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ORIGINAL RESEARCH

Acute Phase Response After Zoledronic Acid is Associated with Long-Term Effects on White Blood Cells

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Abstract We have recently reported a long-lasting decrease in circulating γδ T cells in osteoporotic patients on oral amino-bisphosphonates (N-BPs). Here we verify whether these changes are associated with the occurrence of acute phase response (APR) to intravenous (IV) zoledronic acid (ZOL) or changes of other circulating white blood cells (WBC). WBC count was obtained before and 1 year after a single IV administration of 5 mg ZOL in 36 osteoporotic patients (mean age 72 ± 9, range 45–86 years) without other relevant diseases; 12 of 36 patients developed the classical APR. After 1 year in the patients who experienced an APR, but not in the others, a significant decrease not only of γδ T cells (−30 %), but also of total lymphocytes (−11 %) and eosinophils (−27 %), was observed. The mechanism leading to the observed decrease of circulating lymphocytes and eosinophils remains unclear, but our observation opens a new frontier for the understanding of the immunoeffects of N-BPs.

Keywords Bisphosphonate · White blood cell · Acute phase response · Eosinophil · T cell

Amino-bisphosphonates (N-BPs) are now established therapies for osteoporosis and Paget disease, and they are widely used for the prevention and treatment of skeletal-related events in cancer. The use of intravenous (IV) N-BPs is occasionally associated with the appearance within 24–36 h of fever and musculoskeletal pain [1]; this is referred as the acute phase response (APR) and it is associated with a transitory fall in circulating lymphocyte number [1, 2]. It is known that APR is linked to the

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activation of γδ T cells (in particular their major subpopulation of Vγ9Vδ2 T cells) and the release of pyrogenic cytokines [3]. Studies have revealed that N-BPs act via accumulation in adjacent monocytes of intracellular upstream metabolites, including dimethyl-allyl-pyrophosphate and isopentenyl pyrophosphate, after N-BPs-mediated inhibition of farnesyl pyrophosphate synthase [4, 5]. It has been observed that the proportion of circulating γδ T cells is an important determinant of the occurrence of APR after administration of N-BPs [6, 7], and that both IV or oral N-BPs treatment is associated with a decrease in circulating γδ T cells for at least 1 year [8].

Recently, Kalyan et al. [9] confirmed our results [8] reporting a long-term loss of γδ T cells in osteoporotic patients on oral N-BPs and an even more striking decline in patients administered IV N-BPs. Kalyan et al. [9] observed no differences in the number of circulating monocytes, total T cells, or granulocytes, but no subanalysis was conducted between patients with or without APR.

Here we report an additional subanalysis of our previous study [8]. We aimed to verify whether the long-term changes in circulating γδ T cells are associated with the occurrence of zoledronic acid (ZOL)-related APR or changes of other circulating white blood cells (WBC).

Methods

Counts of peripheral leukocyte and lymphocyte subpopulations were available before and 1 year after a single IV administration of 5 mg ZOL for 36 female patients (mean age 72 ± 9 years, range 45–86 years) with postmenopausal osteoporosis but without other relevant diseases. Patients with cancer, autoimmune or hematological diseases, immunodeficiency, and severe liver or renal insufficiency (serum creatinine >1.0 mg/dl) or recent acute infections were excluded from this study. Patients were not eligible if they had been treated within the last 2 years with cytostatic drugs, statins, corticosteroids, or immunotherapeutics.

WBC and differential cell counts were performed by an automated hematology analyzer (Advia 2120i Siemens, Malvern, PA). Fifty microliters of blood was distributed into each tube by the automated BD FACS Sample Prep Assistant II (Becton Dickinson, Mountain View, CA), a mixture of monoclonal antibodies conjugated with different fluorochromes (FITC, PE, PerCP, PE-Cy7, APC, APC-Cy7; BD Biosciences, San Diego CA) was added, the red blood cells were lysed, and finally the cells were fixed (BD FACS Lysing Solution). Lymphocytes were analyzed by flow cytometer (BD FACSCanto, Becton Dickinson) with BD FACS Diva software. Lymphocytes were isolated using CD45 versus SSC as a gating strategy. Different subsets of T cells were counted using these monoclonal antibodies: APC-conjugated anti-CD3, FITC-conjugated anti-CD4, PE-Cy7-conjugated anti-CD8. The γδ T cells were counted in the samples of $CD3^+$ T lymphocytes stained with anti-TCR γ/δ-PE. Natural killer (NK) cells were counted using APC-CY7-conjugated anti-CD16 and PE-conjugated anti-CD56. B cells were counted using APC-CY7-conjugated anti-CD19. The laboratory used UK NEQAS (www.ukneqas.org.uk) for leukocyte immunophenotyping to ensure external quality.

Body temperature was determined at the skin with digital clinical thermometers immediately before the IV infusion and at 12-h intervals for 3 days. Fever was defined as an increase in body temperature above 37.0 °C. Patients were instructed to register the temperature values on a diary, together with any self-administered acetaminophen dose to treat fever or other symptoms of APR.

Peripheral leukocyte and lymphocyte subpopulations were compared in patients with and without APR, by the Mann–Whitney *U* test for nonparametric independent variables. A two-tailed *P* value of 0.05 was considered significant. SPSS software (version 17.00, SPSS, Chicago, IL) was used for statistical analysis. This study was approved by the local ethic committee, and the subjects' consent was obtained according to the Declaration of Helsinki.

Results

Twelve of 36 patients developed classical APR. The mean age of the APR patients was nonsignificantly lower than non-APR patients (68 ± 11 years vs. 73 ± 7 years, respectively; $P = 0.129$). No significant differences in baseline cell population counts were observed between APR and non-APR patients (Table 1). The percentage changes in circulating γδ T cells or other WBC vs. baseline at 1 year are shown in Fig. 1. In the patients who experienced an APR, but not in the others, a significant decrease not only γδ T cells but also of total WBC, lymphocytes, $CD4^+$ T cells, and eosinophils was observed. In Table 1 the absolute count (cells/μL ±SD) for each WBC subpopulations at baseline and 1 year after IV ZOL administration in patients with and without APR are shown.

Discussion

We observed for the first time that APR is associated with a long-lasting significant decrease not only of γδ T cells, but also of total WBC, lymphocytes, $CD4^+$ T cells, and eosinophils.

Table 1 White blood cell counts (cells/μL ± SD) at baseline and 1 year after a single infusion of zoledronic acid in patients who did or did not experience an APR

APR	Time	WBC	Lymphocytes	T cells ($CD3^+$)	$CD4^+$ T cells	$CD8^+$ T cells	γδ T cells	B cells ($CD19^+$)	NK cells	Eosinophils	Monocytes	Neutrophils
APR	Baseline	6513 ± 2485	1754 ± 535	1251 ± 468	885 ± 408	349 ± 138	44 ± 26	229 ± 111	256 ± 123	200 ± 108	383 ± 160	4027 ± 2062
	1 year	5943 ± 2146	1560 ± 530*	1108 ± 373	765 ± 241	341 ± 200	31 ± 19**	205 ± 131	255 ± 130	144 ± 92**	336 ± 88	3740 ± 2002
No APR	Baseline	5985 ± 1759	1781 ± 526	1304 ± 370	903 ± 326	395 ± 165	33 ± 18	230 ± 161	246 ± 124	169 ± 125	378 ± 157	3510 ± 1232
	1 year	6122 ± 1662	1852 ± 598	1359 ± 415	936 ± 351	424 ± 194	30 ± 16	229 ± 176	269 ± 143	145 ± 110	376 ± 151	3562 ± 1359

The 2 groups did not differ in terms of baseline counts

* $P < 0.05$; ** $P < 0.01$ versus baseline

The clinical implication of a persistent decrease in circulating γδ T cells might be of great interest. The γδ T cells represent only 1–10 % of $CD3^+$ T cells in the human peripheral circulation, although their number is more abundant in epithelial tissues [10]. Like other members of the innate immunoresponse, γδ T cells may rapidly engage life-threatening microbial or host-derived pathogens and have been implicated in response to inflammation, allergy, autoimmunity, infectious disease [10], and certain tumors [10–12]. The decrease in circulating lymphocytes was attributed to the activation, differentiation, and homing at tissue levels of these cells [13], but it may also ensue as a result of the action of N-BPs on osteoclast function and then on the hematopoietic stem cell [14]. Kalyan et al. [9] documented a significant decreases in γδ T cells in patients who had experienced bisphosphonate-associated osteonecrosis of the jaw (BAONJ), and they hypothesized that BAONJ is a consequence of the drug-induced immune long-term effect, but with their hypothesis, the occurrence of ONJ also with denosumab [15], another powerful inhibitor of osteoclastic activity, remains unexplained.

In contrast with our previous experience [6], in this study, we did not observe a significant difference in age and in the baseline number of circulating γδ T cells between APR and non-APR patients, probably as a result the smaller number of patients. Indeed, this study found a very strong trend toward a reduction in basal γδ T cells in patients who did not experience an APR, suggesting that the number of circulating γδ T cells is effectively an important determinant of the occurrence of APR after IV infusion of N-BPs, as previously reported [6, 7].

$CD4^+$ T cells play an important role in the generation and maintenance of inflammation and tolerance; however, the clinical significance of the percentage reduction of their circulating levels remains unclear.

Eosinophils are the most important inflammatory effector cells accumulating at the site of allergic inflammation, e.g., the airway submucosa [16]. The decrease in eosinophils that we observed might be the result of a reduction of $CD4^+$ and Th17-mediated eosinophil activation [17].

Important limitations of this study are the small number of cases and the use of fever as the only criterion for APR.

A number of odd clinical observations made in patients treated with N-BPs, such as an antitumoral effects [18, 19] and reduced pneumonia-related mortality [20], remain puzzling. The observed decrease not only of γδ T cells, but also of other circulating lymphocytes subpopulations and eosinophils, opens a new frontier for our understanding of the immunoeffects of N-BPs.

Disclosure None.

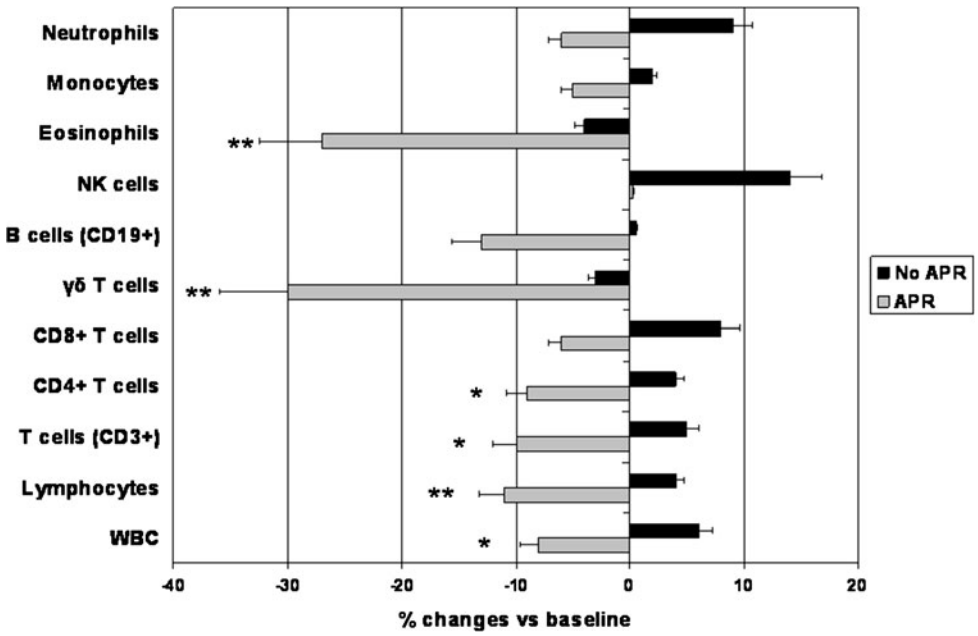

Fig. 1 Percentage changes (±SE) versus baseline of leukocyte and lymphocyte subpopulations at 1 year in patients who did or did not experience an APR. $*P < 0.05$; $**P < 0.01$ versus baseline

References

1. Adami S, Bhalla AK, Dorizzi R, Montesanti F, Rosini S, Salvagno G, Lo Cascio V (1987) The acute-phase response after bisphoshonate administration. Calcif Tissue Int 41:326–333
2. Schweitzer DH, Oostendorp-van de Ruit M, Van der Pluijm G, Lowik CW, Papapoulos SE (1995) Interleukin-6 and the acute phase response during treatment of patients with Paget's disease with the nitrogen-containing bisphosphonate dimethyl-amino-hydroxy-propylidene bisphosphonate. J Bone Miner Res 10:956–962
3. Kunzmann V, Bauer E, Wilhelm M (1999) Gamma/delta T-cell stimulation by pamidronate. N Engl J Med 340:737–738
4. Roelofs AJ, Jauhiainen M, Monkkonen H, Rogers MJ, Monkkonen J, Thompson K (2009) Peripheral blood monocytes are responsible for gamma delta T cell activation induced by zoledronic acid through accumulation of IPP/DMAPP. Br J Haematol 144:245–250
5. Miyagawa F, Tanaka Y, Yamashita S, Minato N (2001) Essential requirement of antigen presentation by monocyte lineage cells for the activation of primary human gamma delta T cells by aminobisphosphonate antigen. J Immunol 166:5508–5514
6. Rossini M, Adami S, Viapiana O, Ortolani R, Vella A, Fracassi E, Gatti D (2012) Circulating γδ T cells and the risk of acute-phase response after zoledronic acid administration. J Bone Miner Res 27:227–230
7. Welton JL, Morgan MP, Martí S, Stone MD, Moser B, Sewell AK, Turton J, Eberl M (2013) Monocytes and γδ T cells control the acute phase response to intravenous zoledronate: insights from a phase IV safety trial. J Bone Miner Res 28:464–471
8. Rossini M, Adami S, Viapiana O, Fracassi E, Ortolani R, Vella A, Zanotti R, Tripi G, Idolazzi L, Gatti D (2012) Long-term effects of amino-bisphosphonates on circulating gammadelta T cells. Calcif Tissue Int 91:395–399
9. Kalyan S, Quabius ES, Wiltfang J, Mönig H, Kabelitz D (2013) Can peripheral blood γδ T cells predict osteonecrosis of the jaw? An immunological perspective on the adverse drug-effects of aminobisphosphonate therapy. J Bone Miner Res 28:728–735
10. Hayday AC (2009) Gammadelta T cells and the lymphoid stress–surveillance response. Immunity 31:184–196
11. Strid J, Roberts SJ, Filler RB, Lewis JM, Kwong BY, Schpero W, Kaplan DH, Hayday AC, Girardi M (2008) Acute upregulation of an NKG2D ligand promotes rapid reorganization of a local immune compartment with pleiotropic effects on carcinogenesis. Nat Immunol 9:146–154
12. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR (1995) Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. Nature 375:155–158
13. Dieli F, Gebbia N, Poccia F, Caccamo N, Montesano C, Fulfaro F, Arcara C, Valerio MR, Meraviglia S, Di Sano C, Sireci G, Salerno A (2003) Induction of γδ T-lymphocyte effector functions by bisphosphonate zoledronic acid in cancer patients in vivo. Blood 102:2310–2311
14. Lymperi S, Ersek A, Ferraro F, Dazzi F, Horwood NJ (2011) Inhibition of osteoclast function reduces hematopoietic stem cell numbers in vivo. Blood 117:1540–1549
15. Van den Wyngaert T, Wouters K, Huizing MT, Vermorken JB (2011) RANK ligand inhibition in bone metastatic cancer and risk of osteonecrosis of the jaw (ONJ): non bis in idem? Support Care Cancer 19:2035–2040
16. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, Ghiran S, Gerard NP, Yu C, Orkin SH, Gerard C (2004) A critical role for eosinophils in allergic airways remodeling. Science 305:1776–1779
17. Cheung PF, Wong CK, Lam CW (2008) Molecular mechanisms of cytokine and chemokine release from eosinophils activated by IL-17A, IL-17F, and IL-23: implication for Th17 lymphocytes-mediated allergic inflammation. J Immunol 180:5625–5635
18. Kamiya N, Suzuki H, Endo T, Takano M, Yano M, Naoi M, Nishimi D, Kawamura K, Imamoto T, Ichikawa T (2011) Additive effect of zoledronic acid on serum prostate-specific antigen changes for hormone-sensitive prostate cancer patients with bone metastasis treated by combined androgen blockade. Int J Urol 19:169–173
19. Rennert G, Pinchev M, Rennert HS, Gruber SB (2011) Use of bisphosphonate and reduced risk of colorectal cancer. J Clin Oncol 29:1146–1150
20. Colón-Emeric CS, Mesenbrink P, Lyles KW, Pieper CF, Boonen S, Delmas P, Eriksen EF, Magaziner J (2010) Potential mediators of the mortality reduction with zoledronic acid after hip fracture. J Bone Miner Res 25:91–97