Immunology Letters 205 (2019) 65–70

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

The link between bone microenvironment and immune cells in multiple myeloma: Emerging role of CD38

Marina Bolzoni[a], Denise Toscani[a], Federica Costa[a], Emanuela Vicario[a,b], Franco Aversa[a,c], Nicola Giuliani[a,c,*]

[a] Department Medicine and Surgery, University of Parma, 43126 Parma, Italy
[b] Biopathology and Medical Biotechnologies, Biology and Genetic Section, University of Palermo, 90133 Palermo, Italy
[c] Hematology and BMT Center, "Azienda Ospedaliero-Universitaria di Parma", 43126 Parma, Italy

ARTICLE INFO

Keywords:
Osteoclast
Osteoblast
Myeloma cells
CD38
Ectoenzyme
Immune-microenvironment

ABSTRACT

The relationship between bone and immune cells is well established both in physiological and pathological conditions. Multiple myeloma (MM) is a plasma cell malignancy characterized by an increase of number and activity of osteoclasts (OCLs) and a decrease of osteoblasts (OBs). These events are responsible for bone lesions of MM patients. OCLs support MM cells survival in vitro and in vivo. Recently, the possible role of OCLs as immunosuppressive cells in the MM BM microenvironment has been underlined. OCLs protect MM cells against T cell-mediated cytotoxicity through the expression of several molecules including programmed death-ligand (PD-L) 1, galectin (Gal) 9, CD200, and indoleamine-2,3-dioxygenase (IDO). Among the molecules that could be involved in the link between immune-microenvironment and osteoclastogenesis the role of CD38 has been hypothesized. CD38 is a well-known adhesion molecule and an ectoenzyme highly expressed by MM cells. Moreover, CD38 is expressed by OCLs and at the surface level on OCL precursors. Targeting CD38 with monoclonal antibodies showed inhibition of both osteoclastogenesis and OCL-mediated suppression of T cell function. This review elucidates this evidence indicating that osteoclastogenesis affect MM immune-microenvironment being a potential target to improve anti-MM immunity and to ameliorate bone disease.

1. Introduction

Multiple myeloma (MM) is a hematological malignancy characterized by plasma cell (PC) accumulation into the bone marrow (BM) leading to both osteolytic bone disease and immunosuppression [1]. Indeed, the presence of MM cells alters the BM microenvironment, affecting bone cells with an increase of the number and activity of osteoclasts (OCLs) and a decrease of osteoblasts (OBs) and those of the immune system. The alteration of the bone and immune-microenvironment was exerted through either the release of soluble factors or the cell-to-cell contact between MM and microenvironment cells, providing a permissive niche that promotes MM cell growth [2,3].

MM patients show several deficit of humoral immunity, immunoparesis and alterations in the activity of effectors [4]. NK cells are decreased and dysfunctional, and T cells are significantly altered both quantitatively and functionally [5]. Moreover, MM patients display dysfunctional dendritic cells (DCs) with a deficit expression of the co-stimulatory molecules, such as CD80 and CD86, needed for the activation of T cells [6]. In addition, high number of myeloid-derived suppressor cells (MDSCs), involved in the immune escape of MM cells, was found in MM patients compared to healthy subjects [7].

This review summarizes the link between bone and immune cells in the context of MM focusing on the key role of osteoclasts.

2. Bone cells as immunocompetent cells: role of OCLs

The close relationship between bone and immune cells dates back to the early 1970s [8]. The term "osteoimmunology" was subsequently developed to indicate the mechanisms behind this interaction [9,10]. Studies on autoimmune disease, such as rheumatoid arthritis (RA), revealed that osteoclastic bone resorption is associated with the prolonged T-cell immune activation [11]. It is well known that activated T cells act directly on OCL precursors by expressing the main pro-osteoclastogenic factor, receptor activator of nuclear factor-kB ligand

Abbreviations: ADO, adenosine; BMSC, bone marrow stromal cell; Gal, galectin; HVEM, anti-herpesvirus entry mediator; IDO, indoleamine-2,3-dioxygenase; IL, interleukin; mAbs, monoclonal antibodiesm; MM, multiple myeloma; NAD, nicotinamide adenine dinucleotide; OB, osteoblast; OCL, osteoclast; OPG, osteoprotegerin; PD, programmed death; PD-L, PD ligand; RANK, receptor activator of nuclear factor-Kb; RANKL, RANK ligand

* Corresponding author at: Department Medicine and Surgery, University of Parma, Via Gramsci 14, 43126, Parma, Italy.
E-mail address: nicola.giuliani@unipr.it (N. Giuliani).

https://doi.org/10.1016/j.imlet.2018.04.007
Received 27 March 2018; Accepted 23 April 2018
Available online 24 April 2018

(RANKL), like BM stromal cells (BMSCs) and OBs [12]. Of note, RANKL and its receptor RANK were first described to play a role in T-cell mediated survival of dendritic cells (DCs), thus confirming the presence of common regulating factors between immune and bone cells [13]. RANKL signaling is also involved in the early T cell development by regulating self-tolerance and auto-immunity in mice models [14]. Interestingly, RANKL$^{-/-}$ mice lack in the development of lymph nodes and have abnormality in the spleen, thus suggesting the time-dependent involvement of RANK/RANKL axis in the regulation of the immune system [15].

In addition, it is known that OCLs differentiate from the same monocytic/macrophage precursor of DCs, according to the influence of macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage CSF (GM-CSF), respectively [16]; and immature DCs can transdifferentiate into functional OCLs in the presence of M-CSF and RANKL, mainly under inflammatory conditions [17].

Despite the well-known common origin with DC, the role of OCLs as antigen-presenting cells (APCs) is debated. Some studies have demonstrated that OCLs express several immune receptors (e.g. DC specific transmembrane protein and immunoreceptor tyrosine-based activation motif signaling receptors) and share mechanisms of regulation with macrophages and DCs [18].

Literature data on murine models also reported that OCLs express only class I MHC, suggesting that they preferentially activate CD8+ T cells [19]. OCLs increased the expression of the T regulatory cells (Treg) marker forkhead box P3 (FoxP3) and suppressed antigen-specific naïve T cell proliferation [19]. Further studies demonstrated that OCL-primed T cells inhibited OC formation through the release of interferon (IFN) γ, interleukin 6 (IL-6) and IL-10 [20]. Similar effects were reported in the inflammatory model of RA [21]. By contrast, Li et al. first showed that human OCLs express both class I and class II MHC molecules, as well as co-stimulatory molecules CD80 and CD86 and can activate both allo- and antigen-specific CD4$^+$ and CD8$^+$ T cell responses [22]. More recently, a study from Ibanez et al. demonstrated that OCL effects on T cell activity depend on their cell origin and the surrounding microenvironment [23]. Specifically it was described that OCLs derived from normal BM express immunosuppressive cytokines (IL-10, transforming growth factor (TGF) β) and induce the activation of immunosuppressive CD4$^+$ Treg cells in an antigen-dependent manner [23]. On the other hand, OCLs derived from an inflammatory milieu enhance the expansion of tumor necrosis factor (TNF) α-producing CD4$^+$ T cells [23]. These data show that OCLs have double effects on immune cells, based on the microenvironment pressure.

Furthermore, the suppression of T cells occurs in the early OCL formation, as confirmed by the upregulation of the expression of the inhibitory molecule CD200, prior to fusion of proliferating monocytes [24]. The binding with CD200 receptor on natural killer and activated T cells then induce the suppression of their activity and proliferation [25].

In addition, it has been recently demonstrated that the primary murine BM OCL precursor belongs to a Lin$^-$CD11b$^{low/-}$CD115$^+$ population [26]. These cells attenuate joint inflammation after adoptive transfer in a T cell dependent model of RA leading to the hypothesis that these OCL precursors may be MDSCs [26]. MDSCs are characterized by expression of CD11b and either Ly6C (monocytic MDSC) or Ly6G (granulocytic MDSC) [27]. Similar to monocytic MDSCs, CD11b$^{low/-}$ Ly6C^{hi} OCL precursors did not express Ly6G and inhibited T cell proliferation *in vitro* mainly through the production of nitric oxide, after T cell derived IFNγ stimulation.[27] Interestingly, the differentiation into OCLs did not change their ability to inhibit T cell proliferation [27]. Moreover, the bone microenvironment seems to be essential for MDSC ability to differentiate into OCLs. As reported by Sawant et al., MDSCs from lung, spleen, blood, and lymph nodes are unable to differentiate into OCLs; [28] however it is not yet clear which factors are involved in polarizing MDSCs toward OCL differentiation. Studies on human cancer with osteolytic bone metastasis also revealed the involvement of MDSCs in the development of bone disease, thus confirming the cross-talk between these two compartments [29].

OBs seem to be also involved in the regulation of the immune system; however, few data are currently available. Several studies reported OB expression of cell surface molecules that activate T-cell signaling, including CD80, CD86, and CD44 with costimulatory functions [30,31]. Lisignoli et al. also demonstrated that T cell recruitment and proliferation is supported by OBs expressing intercellular adhesion molecule 1 (ICAM-1) after stimulation with IL-1, TNFα, IFNγ, and lipopolysaccharide [30]. ICAM-1 is typically expressed by APCs and its binding with CD11a/CD18 on T cells is essential for T-cell activation [32]. It is important to note that T cells are activated after antigen presentation by self-MHC on the surface of the APCs. Interestingly, it was described that IFNγ stimulates the expression of MHC class II on human OBs [31] further suggesting the involvement of these cells in antigen presentation mechanisms. Moreover, OBs express the cytokine receptors, CXCR3 and CXCR5, which control T cell migration and polarization towards T helper (Th) type 1 or Th type 2 (Th2) phenotype [33,34]. However, the functional role of OBs as APCs able to induce strong responses to specific antigens by resting T cells is not yet well understood.

All these data highlight the interplay between the bone and the immune system, thus providing the molecular basis for novel therapeutic strategies against several diseases affecting both cell compartments.

3. Myeloma-induced bone microenvironment alterations

As already mentioned above, the BM of MM patients shows different alterations, including in particular, the alterations of bone remodeling leading to bone destruction. Osteolytic lesions are the main feature of bone involvement in MM patients due to an increase of the osteoclastogenesis and OCL activity and OB suppression [2]. Different cytokines and chemokines involved in OCL activation and/or OB suppression are released at high level into the BM microenvironment including chemokine (C−C motif) ligand (CCL)-3, dickkopf-related protein (DKK)-1, hepatocyte growth factor (HGF), IL-1, IL-3, IL-6, IL-7, secreted frizzled related protein (sFRP)-2, sFRP-3, and TNFα [2,35]. Moreover, cell-to-cell contact between MM cells and BMSCs up-regulates RANKL, the main pro-osteoclastic cytokine, and decreases the release of RANKL decoy receptor osteoprotegerin (OPG) from osteoprogenitor cells [2,36]. The adhesion and interaction between MM cells and the bone microenvironment also causes the inhibition of the expression of the main pro-osteoblastogenic transcription factor, Runt-related transcription factor (Runx) 2, expressed by BMSCs leading to the suppression of OB differentiation [37]. Furthermore, some of the cytokines present into the BM as IL-7, IL-3 and HGF contribute to the inhibition of Runx2 activity and OB suppression [35,38]. Besides its anti-OB activity, IL-3 mediates monocyte secretion of activin A that enhances osteoclastogenesis contributing to MM-induced bone disease [39].

The involvement of immune system in the mechanism involved in MM-induced osteoclastogenesis disease has been underlined. MM T cells secrete high amount of RANKL [40]. The presence of MM cells in co-colture systems increased the expression and secretion of RANKL by activated T lymphocytes.[40] This effect was mediated by MM cell IL-7 secretion [40]. Consistently, MM patients with severe osteolytic lesions showed RANKL up-regulation by BM T cells as compared to MM patients without bone lesions [40]. Beside RANKL, MM T cells produce other pro-osteogenic cytokines as IL-3 which BM levels are higher in MM patients as compared to controls, sustaining MM-induced osteoclastogenesis [41]. The role of T cells was in MM-induced osteoclastogenesis was further confirmed by others showing that MM T cells support OC formation and survival, possibly involving OPG/TRAIL interaction and unbalanced OC expression of TRAIL death and decoy receptors [42].

More recently, it has been reported that MM patients' BM showed an

increase of IL-17-producing Th type 17 (Th17) cells that inhibit cytotoxic T cell activity and promote MM cell growth [43,44]. IL-17 also increases osteoclastogenesis in BM samples cultured with M-CSF and RANKL compared to controls as well as the addition to BM cultures of MM patients' Th17 [43]. Interestingly, a significant relationship between Th17 cell number and the degree of lytic bone lesions has been reported [44]. Consistently, MM cells up-regulated the expression of CCL20 and of its receptor CC chemokine receptor (CCR) 6 into the bone microenvironment with the possible involvement of two pro-inflammatory cytokines IL-1 and TNFα [45]. CCL20 is known to be the main chemokine stimulating Th17 recruitment [43]. and consequently increase osteoclastogenesis. Accordingly, it has been reported that neutralizing antibodies (Abs) against CCL20 or CCR6 significantly inhibit MM-induced OCL formation [45].

Overall these data suggest a significant link between OCL formation and immune system in the pathophysiology of MM-induced bone destruction.

4. Osteoclasts and myeloma cells interplay: the immunosuppressive role of osteoclast

Osteoclastogenesis is highly induced by MM cells, which in turn enhances MM cell survival, angiogenesis and drug resistance, resulting in a vicious loop. *In vitro* evidence indicates that OCLs produce MM pro-survival factors, such as osteopontin (OPN) and IL-6, and adhesion of MM cells to OCLs increased their production [46]. Other factors as B-cell-activating factor (BAFF) and A proliferating-inducing ligand (APRIL) are considered survival factors for MM cells produced by OCLs in the BM microenvironment [47]. Moreover in vivo studies in MM mice models demonstrated that inhibition of OCL formation by amino-bisphosphonates may extent anti-MM activity [48].

Emerging data indicate that OCLs have a possible immunosuppressive role in MM microenvironment. Beside direct positive effect of OCLs on MM cell survival and grow, more recently an indirect effect has also been hypothesized. OCLs modulates T-cell mediated anti-MM immunity protecting MM cells against T cell responses [49]. Both CD4 and CD8 lymphocyte proliferation induced by anti-CD2, CD3, and CD28 beads stimulation was blunted in the presence of OCLs. Interestingly anti-PD-L1 Abs or by indoleamine-2,3-dioxygenase (IDO) inhibitors partially overcame this immunosuppressive effect [49]. The capacity of OCLs to expert an immunosuppressive effect on T cells were due to their APC capacity and the up-regulation of several co-inhibitory molecules including PD-L1, galectin (Gal) 9, anti-herpesvirus entry mediator (HVEM), and CD200 [49].

Immune inhibitory molecules involved in OCL-mediated immunosuppressive effect in MM are also involved in the production of metabolic enzymes particularly IDO. OCLs derived from MM patients highly produced IDO which levels were significantly higher into the BM plasma of MM patients compared to controls [49].

OCL progenitors also expressed the co-inhibitory molecules PD-L1, Gal-9, HEVM and CD200. Upon OCL differentiation, a significant up-regulation of these molecules has been reported [49]. In addition, comparing newly diagnosed MM patients with healthy donors, the expression levels of the co-inhibitory molecules were found to be higher in the MM group [49].

Finally, APRIL promotes MM cell survival and MM progression in vivo [50]. OCLs are the major source of APRIL in the BM microenvironment and the APRIL/BCMA axis regulates the expression of PD-L1 in MM cells via MEK/ERK pathway [50]

Overall, this evidence clearly underlines the immunosuppressive and pro-tumoral role of OCLs in MM. Furthermore, the in vivo mouse models showed that the growth and immunosuppression critically dependent on APRIL/BCMA axis delineating a new therapeutic approach based to target this pathway to restore immune function in MM.

5. Expression profile of ectoenzymes by the bone microenvironment in multiple myeloma

The MM bone microenvironment contains high levels of extracellular nucleotides, such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide $(NAD)^+$, which are metabolized to adenosine (ADO) by the action of cell surface proteins called ectoenzymes [51]. ADO is a nucleoside produced under metabolic stress like hypoxia, which modulates inflammation and immune responses [52]. In melanoma model, ADO suppresses T cell proliferation and their ability to kill cancer cells [53]. Through the canonical adenosinergic pathway, ATP is hydrolyzed by the ecto-nucleoside triphosphate diphosphohydrolase CD39 to adenosine monophosphate (AMP) that can be hydrolyzed by the 5′-nucleotidase CD73, generating ADO. It has been recently characterized an alternative pathway that produces extracellular ADO from NAD^+. This axis involves the nucleotide-metabolizing ectoenzymes NAD+-glycohydrolase CD38, the ecto-nucleotide pyrophosphatase/phosphodiesterase CD203a (also known as PC-1), and the 5′-ectonucleotidase CD73 [54]. Briefly, CD38 generates adenosine diphosphate ribose (ADPR) that is further hydrolyzed by CD203a to produce AMP. The conversion from AMP to ADO is regulated by CD73. Several experimental models confirmed the active function of this alternative pathway in MM bone niche [55–57]. MM and bone cells are equipped with ectoenzyme machinery able to produce ADO by both canonical and alternative pathway. Specifically, MM cell lines established from patients with MM express CD38 and its non-substrate ligand CD31, whereas the expression of CD203a, CD39 and CD73 is undetectable. In the same way, the analysis of the expression of all these ectoenzymes by BMSCs during OB differentiation reveals CD38 decreases during differentiation with a concomitant increase of CD203a. CD39 expression is undetected both in differentiated and undifferentiated cells [56]. The ectoenzyme CD73 displays a stable surface expression during OB differentiation.

The analysis and distribution of the ectoenzymes in MM niche has been recently performed on bone biopsies, primary PCs from BM aspirate and osteogenic cells [55,57]. In line with the data reported above, PCs express high levels of CD38 whereas CD39 and CD73 differ from patient to patient. Interestingly, CD203a was expressed by primary PCs confirming the presence of a complete set of ADO-producing enzymes. The expression profile of BMSCs and OBs reveals that both cell types are $CD38^-/CD39^-$ while expressing CD73 and CD203a [55,57]. These data provide a conclusive evidence that the components of the alternative adenosinergic pathway, CD38, CD203a and CD73, and those of the canonical pathway CD39 and CD73, are expressed by bone microenvironment cells. Subsequently, the same authors sought to link the expression of all these ectoenzymes to the production of ADO. In a first preliminary paper, they demonstrated that ADO is present in BM plasma aspirates from MM patients [51]. Interestingly, ADO levels were found to be higher in MM patients than in asymptomatic patients and in patients with an ISS = III compared with ISS = I and II [57]. Noteworthy, primary PCs isolated from BM aspirates do not produce detectable levels of ADO possibly because of the low levels of CD73, the enzymes responsible for the final production of ADO. To clarify, the authors analyzed the generation of ADO in co-culture system between MM cell line and OCLs, OBs or BMSCs after treatment with different substrates (ATP, AMP or NAD^+). The results indicate that ADO production increases because of cell-to-cell contact supporting the growth of tumoral cells within the niche [57]. More recently, it has been demonstrated that microvesicles (MVs) isolated from MM BM express high levels of all ectoenzymes compared with those isolated from controls. Moreover, the production of ADO is higher in MVs from MM compared with controls suggesting that MVs may contribute to the production of ADO in BM niche [58].

All these observations support the notion that the canonical CD39/CD73 pathway to generate ADO from ATP is supphorted by the alternative CD38/CD203a/CD73 that uses NAD^+ as substrate. Both are

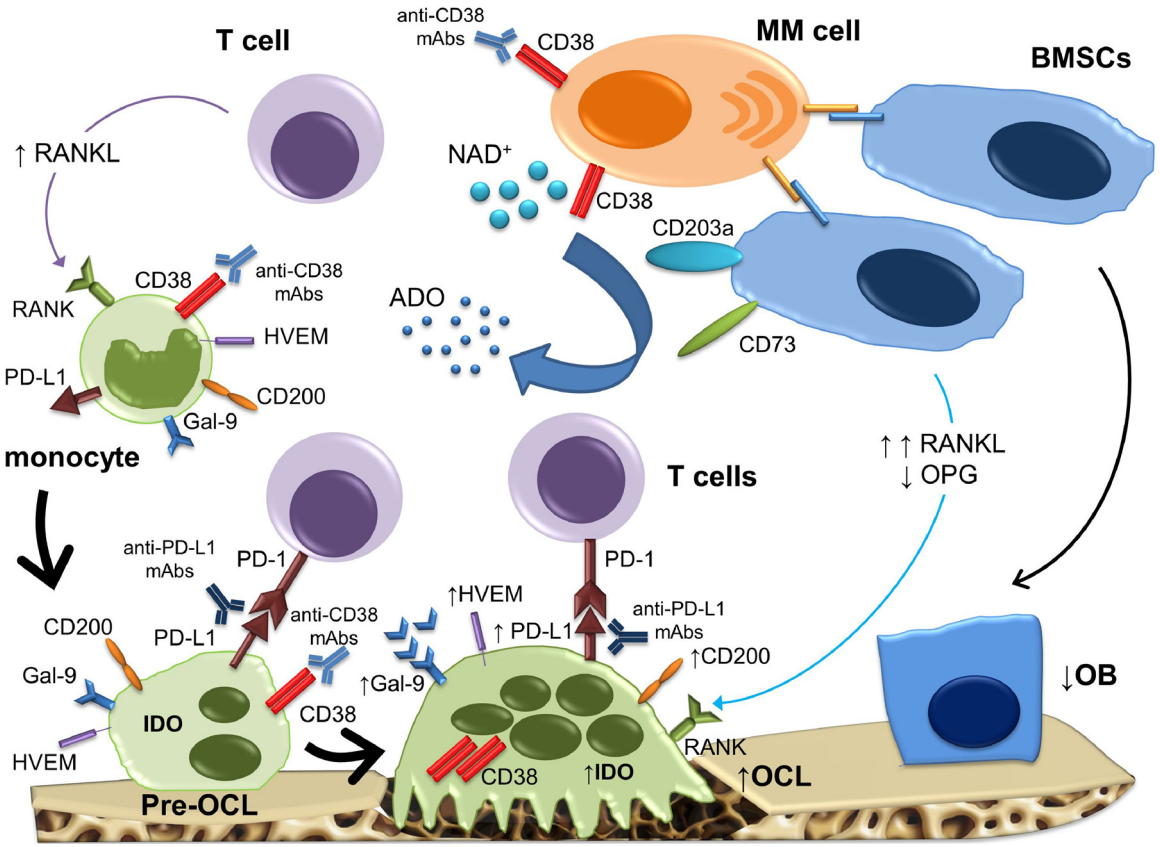

Fig. 1. The link between immune cells and OCLs in MM microenvironment.
The presence of MM cells in the microenvironment upregulates the critical pro-osteoclastogenic factor RANKL by both T lymphocytes and BMSC inhibiting its decoy receptor OPG. The increase of OCL formation and activation induces an immune suppressive effect in the BM microenvironment supporting the growth of MM cells. Several co-inhibitory molecules including PD-L1, Gal-9, HVEM, and CD200 are up-regulated on OCLs that produce high levels of IDO. All these molecules display an inhibitory effect on T lymphocytes. CD38 contribute to immunosuppression in MM microenvironment acting as key ectoenzyme of the non-canonical pathway (together with CD203a and CD73) through the production of ADO, a potent T lymphocyte inhibitor. Moreover, CD38 expressed at surface level on monocyte and early OCL progenitors is involved in the osteoclastogenesis.
Both CD38 and PD-L1 are suitable targets for Ab-based immunotherapy to block immunomosuppressive effect of OCLs. Moreover, the recently developed anti-CD38 mAbs has shown in pre-clinical models both anti-osteoclastogenic and immunomodulatory effects. This evidence indicate that this approach could be also suitable to target bone disease and to restore the immunosuppressive microenvironment in MM.

responsible for the accumulation of ADO within the niche. Intriguingly, ADO suppresses T cell proliferation and their ability to kill cancer cells suggesting that MM bone niche may represent the ideal setting where MM cells take advantage of the products of the adenosinergic ectoenzymes to build up a microenvironment for their survival and protection from host immune system.

6. Role of CD38 in myeloma-induced osteoclastogenesis

In the last years, CD38, a 45-kDa type II transmembrane glycoprotein, has achieved considerable importance in MM setting because of its dual role and ubiquitous distribution. Indeed, beside its role as ectoenzyme, CD38 also acts as surface molecule by regulating the intracellular levels of calcium and downstream signaling pathways through its ADP-ribosyl cyclase activity. The wide knowledge of its function in PCs led to the development of different CD38-targeting Abs with important clinical benefit [59].

Several studies have also reported the CD38 involvement in mice and rabbit bone resorption [60,61]. A study from Sun et al., reported that enzymatically active CD38 localizes in the plasma membrane of rabbit OCL [60]. The activation of CD38 by the agonist molecule triggers cytosolic Ca^{2+} signal and, consequently, inhibits bone resorption. In vivo experiment showed that $CD38^{-/-}$ mice have reduced bone mineral density and their hematopoietic stem cells displays a strong ability to differentiate into highly resorptive OCL [61]. The authors speculated that CD38 might act as sensor able to couple the metabolic activity of OCL with its $Ca2^{+}$ signaling pathway.

More recently, Costa et al. observed that human OCL progenitors present CD38 on their surface but it decreases during differentiation toward OCL phenotype. The treatment with the high-affinity human IgG1κ anti-CD38 mAb, Daratumumab (DARA), shows inhibitory effects on OCL formation and activity targeting OCL progenitors [55]. The role of CD38 as immune checkpoint molecules has been recently explored [49]. First, the authors showed that cytoplasmic CD38 is induced during osteoclastogenesis and the treatment with the anti CD38 mAbs SAR650984 (SAR) reduces CD38 expression in an ex vivo culture system from monocyte without affecting OCL formation [49]. Co-culture between OCL and activated T cells increases the death of OCL through IFN-γ production and SAR enhances this cytotoxic effect. Moreover, OCL blocks the proliferation of T cell in co-culture system whereas the treatment with SAR restores T-cell response.

Overall, these data suggest that anti-CD38 therapy, either by restoring T cell function or inhibition early osteoclastogenesis, may mitigate bone disease.

7. Conclusions

The possible role of T lymphocytes in MM-induced

osteoclastogenesis and OCL activity is well established. Growing evidence indicates that OCLs have an immunosuppressive role in MM microenvironment also supporting the indirectly growth of MM cells. The mechanism by which OCLs exert this immune effect is currently under investigation. It is known that OCLs display APC features and upregulate several co-inhibitory molecules including PD-L1, Gal-9, HVEM, and CD200. In addition, OCLs in MM microenvironment produce high levels of IDO. All these molecules are involved in the inhibitory effect on T lymphocytes (Fig. 1).

Recently, a dual role of CD38 in MM OCLs and OCL progenitors has been underlined as both co-immunosuppressive factor and pro-osteoclastogenic molecule (Fig. 1). CD38 is a new therapeutic target in MM patients and anti-CD38 Ab-based immunotherapy has shown in preclinical models anti-osteoclastogenic and immunomodulatory effects (Fig. 1). These preliminary data indicate that this approach could be also suitable to target bone disease and to restore the immunosuppressive microenvironment in MM.

Conflict of interest

None of the authors has any conflict of interest to declare.

Acknowledgement

This work was supported by the “Associazione Italiana per la Ricerca sul Cancro” under IG2017 Grant (id. 20299), the International Myeloma Foundation under 2018 Brian D. Novis Senior Research Grant, and the “Ministero della Salute” under Ricerca finalizzata PE-2016 Grant (NG). MB was supported by “Fondazione Italiana per la Ricerca sul Cancro” fellowship (id. 18152).

References

[1] A. Palumbo, K. Anderson, Multiple myeloma, N. Engl. J. Med. 364 (11) (2011) 1046–10460.
[2] G.D. Roodman, Pathogenesis of myeloma bone disease, Leukemia 23 (3) (2009) 435–441.
[3] S. Yaccoby, Advances in the understanding of myeloma bone disease and tumour growth, Br. J. Haematol. 149 (3) (2010) 311–321.
[4] S.M. Tete, M. Bijl, S.S. Sahota, N.A. Bos, Immune defects in the risk of infection and response to vaccination in monoclonal gammopathy of undetermined significance and multiple myeloma, Front. Immunol. 5 (2014) 257.
[5] T. Dosani, M. Carlsten, I. Maric, O. Landgren, The cellular immune system in myelomagenesis: NK cells and T cells in the development of myeloma [corrected] and their uses in immunotherapies, Blood Cancer J. 5 (2015) e306.
[6] R.D. Brown, B. Pope, A. Murray, D.M. Esdale, J. Gibson, P.J. Ho, D. Hart, D. Joshua, Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to up-regulate CD80 (B7-1) expression after huCD40LT stimulation because of inhibition by transforming growth factor-beta1 and interleukin-10, Blood 98 (10) (2001) 2992–2998.
[7] G.T. Gorgun, G. Whitehill, J.L. Anderson, T. Hideshima, C. Maguire, J. Laubach, N. Raje, N.C. Munshi, P.G. Richardson, K.C. Anderson, Tumor-promoting immune-suppressive myeloid-derived suppressor cells in the multiple myeloma microenvironment in humans, Blood 121 (15) (2013) 2975–2987.
[8] J.E. Horton, L.G. Raisz, H.A. Simmons, J.J. Oppenheim, S.E. Mergenhagen, Bone resorbing activity in supernatant fluid from cultured human peripheral blood leukocytes, Science 177 (4051) (1972) 793–795.
[9] J.R. Arron, Y. Choi, Bone versus immune system, Nature 408 (6812) (2000) 535–536.
[10] H. Takayanagi, Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems, Nat. Rev. Immunol. 7 (4) (2007) 292–304.
[11] H. Takayanagi, Osteoimmunology and the effects of the immune system on bone, Nat. Rev. Rheumatol. 5 (12) (2009) 667–676.
[12] H. Takayanagi, K. Ogasawara, S. Hida, T. Chiba, S. Murata, K. Sato, A. Takaoka, T. Yokochi, H. Oda, K. Tanaka, K. Nakamura, T. Taniguchi, T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma, Nature 408 (6812) (2000) 600–605.
[13] D.M. Anderson, E. Maraskovsky, W.L. Billingsley, W.C. Dougall, M.E. Tometsko, E.R. Roux, M.C. Teepe, R.F. DuBose, D. Cosman, L. Galibert, A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function, Nature 390 (6656) (1997) 175–179.
[14] G.E. Desanti, J.E. Cowan, S. Baik, S.M. Parnell, A.J. White, J.M. Penninger, P.J. Lane, E.J. Jenkinson, W.E. Jenkinson, G. Anderson, Developmentally regulated availability of RANKL and CD40 ligand reveals distinct mechanisms of fetal and adult cross-talk in the thymus medulla, J. Immunol. 189 (12) (2012) 5519–5526.
[15] Y.Y. Kong, H. Yoshida, I. Sarosi, H.L. Tan, E. Timms, C. Capparelli, S. Morony, A.J. Oliveira-dos-Santos, G. Van, A. Itie, W. Khoo, A. Wakeham, C.R. Dunstan, D.L. Lacey, T.W. Mak, W.J. Boyle, J.M. Penninger, OPGL is a key regulator of osteoclastogenesis lymphocyte development and lymph-node organogenesis, Nature 397 (6717) (1999) 315–323.
[16] T. Miyamoto, O. Ohneda, F. Arai, K. Iwamoto, S. Okada, K. Takagi, D.M. Anderson, T. Suda, Bifurcation of osteoclasts and dendritic cells from common progenitors, Blood 98 (8) (2001) 2544–2554.
[17] A. Rivollier, M. Mazzorana, J. Tebib, M. Piperno, T. Aitsiselmi, C. Rabourdin-Combe, P. Jurdic, C. Servet-Delprat, Immature dendritic cell transdifferentiation into osteoclasts: a novel pathway sustained by the rheumatoid arthritis microenvironment, Blood 104 (13) (2004) 4029–4037.
[18] Y. Wu, M.B. Humphrey, M.C. Nakamura, Osteoclasts – the innate immune cells of the bone, Autoimmunity 41 (3) (2008) 183–194.
[19] J.R. Kiesel, Z.S. Buchwald, R. Aurora, Cross-presentation by osteoclasts induces FoxP3 in CD8+ T cells, J. Immunol. 182 (9) (2009) 5477–5487.
[20] Z.S. Buchwald, J.R. Kiesel, C. Yang, R. DiPaolo, D.V. Novack, R. Aurora, Osteoclast-induced Foxp3+ CD8 T-cells limit bone loss in mice, Bone 56 (1) (2013) 163–173.
[21] D. Jones, L.H. Glimcher, A.O. Aliprantis, Osteoimmunology at the nexus of arthritis osteoporosis, cancer, and infection, J. Clin. Invest. 121 (7) (2011) 2534–2542.
[22] H. Li, S. Hong, J. Qian, Y. Zheng, J. Yang, Q. Yi, Cross talk between the bone and immune systems: osteoclasts function as antigen-presenting cells and activate CD4+ and CD8+ T cells, Blood 116 (2) (2010) 210–217.
[23] L. Ibanez, G. Abou-Ezzi, T. Ciucci, V. Amiot, N. Belaid, D. Obino, A. Mansour, M. Rouleau, A. Wakkach, C. Blin-Wakkach, Inflammatory osteoclasts prime TNFalpha-Producing CD4(+) t cells and express CX3 CR1, J. Bone Miner. Res. 31 (10) (2016) 1899–1908.
[24] W. Cui, E. Cuartas, J. Ke, Q. Zhang, H.B. Einarsson, J.D. Sedgwick, J. Li, A. Vignery, CD200 and its receptor CD200R, modulate bone mass via the differentiation of osteoclasts, Proc. Natl. Acad. Sci. U. S. A. 104 (36) (2007) 14436–14441.
[25] S.J. Coles, M.N. Gilmour, R. Reid, S. Knapper, A.K. Burnett, S. Man, A. Tonks, R.L. Darley, The immunosuppressive ligands PD-L1 and CD200 are linked in AML T-cell immunosuppression: identification of a new immunotherapeutic synapse, Leukemia 29 (9) (2015) 1952–1954.
[26] J.F. Charles, L.Y. Hsu, E.C. Niemi, A. Weiss, A.O. Aliprantis, M.C. Nakamura, Inflammatory arthritis increases mouse osteoclast precursors with myeloid suppressor function, J. Clin. Invest. 122 (12) (2012) 4592–4605.
[27] P. Serafini, Myeloid derived suppressor cells in physiological and pathological conditions: the good, the bad, and the ugly, Immunol. Res. 57 (1–3) (2013) 172–184.
[28] A. Sawant, J. Deshane, J. Jules, C.M. Lee, B.A. Harris, X. Feng, S. Ponnazhagan, Myeloid-derived suppressor cells function as novel osteoclast progenitors enhancing bone loss in breast cancer, Cancer Res. 73 (2) (2013) 672–682.
[29] A. Sawant, S. Ponnazhagan, Myeloid-derived suppressor cells as a novel target for the control of osteolytic bone disease, Oncoimmunology 2 (5) (2013) e24064.
[30] G. Lisignoli, S. Toneguzzi, A. Piacentini, S. Cristino, L. Cattini, F. Grassi, A. Facchini, Recruitment and proliferation of T lymphocytes is supported by IFNgamma- and TNFalpha-activated human osteoblasts: involvement of CD54 (ICAM-1) and CD106 (VCAM-1) adhesion molecules and CXCR3 chemokine receptor, J. Cell. Physiol. 198 (3) (2004) 388–398.
[31] H. Skjodt, T. Moller, S.F. Freiesleben, Human osteoblast-like cells expressing MHC class II determinants stimulate allogeneic and autologous peripheral blood mononuclear cells and function as antigen-presenting cells, Immunology 68 (3) (1989) 416–420.
[32] A.K. Hubbard, R. Rothlein, Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades, Free Radic. Biol. Med. 28 (9) (2000) 1379–1386.
[33] G. Lisignoli, S. Toneguzzi, A. Piacentini, L. Cattini, A. Lenti, M. Tschon, S. Cristino, F. Grassi, A. Facchini, Human osteoblasts express functional CXC chemokine receptors 3 and 5: activation by their ligands CXCL10 and CXCL13, significantly induces alkaline phosphatase and beta-N-acetylhexosaminidase release, J. Cell. Physiol. 194 (1) (2003) 71–79.
[34] C.H. Kim, L. Rott, E.J. Kunkel, M.C. Genovese, D.P. Andrew, L. Wu, E.C. Butcher, Rules of chemokine receptor association with T cell polarization in vivo, J. Clin. Invest. 108 (9) (2001) 1331–1339.
[35] N. Giuliani, V. Rizzoli, G.D. Roodman, Multiple myeloma bone disease: pathophysiology of osteoblast inhibition, Blood 108 (13) (2006) 3992–3996.
[36] N. Giuliani, S. Colla, V. Rizzoli, New insight in the mechanism of osteoclast activation and formation in multiple myeloma: focus on the receptor activator of NF-kappaB ligand (RANKL), Exp. Hematol. 32 (8) (2004) 685–691.
[37] N. Giuliani, S. Colla, F. Morandi, M. Lazzaretti, R. Sala, S. Bonomini, M. Grano, S. Colucci, M. Svaldi, V. Rizzoli, Myeloma cells block RUNX2/CBFA1 activity in human bone marrow osteoblast progenitors and inhibit osteoblast formation and differentiation, Blood 106 (7) (2005) 2472–2483.
[38] D. Toscani, M. Bolzoni, F. Accardi, F. Aversa, N. Giuliani, The osteoblastic niche in the context of multiple myeloma, Ann. N. Y. Acad. Sci. 1335 (2015) 45–62.
[39] R. Silbermann, M. Bolzoni, P. Storti, D. Guasco, S. Bonomini, D. Zhou, J. Wu, J.L. Anderson, J.J. Windle, F. Aversa, G. David Roodman, N. Giuliani, Bone marrow monocyte-/macrophage-derived activin A mediates the osteoclastogenic effect of IL-3 in multiple myeloma, Leukemia 28 (4) (2014) 951–954.
[40] N. Giuliani, S. Colla, R. Sala, M. Moroni, M. Lazzaretti, S. La Monica, S. Bonomini, M. Hojden, G. Sammarelli, S. Barille, R. Bataille, V. Rizzoli, Human myeloma cells stimulate the receptor activator of nuclear factor-kappa B ligand (RANKL) in T lymphocytes: a potential role in multiple myeloma bone disease, Blood 100 (13) (2002) 4615–4621.
[41] N. Giuliani, F. Morandi, S. Tagliaferri, S. Colla, S. Bonomini, G. Sammarelli, V. Rizzoli, Interleukin-3 (IL-3) is overexpressed by T lymphocytes in multiple

myeloma patients, Blood 107 (2) (2006) 841–842.
[42] S. Colucci, G. Brunetti, R. Rizzi, A. Zonno, G. Mori, G. Colaianni, D. Del Prete, R. Faccio, A. Liso, S. Capalbo, V. Liso, A. Zallone, M. Grano, T cells support osteoclastogenesis in an in vitro model derived from human multiple myeloma bone disease: the role of the OPG/TRAIL interaction, Blood 104 (12) (2004) 3722–3730.
[43] K. Noonan, L. Marchionni, J. Anderson, D. Pardoll, G.D. Roodman, I. Borrello, A novel role of IL-17-producing lymphocytes in mediating lytic bone disease in multiple myeloma, Blood 116 (18) (2010) 3554–3563.
[44] S. Dhodapkar, P. Barbuto, A. Matthews, A. Kukreja, D. Mazumder, S. Vesole, M.V. Jagannath, Dendritic cells mediate the induction of polyfunctional human IL17-producing cells (Th17-1 cells) enriched in the bone marrow of patients with myeloma, Blood 112 (7) (2008) 2878–2885.
[45] N. Giuliani, G. Lisignoli, S. Colla, M. Lazzaretti, P. Storti, C. Mancini, S. Bonomini, C. Manferdini, K. Codeluppi, A. Facchini, V. Rizzoli, CC-chemokine ligand 20/macrophage inflammatory protein-3alpha and CC-chemokine receptor 6 are overexpressed in myeloma microenvironment related to osteolytic bone lesions, Cancer Res. 68 (16) (2008) 6840–6850.
[46] M. Abe, K. Hiura, J. Wilde, A. Shioyasono, K. Moriyama, T. Hashimoto, S. Kido, T. Oshima, H. Shibata, S. Ozaki, D. Inoue, T. Matsumoto, Osteoclasts enhance myeloma cell growth and survival via cell–cell contact: a vicious cycle between bone destruction and myeloma expansion, Blood 104 (8) (2004) 2484–2491.
[47] S. Abe, M. Kido, A. Hiasa, A. Nakano, H. Oda, T. Amou, BAFF and APRIL as osteoclast-derived survival factors for myeloma cells: a rationale for TACI-Fc treatment in patients with multiple myeloma, Leukemia 20 (7) (2006) 1313–1315.
[48] P.I. Croucher, R. De Hendrik, M.J. Perry, A. Hijzen, C.M. Shipman, J. Lippitt, J. Green, E. Van Marck, B. Van Camp, K. Vanderkerken, Zoledronic acid treatment of 5T2MM-bearing mice inhibits the development of myeloma bone disease: evidence for decreased osteolysis tumor burden and angiogenesis, and increased survival, J. Bone Miner. Res. 18 (3) (2003) 482–492.
[49] G. An, C. Acharya, X. Feng, K. Wen, M. Zhong, L. Zhang, N.C. Munshi, L. Qiu, Y.T. Tai, K.C. Anderson, Osteoclasts promote immune suppressive microenvironment in multiple myeloma: therapeutic implication, Blood 128 (12) (2016) 1590–1603.
[50] C. Tai, G. Acharya, M. An, M.Y. Moschetta, X. Zhong, M. Feng, A. Cea, K. Cagnetta, H. Wen, A. van Eenennaam, L. van Elsas, P. Qiu, N. Richardson, K.C. Munshi, APRIL and BCMA promote human multiple myeloma growth and immunosuppression in the bone marrow microenvironment, Blood 127 (25) (2016) 3225–3236.
[51] A.L. Horenstein, A. Chillemi, V. Quarona, A. Zito, I. Roato, F. Morandi, D. Marimpietri, M. Bolzoni, D. Toscani, R.J. Oldham, M. Cuccioloni, A.K. Sasser, V. Pistoia, N. Giuliani, F. Malavasi, NAD(+)-metabolizing ectoenzymes in remodeling tumor-host interactions: the human myeloma model, Cells 4 (3) (2015) 520–537.
[52] L. Antonioli, C. Blandizzi, P. Pacher, G. Hasko, Immunity inflammation and cancer: a leading role for adenosine, Nat. Rev. Cancer 13 (12) (2013) 842–857.
[53] F. Morandi, B. Morandi, A.L. Horenstein, A. Chillemi, V. Quarona, G. Zaccarello, P. Carrega, G. Ferlazzo, M.C. Mingari, L. Moretta, V. Pistoia, F. Malavasi, A non-canonical adenosinergic pathway led by CD38 in human melanoma cells induces suppression of T cell proliferation, Oncotarget 6 (28) (2015) 25602–25618.
[54] A.L. Horenstein, A. Chillemi, G. Zaccarello, S. Bruzzone, V. Quarona, A. Zito, S. Serra, F. Malavasi, A CD38/CD203a/CD73 ectoenzymatic pathway independent of CD39 drives a novel adenosinergic loop in human T lymphocytes, Oncoimmunology 2 (9) (2013) e26246.
[55] F. Costa, D. Toscani, A. Chillemi, V. Quarona, M. Bolzoni, V. Marchica, R. Vescovini, C. Mancini, E. Martella, N. Campanini, C. Schifano, S. Bonomini, F. Accardi, A.L. Horenstein, F. Aversa, F. Malavasi, N. Giuliani, Expression of CD38 in myeloma bone niche: a rational basis for the use of anti-CD38 immunotherapy to inhibit osteoclast formation, Oncotarget 8 (34) (2017) 56598–56611.
[56] V. Quarona, V. Ferri, A. Chillemi, M. Bolzoni, C. Mancini, G. Zaccarello, I. Roato, F. Morandi, D. Marimpietri, G. Faccani, E. Martella, V. Pistoia, N. Giuliani, A.L. Horenstein, F. Malavasi, Unraveling the contribution of ectoenzymes to myeloma life and survival in the bone marrow niche, Ann. N. Y. Acad. Sci. 1335 (2015) 10–22.
[57] A.L. Horenstein, V. Quarona, D. Toscani, F. Costa, A. Chillemi, V. Pistoia, N. Giuliani, F. Malavasi, Adenosine generated in the bone marrow niche through a CD38-mediated pathway correlates with progression of human myeloma, Mol. Med. 22 (2016).
[58] F. Morandi, D. Marimpietri, A.L. Horenstein, M. Bolzoni, D. Toscani, F. Costa, B. Castella, A.C. Faini, M. Massaia, V. Pistoia, N. Giuliani, F. Malavasi, Microvesicles released from multiple myeloma cells are equipped with ectoenzymes belonging to canonical and non-canonical adenosinergic pathways and produce adenosine from ATP and NAD+, Oncoimmunology (March) (2018), http://dx.doi.org/10.1080/2162402X.2018.1458809.
[59] N.W. van de Donk, M.L. Janmaat, T. Mutis, J.J. Lammerts van Bueren, T. Ahmadi, A.K. Sasser, H.M. Lokhorst, P.W. Parren, Monoclonal antibodies targeting CD38 in hematological malignancies and beyond, Immunol. Rev. 270 (1) (2016) 95–112.
[60] L. Sun, O.A. Adebanjo, B.S. Moonga, S. Corisdeo, H.K. Anandatheerthavarada, G. Biswas, T. Arakawa, Y. Hakeda, A. Koval, B. Sodam, P.J. Bevis, A.J. Moser, F.A. Lai, S. Epstein, B.R. Troen, M. Kumegawa, M. Zaidi, CD38/ADP-ribosyl cyclase: a new role in the regulation of osteoclastic bone resorption, J. Cell Biol. 146 (5) (1999) 1161–1172.
[61] L. Sun, J. Iqbal, S. Dolgilevich, T. Yuen, X.B. Wu, B.S. Moonga, O.A. Adebanjo, P.J. Bevis, F. Lund, C.L. Huang, H.C. Blair, E. Abe, M. Zaidi, Disordered osteoclast formation and function in a CD38 (ADP-ribosyl cyclase)-deficient mouse establishes an essential role for CD38 in bone resorption, FASEB J. 17 (3) (2003) 369–375.