The effect of zoledronic acid on the function and differentiation of myeloid cells

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Background and Objectives. Bisphosphonates are widely used for treatment of osteoporosis and metastases of the skeletal system. Recent data suggest that bisphosphonates may not only reduce bone loss but also exert direct anti-tumor, anti-angiogenic and γδ T-cell activating effects, properties which depend at least partially on their affinity to phagocytosing and antigen-presenting cells (i.e. osteoclasts and monocytes). The latter represent the major source of dendritic cells (DC). Thus, we determined the immunomodulatory properties of zoledronic acid (ZA), a member of the latest generation of bisphosphonates.

Design and Methods. Primary human monocytes, macrophages, immature and mature dendritic cells were incubated with increasing doses of ZA for subsequent analysis of cell surface marker expression and cytokine production. In addition, phagocytic and allo-stimulatory properties, differentiation capacity, and NF-κB activation were determined.

Results. Therapeutic doses of ZA inhibited the *in vitro* generation of DC from monocytes, as shown by an impaired up-regulation of maturation markers. In parallel, ZA also impaired lipopolysaccharide-induced activation of NF-κB, which represents a critical factor for DC differentiation. Accordingly, the activation of allogeneic T cells by ZA-treated DC in a mixed-lymphocyte reaction was significantly reduced. Finally, ZA inhibited the production of tumor necrosis factor-α in monocyte-derived cells and impaired the phagocytic capacity of macrophages and immature DC.

Interpretation and Conclusions. Therapeutic doses of ZA modulate monocyte, macrophage and DC function and might thereby modulate immune function.

Key words: monocytes, dendritic cells, zoledronic acid

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Bisphosphonates are analogs of endogenous pyrophosphates in which the central oxygen atom is replaced by a carbon atom. This chemical modification renders these compounds resistant to hydrolysis and allows two additional chains to be substituted. One of the chains generally contains a hydroxyl moiety that has high affinity for calcium crystals of the bone. The other is responsible for the pharmacokinetic potency of the drug. Newer generation bisphosphonates, such as zoledronic acid (ZA) are 10,000 to 100,000-fold more potent than the older generation bisphosphonates.[1]

Bisphosphonates are currently widely used for the prevention and treatment of osteoporosis as well as skeletal metastases in patients suffering from malignant diseases, such as breast cancer or multiple myeloma, both of which are characterized by osteolytic lesions within the skeletal system. In addition, recent pre-clinical *in vitro* and *in vivo* models provided evidence that high doses of bisphosphonates may not only reduce bone loss by inhibition of osteoclast activity, but may also exert direct anti-tumor and anti-angiogenic effects.[2-4] Bisphosphonates and their metabolites have been shown to share structural homologies with recently identified γδ T-cell-ligands, leading to potent activation of γδ T-cells.[5,6] These effects may represent a potential novel anti-tumor mechanism induced by aminobisphosphonates, which has been linked to the selective expansion of Vγ9Vδ2 T-cells. Indeed, Vγ9Vδ2 T-cells exert potent anti-tumor activity *in vitro* by killing malignant plasma cells isolated from patients with multiple myeloma.[7] In support of this concept, it was found that the combination of pamindronate and low-dose interleukin-2 induced the *in vivo* expansion of Vγ9Vδ2 T-cells in a small cohort of patients with non-Hodgkin's lymphoma.[8] A recent study further demonstrated that application of ZA is able to induce γδ T-cell expansion and function in patients suffering from epithelial malignancies.[9]

Both the anti-osteolytic as well as the T-cell activating properties of biphosphonates have been shown to depend at least partially on the affinity of these drugs for phagocytosing and/or antigen-presenting cell types, such as osteoclasts and monocytes.[10,11] The latter represent key players in the regulation of the immune response and are the major source of dendritic cells (DC). Hence, it is tempting to speculate that ZA, a widely used member of the latest generation of bisphosphonates, exerts immuno-regulatory properties by modulating monocytic cell function and differentiation. The aim of this study was to investigate the effects of therapeutic

doses of ZA on monocyte/macrophage function and DC differentiation from monocyte precursors.

Design and Methods

Reagents

Lipopolysaccharide (LPS) (from *Escherichia coli* 055:B5) and fluoroscein isothiocyanate (FITC)-dextran were purchased from Sigma-Aldrich (Vienna, Austria). The monoclonal antibodies directed against CD1a, CD14, CD16, HLA-DR, CD11c, CD40, CD83, CD86, and CD206, and the corresponding isotype control monoclonal antibodies were purchased from Becton Dickinson (San José, CA, USA). ZA was a kind gift from Novartis (Austria) and was dissolved in distilled water.

Generation of monocytes, DC and macrophages

DC and macrophages were generated from monocytes of healthy individuals. In brief, $CD14^+$ monocytes were isolated from peripheral blood by magnetic bead separation using anti-CD14 microbeads (Miltenyi Biotech, Bergisch-Gladbach, Germany) to a purity that was generally >90%, as determined by FACS analysis. The purified monocytes were either used immediately or cultured at 1×10^6 cells/mL in RPMI 1640 supplemented with 10% fetal calf serum. For DC generation 1000 U/mL human recombinant granulocyte-colony stimulating factor (rGM-CSF), and 800 U/mL human recombinant interleukin-4 (Peprotech, London, UK) were added. Macrophages were generated with 1000 U/mL human rGM-CSF for 10 days. The medium was changed on day 3 and day 6, respectively. For the induction of DC maturation, immature DC were incubated with LPS (100 ng/mL) on day 6 for 24 hours. To determine the impact of ZA on myeloid cell phenotye and function, ZA or solvent was added at 0.1 μM, 1.0 μM or 10 μM for 24 hours to monocytes, immature DC (iDC) or fully mature DC (mDC). To determine the influence of ZA on DC maturation, ZA was added to iDC during LPS-induced maturation.

Cytokine production

In order to detect tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) in supernatants, cells were pre-incubated with either the indicated concentrations of ZA or solvent and subsequently stimulated with LPS for an additional 6 hours. Cytokines were assayed in supernatants by ELISA strictly according to the manufacturer's instructions (BD Pharmingen, San José, CA, USA).

Flow cytometric analysis of monocytes, macrophages and DC

The death of monocytes, macrophages or DC was quantified using the annexin V-FITC apoptosis detection kit (Roche, Vienna, Austria). Cells undergoing apoptosis expose phosphatidylserine, which is normally present on the inner cell membrane leaflet, to the outer leaflet, allowing annexin V to bind to the phosphatidylserine at the cell surface. Cell aliquots were stained with FITC-labeled annexin V and counterstained with propidium iodide, which stains primary necrotic cells.

In order to determine changes of cell phenotype, ZA- or solvent-treated cells were washed and subsequently incubated with either the indicated monoclonal antibodies or the corresponding isotype controls dissolved in phosphate buffered saline (PBS) containing 2% fetal bovine serum (FBS) and 0.01% NaN_3 for 20 min at room temperature. Cells were then acquired on a FACSCalibur (Becton Dickinson) and data were analyzed using Cell Quest Software.

Quantification of endocytosis

ZA- or solvent-treated monocytes, macrophages, iDC, and mDC were washed and subsequently resuspended in PBS for incubation with 2 mg/mL FITC-dextran (Sigma, Vienna, Austria) at 37°C or at 4°C (negative control). After 1 (macrophages) to 6 (monocytes) hours, uptake was stopped by adding ice-cold PBS containing 2% FBS and 0.01% NaN3. Cells were washed a further three times and analyzed on a FACSCalibur. Surface binding values obtained when incubating cells at 4°C were subtracted from values measured at 37°C.

Mixed lymphocyte reaction

For the primary mixed lymphocyte reaction assay allogeneic $CD3^+$ T cells were magnetically isolated from healthy donors after Ficoll-separation of peripheral blood mononuclear cells. iDC and mDC were pre-treated with ZA as indicated, subsequently washed and then cultured in 96-well microculture plates along with allogeneic T cells in complete RPMI 1640/10% FCS. On day 5 proliferation was measured by the metabolic activity of the cells using the EZ4U system (Easy for You, Biomedica GmbH, Austria). The red soluble formazans released into the culture medium were determined by extinction measurement using the ELISA reader.

Detection of NK-κβ activation

LPS-induced activation of NF-κβ in DC was determined by the chemiluminiscent EZ Detect p65 Transcription Factor Kit (Pierce, Rockford, USA) strictly according to the manufacturer's instructions. In brief, whole cell fractions of LPS-stimulated DC (stimulation duration 0.5 h) were prepared and incubated in EZ-Detect assay plates together with 50 μL of binding buffer for 1h. After rinsing, the primary antibody was detected using a horseradish peroxidase-conjugated secondary antibody. The chemiluminiscent signal was detected using a Luminometer (Wallac, Vienna, Austria). Positive (HeLa extracts) and negative controls (HeLa extracts plus wild type NF-κβ competitor duplex) were included in each assay.

Statistical analysis

After analysis of variance, the Student's t-test was used. p values <0.05 were considered statistically significant. The statistical analyses were performed using GraphPadPrism software.

Results

Low dose ZA does not induce apoptosis in monocytes and dendritic cells

To determine the direct cytotoxic effect of ZA on myeloid cells, $CD14^+$-selected monocytes and iDC were treated with increasing concentrations of ZA (0.1 μM, 1.0 μM or 10 μM) for 24 hours. As shown by annexin/propidium iodide staining, low doses of ZA did not induce a significant increase of the rate of apoptosis and/or necrosis in monocytes (Figure 1A) and dendritic cells (Figure 1B) as compared to the rate in solvent-treated cells. Similar results were obtained at 48 and 72 hours (*data not shown*).

Inhibition of TNF-α-production by monocytes, dendritic cells and macrophages

Incubation of monocytes, iDC, mDC or primary macrophages with increasing doses of ZA for 24 hours inhibited the LPS-induced production of TNF-α only upon pre-incubation with 10 μM ZA (Figure 2A-D). In contrast, LPS-induced production of the anti-inflammatory cytokine IL-10 was not significantly modulated in ZA-treated myeloid cells (Figure 2E-H).

Dose-dependent inhibition of phagocytosis by ZA

Phagocytosis enables the uptake of soluble antigens for subsequent processing and MHC class II-dependent or MHC class I-dependent cross-presentation. To test the effects of low dose ZA on phagocytic properties, cells were pre-treated for 24h with 0.1 or 1.0 or 10 μM ZA. As shown in Figure 3, ZA dose-dependently inhibited the phagocytosis of FITC-dextran by iDC and macrophages but not by monocytes, ZA-matured DC, or mDC. Of note, mDC and monocytes are known to have low phagocytic capacity.

Impaired differentiation of ZA-treated monocytes into DC

To investigate the effect of ZA on monocyte differentiation into DC and on final LPS-induced DC-maturation as well as on myeloid cell phenotype, CD14-selected monocytes, iDC, mDC and macrophages were exposed to 0.1, 1.0 and 10 μM of ZA. Short-term incubation of $CD14^+$ monocytes with ZA did not significantly affect the phenotype of these cells (Figure 4A and B). In contrast, addition of ZA to iDC (Figure 4C and D) or ZA-matured DC (Figure 4E and F) significantly inhibited up-regulation of DC-specific surface markers such as CD83, CD86, CD40, and CD206. In contrast, the phenotype of already fully mature DC was only marginally affected by ZA (Figure 4G and H).

ZA-treated DC have impaired allo-stimulatory capacity

T-cell activating properties of ZA-treated DC were determined by stimulating allogeneic T-cells with iDC, ZA-matured DC and mDC. Figure 5A illustrates that ZA-matured DC had a significantly impaired allo-stimulatory capacity as compared to untreated DC when ZA was added either before or during the final maturation phase. In contrast, fully mature DC appeared to be relatively resistant to the effects of ZA (Figure 5A).

ZA downregulates LPS-induced activation of NF-κβ in DC

The activation of NF-κB has been demonstrated to be critical for the development and survival of DC. Activation of NF-κB in DC derived from ZA-treated monocytes was markedly reduced upon short-term stimulation for 30 minutes with 100 ng/mL LPS (Figure 5B).

Discussion

Exposure to bisphosphonates may not only reduce bone loss by inhibition of osteoclast activity but may also induce profound, direct anti-tumor effects.[12] Recent reports underscored the apoptosis-inducing properties of bisphosphonates, especially the new aminobisphosphonate zoledronic acid.[13] However, the doses used in the presented studies were far from plasma levels achieved after administration of the standard dose of ZA, i.e. 4 mg per month. Doses above 50 μM exert a direct cytotoxic effect on a wide variety of tumor cell lines *in vitro*.[14] However, after application of the standard dose of ZA, maximum plasma levels are 1.0 μM, which subsequently decline to 0.01 μM within 24 hours.[15] In this study, we, therefore, used low doses of ZA ranging from 0.1 μM to 10 μM and analyzed their *in vitro* immuno-modulatory effects. We primarily focused on myeloid cell types, as the major target cell type of bisphosphonates are osteoclasts, which belong to this common cell family derived from a myeloid progeny.

There is already evidence that older generation bisphosphonates may alter the function of myeloid cells. However, probably due to the use of different cell types, different compounds and varying experimental setups, the exact effect of bisphosphonates on myeloid cells remains contradictory.[11,16]

Here we describe for the first time that incubation of monocytes and monocyte-derived cells (i.e. macrophages and DC) with low doses of ZA impaired myeloid cell function and reduced the production of the major monocyte-derived cytokine, TNF-α. In contrast

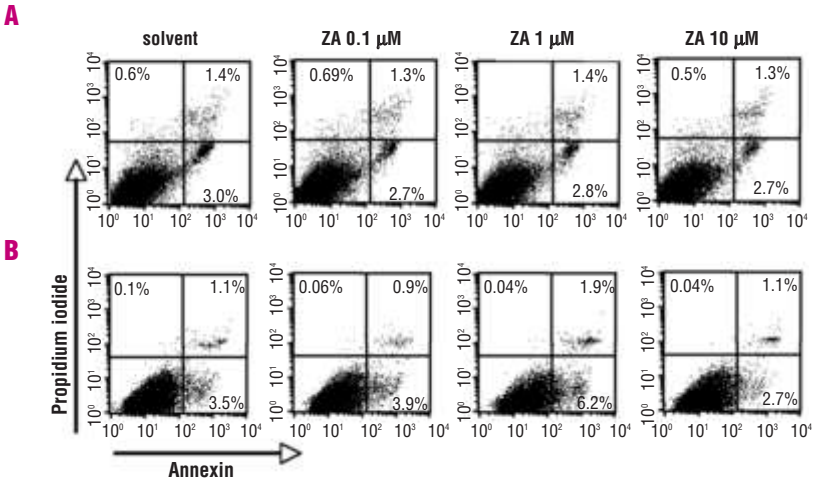

Figure 1. ZA does not induce cell death in monocytes or DC. Freshly isolated CD14+-monocytes (A) and monocyte-derived iDC (B) were incubated for 24 hours with either 0.1 μM, 1.0 μM or 10 μM ZA or solvent alone. A representative staining of three independent experiments is shown.

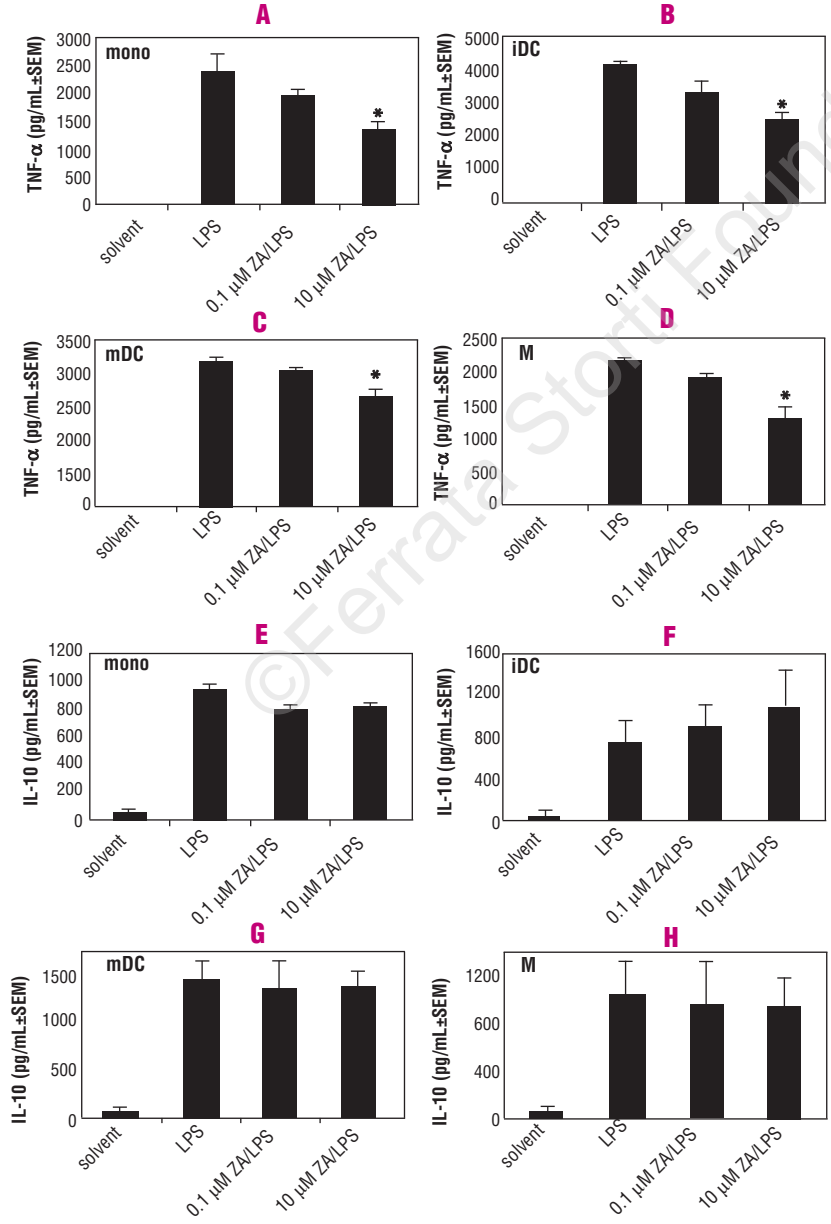

Figure 2. ZA inhibits the production of TNF-α by monocytes, DC and macrophages. Monocytes, monocyte-derived iDC, mDC or primary macrophages were incubated for 24 hours with either 0.1 μM, 1.0 μM, 10 μM ZA or with solvent followed by stimulation with LPS for 6 hours. TNF-α levels were determined in supernatants of LPS-stimulated (A) monocytes (n=3), (B) iDC (n=5), (C) mDC (n=3) and (D) macrophages (n=4). In contrast, IL-10 production was not altered in ZA-treated (E) monocytes (n=3), (F) iDC (n=5), (G) mDC (n=3) or (H) macrophages (n=4) (*$p \leq 0.05$).

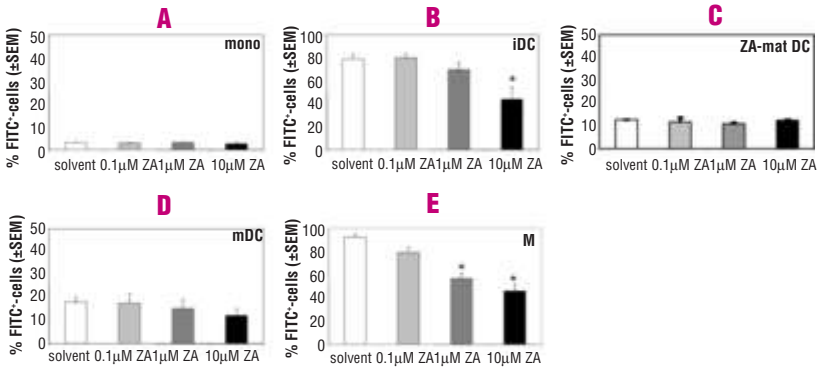

Figure 3. *ZA inhibits phagocytosis of iDC and macrophages.* (A) Monocytes, (B) iDC, (C) ZA-matured DC, (D) mDC and (E) macrophages were incubated with either 0.1 μM, 1.0 μM, 10 μM ZA or solvent for 24 hours followed by addition of FITC-dextran and incubation at 37°C for 1 (macrophages) to 6 (monocytes) hours. As a control, cells were incubated at 4°C (filled line). ZA-treatment dose-dependently decreased FITC-dextran uptake of macrophages and iDC (C+E) (*$p \leq 0.05$). (B+D) ZA treatment had no effect on the phagocytic capacities of monocytes, ZA-matured DC or mDC.

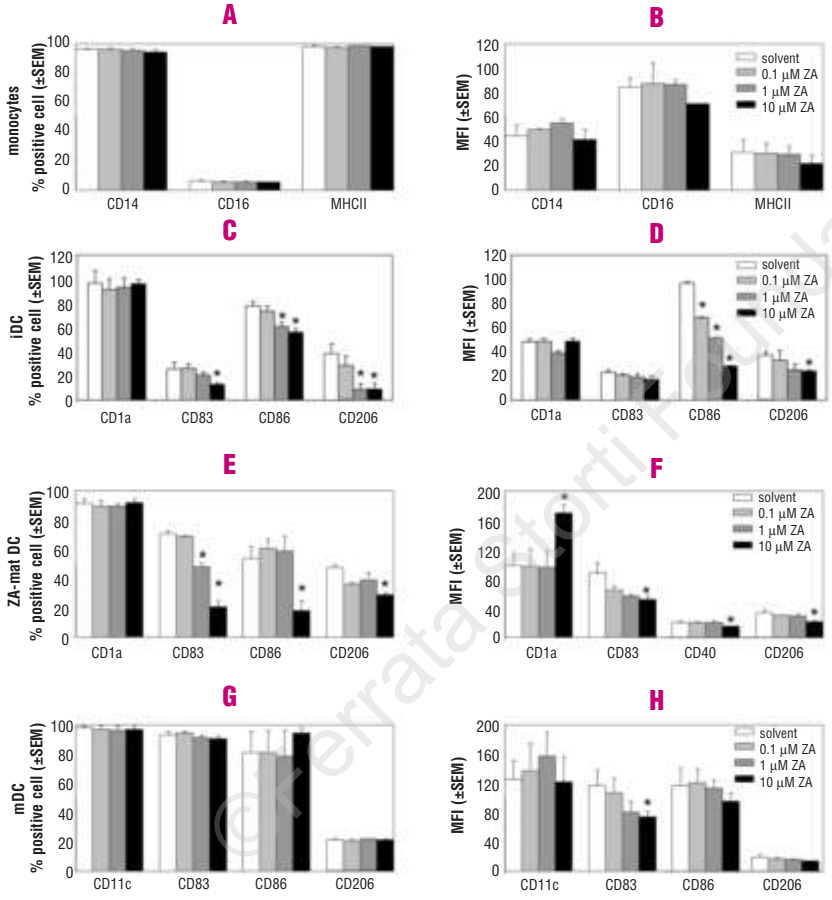

Figure 4. *ZA impairs the maturation of DC.* Surface marker expression of ZA-treated (A+B) CD14⁺-monocytes, (C+D) iDC, (E+F) ZA-matured DC and (G+H) mDC was determined by flow-cytometry. Mean values of positive cells (left panels) and mean fluorescent intensities (right panels) of three independent experiments are shown (*$p \leq 0.05$).

ZA did not affect the production of the anti-inflammatory mediator IL-10. These observations demonstrate that, at least at the dose of 10 μM, ZA impaired myeloid cell sensitivity towards stimulation with LPS. This is in line with a recent report showing that ZA reduced bone loss in a model of TNF-α-mediated arthritis.[17] In addition, phagocytosis of FITC-dextran, which is a characteristic feature of macrophages and iDC is dose-dependently inhibited by ZA. This observation might be due to alterations of the cytoskeleton, i.e. disturbed actin polymerization by inhibition of Rho kinases, which are induced by bisphosphonates in tumor cell lines.[18] This would alter the rigidity of the cell membrane, thereby modulating the phagocytic capacity of macrophages and iDC. In addition, ZA-induced downregulation of CD206, which is the common receptor for FITC-dextran uptake, might also be involved in the reduced phagocytosis.[19] In contrast, in line with previous data,[19] mDC and ZA-matured DC displayed only low phagocytic capacities.

Monocytes represent an important precursor cell population for the generation of DC. We therefore next focused on the effects of ZA on the *in vitro* differentiation of DC from monocytes. Up-regulation of typical maturation markers of DC (i.e. CD83, CD86, and CD40) was markedly impaired, which is in line with *in vitro* and *in vivo* data showing that mature DC have to express the full set of co-stimulatory molecules for

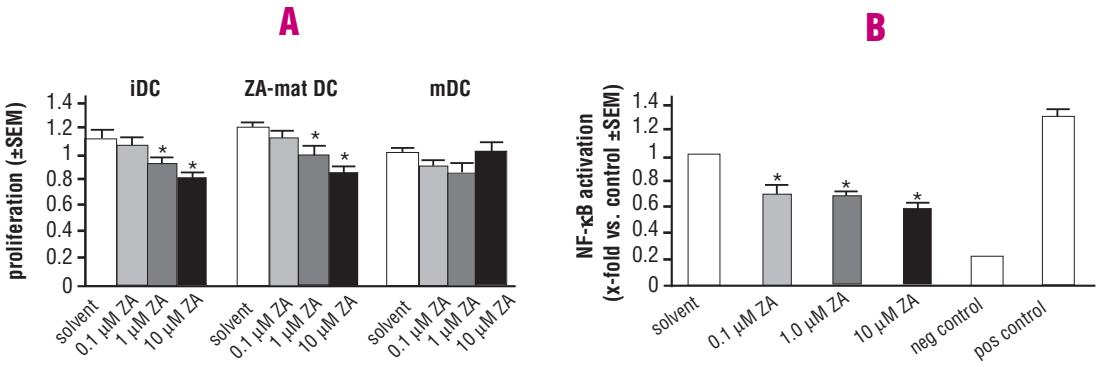

Figure 5. A. ***DC derived from ZA-treated monocytes have an impaired allostimulatory capacity.*** **Immature DC, ZA-matured DC and mDC were used as stimulator cells in a mixed-lymphocyte reaction using CD3-selected T-cells as the responder cells. The proliferation was determined in triplicate cultures (*$p \leq 0.05$). B.** ***DC derived from ZA-treated monocytes*** **caused decreased activation of NF-κB. $CD14^+$-monocytes were freshly isolated from peripheral blood mononuclear cells by magnetic beads and then incubated for 24 hours with 0.1 µM, 1.0 µM or 10 µM ZA or solvent alone. The cells were then stimulated with standard dose GM-CSF together with IL-4 for 6 days. After maturation with 100 ng/mL LPS for 1 hour, protein extracts were prepared for the detection of NF-κB activation (*$p \leq 0.05$, n=3).**

induction of an efficient T-cell response.[20-23] Accordingly, the ability of ZA-treated DC to activate allogeneic T-cells was also impaired.

Activation and DNA-binding of NF-κB are critical events for proper maturation of DC.[24] Interestingly, ZA also affected LPS-induced activation of NF-κβ in DC. These observations are in line with recent reports showing that the anti-angiogenic effects of ZA are due to inhibition of matrix metalloproteinase-9 (MMP-9) expression by tumor-infiltrating macrophages,[25] further corroborating our hypothesis that monocytes/macrophages are target cells for ZA. MMP-9 has also been shown to be critically regulated by NF-κB, which might explain, at least in part, the inhibitory effect of ZA on MMP-9 expression.[26]

In summary, our current report provides the first evidence that pre-treatment of monocytes with therapeutic doses of the new generation bisphosphonate ZA inhibits monocyte/macrophage function and DC maturation *in vitro*. Notably, we used low doses of ZA (0.1 to 10 µM), which more closely reflect the *in vivo* pharmacokinetics after ZA application, as the dose range is far lower than that used in a variety of *in vitro* studies demonstrating the apoptosis-inducing properties of ZA (50 to 100 µM). Our data suggest that the effects of therapeutic doses of ZA in inflammatory diseases as well as in cancer therapy might be mediated at least in part by modulation of myeloid cell function and differentiation. Especially under conditions of bone loss due to chronic inflammation (e.g. Crohn's disease) ZA treatment might provide additional beneficial effects by modulating the exaggerated inflammatory autoimmune response. The clinical significance of ZA-induced immune-modulation in cancer patients with osteolytic bone lesions remains to be elucidated, because these patients already suffer from severe immune defects due to their underlying disease.[27]

AMW: study design, designed and performed experiments, wrote the manuscript; HR: performed experiments, provided infrastructure; HT, GG: study concept, provided money and infrastructure; EG: provided money and infrastructure; DW: study design, profound revision of the manuscript.

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References

1. Anderson KC, Kyle RA, Dalton WS. Landowski T, Shain K, Jove R, et al. Multiple myeloma: new insights and therapeutic approaches. Hematology Am Soc Hematol Educ Program 2000; 147-65.
2. Corey E, Brown LG, Quinn JE, Poot M, Roudier MP, Higano CS, et al. Zoledronic acid exhibits inhibitory effects on osteoblastic and osteolytic metastases of prostate cancer. Clin Cancer Res 2003;9:295-306.
3. Lee YP, Schwarz EM, Davies M, Jo M, Gates J, Zhang X, et al. Use of zoledronate to treat osteoblastic versus osteolytic lesions in a severe-combined-immunodeficient mouse model. Cancer Res 2002;62:5564-70.
4. Wood J, Bonjean K, Ruetz S, Bellahcene A, Devy L, Foidart JM, et al. Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. J Pharmacol Exp Ther 2002; 302:1055-61.
5. Gober HJ, Kistowska M, Angman L, Jeno P, Mori L, De Libero G. Human T cell receptor γδ cells recognize endogenous mevalonate metabolites in tumor cells. J Exp Med 2003;197:163-8.
6. Hayday AC. γδ cells: a right time and a right place for a conserved third way of protection. Annu Rev Immunol 2000; 18:975-1026.
7. Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M. Stimulation of γδ T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. Blood 2000;96:384-92.
8. Wilhelm M, Kunzmann V, Eckstein S, Reimer P, Weissinger F, Ruediger T, et al. γδ T cells for immune therapy of patients with lymphoid malignancies. Blood 2003;102:200-6.

9. Dieli F, Gebbia N, Poccia F, Caccamo N, Montesano C, Fulfaro F, et al. Induction of γδ T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients in vivo. Blood 2003;102:2310-1.
10. Miyagawa F, Tanaka Y, Yamashita S, Minato N. Essential requirement of antigen presentation by monocyte lineage cells for the activation of primary human γδ T cells by aminobisphosphonate antigen. J Immunol 2001;166: 5508-14.
11. de Vries E, van der Weij JP, van der Veen CJ, van Paassen HC, Jager MJ, Sleeboom HP, et al. In vitro effect of (3-amino-1-hydroxypropylidene)-1,1-bisphosphonic acid (APD) on the function of mononuclear phagocytes in lymphocyte proliferation. Immunology 1982;47:157-63.
12. Clezardin P, Ebetino FH, Fournier PG. Bisphosphonates and cancer-induced bone disease: beyond their antiresorptive activity. Cancer Res 2005;65:4971-4.
13. Tassone P, Forciniti S, Galea E, Morrone G, Turco MC, Martinelli V, et al. Growth inhibition and synergistic induction of apoptosis by zoledronate and dexamethasone in human myeloma cell lines. Leukemia 2000;14:841-4.
14. Ural AU, Yilmaz MI, Avcu F, Pekel A, Zerman M, Nevruz O, et al. The bisphosphonate zoledronic acid induces cytotoxicity in human myeloma cell lines with enhancing effects of dexamethasone and thalidomide. Int J Hematol 2003;78:443-9.
15. Legay F, Gauron S, Deckert F, Gosset G, Pfaar U, Ravera C, et al. Development and validation of a highly sensitive RIA for zoledronic acid, a new potent heterocyclic bisphosphonate, in human serum, plasma and urine. J Pharm Biomed Anal 2002; 30:897-911.
16. Makkonen N, Salminen A, Rogers MJ, Frith JC, Urtti A, Azhayeva E, et al. Contrasting effects of alendronate and clodronate on RAW 264 macrophages: the role of a bisphosphonate metabolite. Eur J Pharm Sci 1999;8:109-18.
17. Herrak P, Gortz B, Hayer S, Redlich K, Reiter E, Gasser J, et al. Zoledronic acid protects against local and systemic bone loss in tumor necrosis factor-mediated arthritis. Arthritis Rheum 2004;50:2327-37.
18. Denoyelle C, Hong L, Vannier JP, Soria J, Soria C. New insights into the actions of bisphosphonate zoledronic acid in breast cancer cells by dual RhoA-dependent and -independent effects. Br J Cancer 2003;88:1631-40.
19. Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med 1995;182:389-400.
20. Fu F, Li Y, Qian S, Lu L, Chambers F, Starzl TE, Fung JJ, et al. Costimulatory molecule-deficient dendritic cell progenitors prolong cardiac allograft survival in nonimmunosuppressed recipients. Transplantation 1996;62:659-65.
21. Romani N, Koide S, Crowley M, Witmer-Pack M, Livingstone AM, Fathman CG, et al. Presentation of exogenous protein antigens by dendritic cells to T cell clones. Intact protein is presented best by immature, epidermal Langerhans cells. J Exp Med 1989;169:1169-78.
22. Steinbrink K, Wolfl M, Jonuleit H, Knop J, Enk AH. Induction of tolerance by IL-10-treated dendritic cells. J Immunol 1997;159:4772-80.
23. Zhang QW, Kish DD, Fairchild RL. Absence of allograft ICAM-1 attenuates alloantigen-specific T cell priming, but not primed T cell trafficking into the graft, to mediate acute rejection. J Immunol 2003;170:5530-7.
24. Ouaaz F, Arron J, Zheng Y, Choi Y, Beg AA. Dendritic cell development and survival require distinct NF-κB subunits. Immunity 2002;16:257-70.
25. Giraudo E, Inoue M, Hanahan D. An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Invest 2004;114:623-33.
26. Yoshida M, Korfhagen TR, Whitsett JA. Surfactant protein D regulates NFκB and matrix metalloproteinase production in alveolar macrophages via oxidant-sensitive pathways. J Immunol 2001;166:7514-9.
27. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer 2005;5:263-74.