed to be MyD88-independent, remains unclear. Even though NF-κB was proposed to play a role in IRF-3 transcriptional activity,58 it seems unlikely that the sole NF-κB relieving by bortezomib also accounts for inhibition of IRF-3 and IRF-8, based on the present understanding of IRFs signaling.22,33 Thus, more investigation is required to elucidate this effect. Yet, these data altogether delineate profound effects of bortezomib on key signal transduction cascades and suggest a
common mechanism that may account for DC refractoriness to activation signals.

In conclusion, the present work shows that DC function is profoundly affected by bortezomib, probably via interference with NF-κB and other pivotal signal transduction pathways. To the extent to which the results of this study can be extrapolated to in vivo setting, they suggest a novel mechanism of immunomodulation by proteasome inhibitors and foster the evaluation of these compounds as therapeutics capable of modulating TLR signaling and possibly inflammatory and immune responses.

Acknowledgments
We thank Sylvia Stephan and Brunni Schuster for the excellent technical assistance.

References
24. For personal use only.on May 29, 2017. by guest
25. Weighardt H, Jusek G, Mages J, et al. Identifica-


Proteasome inhibitor bortezomib modulates TLR4-induced dendritic cell activation

Alessio Nencioni, Karin Schwarzenberg, Katharina M. Brauer, Susanne M. Schmidt, Alberto Ballestrero, Frank Grünebach and Peter Brossart