CHAPTER 13
THE ENIGMATIC FUNCTION OF TREM-2 IN OSTEOCLASTOGENESIS

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1. ABSTRACT

The triggering receptor expressed on myeloid cells 2 (TREM-2) is a member of family of receptors that play a central role in regulating function of myeloid cells. TREM-2 is expressed on macrophages, microglia and pre-osteoclasts and transduces intracellular signals through the adaptor DAP12. In human, genetic defects of TREM-2 and DAP12 result in a rare syndrome characterized by presenile dementia and bone cysts. This syndrome and the tissue distribution of TREM-2 have indicated a role of the TREM-2/DAP12 complex in brain function and bone modeling, particularly osteoclastogenesis. Accordingly, human TREM-2- and DAP12-deficient pre-osteoclast precursors failed to differentiate *in vitro* into mature osteoclasts endowed with bone resorptive activity. In mouse, DAP12-deficiency also resulted in impaired osteoclastogenesis *in vitro* and a mild osteopetrosis *in vivo* although bone cysts were not observed. Surprisingly, TREM-2-deficiency in mouse led to accelerated osteoclastogenesis *in vitro* without osteopetrosis or bone cysts *in vivo*, revealing an unexpected inhibitory function of mouse TREM-2. These data demonstrate that TREM-2 function is essential for normal osteoclastogenesis. The conflicting results as to the relationship between TREM-2, DAP12 and osteoclastogenesis and bone modeling in human and mouse suggest that TREM-2 contribution to osteoclast biology may vary depending on the influence of additional DAP12-associated receptors and on the presence of TREM-2 ligands with variable avidity/affinity, which may induce either activating or an inhibitory signals through TREM-2/DAP12.

2. THE ITAM SIGNALING PATHWAY IN OSTEOCLASTOGENESIS

Osteoclasts are multinucleated giant cells of myeloid origin involved in bone resorption and homeostasis (Teitelbaum 2000). Development of osteoclasts from myeloid progenitors involves multiple signaling pathways. The M-CSF/M-CSF-R and RANK/RANKL pathways

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have been extensively characterized and shown to be essential in osteoclastogenesis (Takayanagi 2005; Teitelbaum 2000; Walsh, Kim, Kadono, et al. 2006). More recently, attention has focused on the pathways triggered by cell surface receptors that signal through the DNAX-activating protein 12 (DAP12, also called KARAP) and Fc receptor-common gamma chain (FcRγ) (Humphrey, Lanier, and Nakamura 2005; Koga, Inui, Inoue, et al. 2004; Mocsai, Humphrey, Van Ziffle, et al. 2004).

DAP12 and FcRγ are transmembrane adaptors expressed as disulfide bonded homodimers. They associate with a variety of cell-surface receptors via complementary charged transmembrane domains that form a salt-bridge in the context of the hydrophobic lipid bilayer. DAP12 and FcRγ cytoplasmic domains contain immunoreceptor tyrosine-based activation motif (ITAM) (Lanier 2003; McVicar and Burshtyn 2001; Nadler, Matthews, Turner, et al. 2000; Vivier, Nunes, and Vely 2004). In response to receptor ligation, Src kinases phosphorylate the tyrosines in the ITAM, forming docking site for the protein tyrosine kinases Syk and ZAP70. Syk and ZAP70 then phosphorylate the scaffolding molecules LAT and NTAL, recruiting proximal signaling molecules phosphatidylinositol 3-kinase (PI3-K), phospholipase C gamma, the SLP76/Vav complex, the Grb2/Sos complex and c-Cbl. These activate downstream signaling cascades resulting in intracellular Ca^{2+} mobilization, activation of PKC, activation of MAP-kinases and rearrangement of the actin cytoskeleton.

The importance of the ITAM signaling pathway in osteoclastogenesis has been recently demonstrated (Koga, Inui, Inoue, et al. 2004; Mocsai, Humphrey, Van Ziffle et al. 2004). In these studies, mice lacking both DAP12 and FcRγ exhibited impaired osteoclast differentiation, leading to severe osteopetrosis. Specifically, DAP12 and FcRγ are required for the generation of calcium signals that lead to induction of nuclear factor of activated T cells c1 (NFATc1), which is a key transcription factor for osteoclastogenesis (Asagiri, Sato, Usami, et al. 2005). Thus, although M-CSF/M-CSF-R and RANKL/RANK pathways are necessary for osteoclastogenesis, they are not sufficient, as the ITAM pathway is also required to activate osteoclastogenesis. In this article, we will focus on the function of one DAP12-associated receptor, the triggering receptor expressed on myeloid cells 2 (TREM-2), which has been recently shown to play a critical role in osteoclastogenesis.

3. THE TREM-2/DAP12 COMPLEX

DAP12 and FcRγ associate with multiple receptors, which are expressed inside and outside the immune system, including natural killer (NK) cells, B cells, monocytes, macrophages, dendritic cells, microglia and osteoclasts (Lanier 2003; McVicar and Burshtyn 2001; Nadler, Matthews, Turner, et al. 2000; Vivier, Nunes, and Vely, et al. 2004). We have concentrated our attention on a group of DAP12-associated receptors called triggering receptors expressed on myeloid cells (TREM). TREM receptors are cell surface glycoproteins encoded by a cluster of genes on human chromosome 6p21 and mouse chromosome 17C3 (Allcock, Barrow, Forbes, et al. 2003; Klesney-Tait, Turnbull, and Colonna 2006). TREM receptors include TREM-1, TREM-2, and in the mouse, TREM-3, as well as at least two other TREM-related molecules, called TREM-like Transcript (TLT)-1 and TLT-2. All of these receptors are members of the immunoglobulin super family and contain a single V type Ig domain. The closest TREM relative is NKp44, an activating NK cell receptor encoded by a gene closely linked to the TREM gene cluster (Cantoni, Bottino, Vitale, et al. 1999). More distant relatives of TREMs include CD300 family members, as well as the polymeric Ig receptor (Aguilar, Alvarez-Errico, Garcia-Montero, et al. 2004; Chung, Humphrey, Nakamura, et al. 2003; Jackson, Hart, Starling, et al. 1992).

Among TREM receptors, TREM-1 and TREM-2 have been more extensively characterized. The first TREM identified, TREM-1, is an amplifier of the inflammatory response that strongly potentiates the activation of granulocytes and macrophages to microbial products. The function

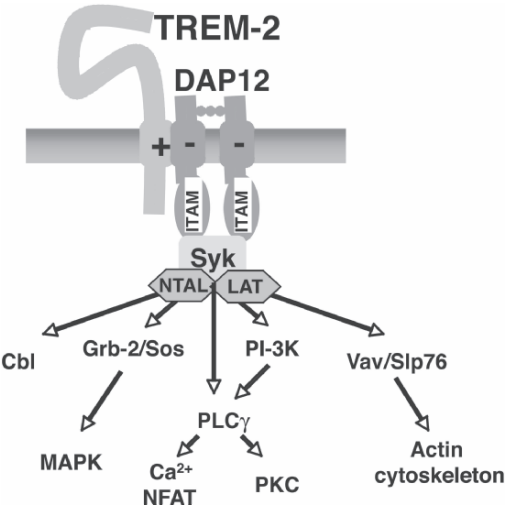

Figure 1. TREM-2/DAP12 signaling. TREM-2 associates with DAP12 for cell surface expression and signaling. Engagement of TREM-2 leads to tyrosine phosphorylation of DAP12 ITAM and subsequent recruitment of Syk. Syk promotes phosphorylation and recruitment of the signaling adaptors NTAL and LAT which trigger a signaling cascade leading to Ca2+ mobilization, PKC and MAP kinase activation and rearrangement of actin cytoskeleton (*See Color Plate*).

of TREM-1 has been recently reviewed (Klesney-Tait, Turnbull, and Colonna 2006). Because of its involvement in osteoclastogenesis, here we will focus on TREM-2. TREM-2 consists of a single V type Ig ectodomain, a transmembrane region and a short cytoplasmic tail lacking any signaling motifs. The transmembrane domain of TREM-2 has a positively charged residue, which mediates formation of a com plex with DAP12 (Figure 1). DAP12 is essential for surface expression and signaling by TREM-2.

TREM-2 was originally identified in human monocyte derived DC and shown to promote DC migration through the upregulation of the chemokine receptor CCR7 (Bouchon, Hernandez-Munain, Cella, et al. 2001). However TREM-2 has not been detected in primary DC and therefore it role in DC biology remains unclear. TREM-2 expression has been also demonstrated in macrophage cell lines (Daws, Lanier, Seaman, et al. 2001), bone marrow derived macrophages (Humphrey, Daws, Spusta, et al. 2006), macrophages recruited to the peritoneum following treatment with thioglycollate and on alternatively activated macrophages (Turnbull, Gilfillan, Cella, et al. 2006), suggesting a role for TREM-2 in macrophage biology. Beyond DC and macrophages, TREM-2 expression has been reported outside the immune system, in pre-osteoclasts (Cella, Buonsanti, Strader, et al. 2003; Paloneva Manninen, Christman, et al. 2002) and microglia (Schmid, Sautkulis, Danielson, et al. 2002; Sessa, Podini, Mariani, et al. 2004), suggesting a function for TREM-2 in bone and brain biology. Indeed, such as role has been demonstrated by recent studies of a rare genetic disorder, called Nasu-Hakola disease (NHD).

4. THE NASU-HAKOLA DISEASE

NHD, also called polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), is a recessively inherited disorder characterized by systemic bone cysts and progressive presenile dementia associated with sclerosing encephalopathy (Hakola, Jarvi, and Sourander 1970; Nasu, Tsukahara, and Terayama 1973; Paloneva, Autti, Raininko, et al. 2001).

The disease has been initially reported in Finland and in Japan, but has a worldwide distribution, with cases reported in South America and Italy. NHD patients develop joint swelling and/or fractures following minor accidents between the ages of 20–30. Multiple bone cysts are visible on x-ray and (in the absence of neoplasia) are pathognomic of Nasu-Hakola disease in affected populations. More insidious than the bone pathology, NHD patients develop a presenile dementia that is progressive and ultimately fatal. The central nervous system (CNS) of NHD patients contains sclerotic lesions including activated microglial cells particularly affecting the white matter of the brain.

The original study of Finnish NHD reported linkage of the disease to a region on chromosome 19. Analysis of the potential candidate genes in this chromosomal region revealed a homozygous deletion of exons 1–4 of the DAP12 gene (Paloneva, Kestila, Wu, et al. 2000). Confirming the role of DAP12, several Japanese and South American NHD cases were found to have a loss-of-function DAP12 mutation (Bianchin, Capella, Chaves, et al. 2004; Kondo, Takahashi, Kohara, et al. 2002). Subsequent analysis of NHD patients with intact DAP12 genes and normal levels of DAP12 expression revealed loss of function mutations in TREM-2 (Paloneva, Manninen, Christman, et al. 2002; Soragna, Papi, Ratti, et al. 2003). TREM-2- and DAP12-deficient patients develop identical disease, suggesting that the clinical manifestations of bone cysts and CNS disease result from the failure of TREM-2 signaling, either due to a defect in the TREM-2 receptor itself or in the associated DAP12 signaling chain. Given that TREM-2 is expressed in pre-osteoclasts and microglia, it is likely that the defects in the bone and the CNS observed in NHD patients result from a functional failure of these cells in the bone and brain respectively.

5. TREM-2 IS REQUIRED FOR OSTEOCLASTOGENESIS

To determine the potential role of the TREM-2/DAP12 complex in osteoclast function, studies were performed in human TREM-2- and DAP12-deficient osteoclasts in vitro and in DAP12-deficient mice both in vitro and in vivo. Osteoclasts can be derived in-vitro from blood monocytes or bone marrow cells by culture with M-CSF and RANKL, leading to fusion and differentiation of cultured cells into multinucleate osteoclasts capable of resorbing bone in vitro. In these culture conditions, blood monocyte derived from TREM-2- or DAP12-deficient patients were incapable of fusing into multinucleated osteoclasts endowed with bone resorptive activity (Table 1) (Cella, Buonsanti, Strader, et al. 2003; Paloneva, Mandelin, Kiialainen, et al. 2003). These data were validated using DAP12−/− mice. Murine DAP12−/− bone marrow cells failed to form bone-resorbing osteoclasts in culture, consistent with the human phenotype (Table 1) (Kaifu, Nakahara, Inui, et al. 2003). Similar conclusions were reached by a recent study by Humphery et al. in which TREM-2 expression in osteoclast precursors is attenuated by siRNA (Humphrey, Daws, Spusta, et al. 2006). These authors found that decreasing TREM-2 levels resulted in defective

Table 1. Phenotype of TREM-2/DAP12 deficiencies in human and mouse

		Phenotype	
		In vitro	*In vivo*
Human	DAP12−/−	Defect of osteoclast formation	Bone cysts
	TREM-2−/−	Defect of osteoclast formation	Bone cysts
Mouse	DAP12−/−	Defect of osteoclast formation	Mild osteopetrosis, no bone cysts
	TREM-2−/−	Accelerated osteoclastogenesis	No osteopetrosis or bone cysts

osteoclastogenesis in-vitro. Together, these results suggest that TREM-2 is required for successful osteoclastogenesis. Finally, in vivo studies of DAP12−/− mice revealed the development of a mild increase in bone mass, consistent with a defect in osteoclast function in-vivo (Table 1) (Faccio, Zou, Colaianni, et al. 2003; Humphrey, Ogasawara, Yao, et al. 2004; Kaifu, Nakahara, Inui, et al. 2003).

All these studies demonstrate a role of TREM-2/DAP12 in osteoclastogenesis and reveal that a deficit of TREM-2/DAP12 may lead to a mild osteopetrosis. However, it is noteworthy that unlike the DAP12−/− mice, NHD patients have normal numbers of osteoclasts and do not have an obvious increase of bone mass but instead develop bone cysts (Table 1). Thus, it is possible that TREM-2/DAP12 have additional functions on bone modeling that have yet not been recognized in vitro and/or in vivo.

6. THE PARADOXICAL INHIBITORY FUNCTION OF TREM-2

Although the DAP12 signaling pathway is generally considered to trigger cell activation, recent data demonstrate that in some context DAP12-signaling downstream of TREM-2 may have an inhibitory effect on cell activation. Initial studies by Hamerman et al. demonstrated that DAP12−/− macrophages have an exaggerated response to TLR ligation, as if DAP12 inhibits macrophage responses to microbial components (Hamerman, Tchao, Lowell, et al. 2005). Since DAP12 associates with multiple receptors, it was unclear which DAP12-associated receptor mediates such inhibition. Recently, Hamerman et al. demonstrated that siRNA attenuation of TREM-2 expression in macrophages results in increased TLR responses (Hamerman, Jarjoura, Humphrey, et al. 2006). The generation of TREM-2-deficient mice has allowed the conclusive validation of the inhibitory function of TREM-2 on macrophages. Bone marrow derived macrophages from TREM-2−/− mice produce higher levels of the inflammatory cytokines TNF-alpha and IL-6 in response to the TLR agonists LPS, zymosan and CpG, demonstrating that TREM-2 inhibits the response to macrophages to TLR ligation (Turnbull, Gilfillan, Cella, et al. 2006). Moreover, parallel comparison of TREM-2−/− and DAP12−/− macrophages showed that TREM-2 deficiency largely accounted for the increased cytokine production observed in DAP12−/− mice, demonstrating that it is TREM-2 that is the operative receptor in the phenotype reported for DAP12−/− mice (Turnbull, Gilfillan, Cella, et al. 2006). The mechanisms by which DAP12 and TREM-2 inhibit cellular activation are unclear, although several interesting hypothesis have been proposed recently (Hamerman and Lanier 2006). In one model, the type of signal delivered by TREM-2/DAP12 depends on the avidity/affinity of TREM-2 ligands. High avidity/affinity ligands may induce extensive TREM-2/DAP12 clustering, leading to full activation of the DAP12 signaling cascade. In contrast, low avidity/affinity ligands may induce an abortive DAP12 pathway, which may cause recruitment without activation of downstream signaling mediators, effectively resulting in their sequestration. Alternatively, this abortive pathway may lead to recruitment of protein tyrosine phosphatases that actively inhibit cell activation (Figure 2). Although this model has not been demonstrated for TREM-2 or any other DAP12 associated receptors, it has been previously established for other activating receptors such as the Fc receptor alpha and the T cell receptor (Pasquier, Launay, Kanamaru, et al. 2005; Stefanova, Hemmer, Vergelli, et al. 2003).

Consistent with an inhibitory function of TREM-2, preliminary data from TREM-2−/− mice suggests that in mice, TREM-2 may inhibit osteoclastogenesis. When osteoclasts were derived in-vitro from TREM-2−/− mice, we observed accelerated osteoclastogenesis with more rapid fusion of the cells to form functional osteoclasts capable of resorbing bone ex-vivo (Table 1)

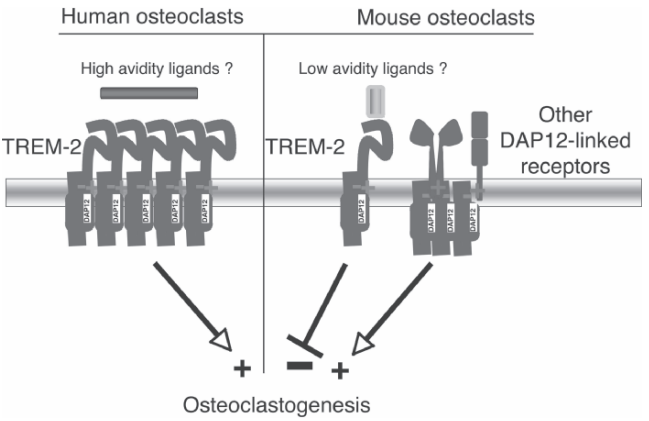

Figure 2. A model for the opposing functions of TREM-2/DAP12 in osteoclastogenesis. In human, TREM-2 is engaged by a high avidity/affinity ligand that induces extensive TREM-2/DAP12 clustering, leading to an activating signaling cascade. In mouse, TREM-2 is engaged by a low avidity/affinity ligand that induces abortive DAP12 signaling, leading to inhibition of osteoclastogenesis. In vivo, the activating signals mediated other DAP12 associated receptors overcome the inhibitory function of TREM-2. Thus, while TREM-2 deficiency accelerates osteoclastogenesis, DAP12 deficiency impairs it (*See Color Plate*).

(Colonna, unpublished results). Additionally, these mice do not have the osteopetrosis observed in DAP12−/− mice (Table 1). These data conflict directly with osteoclastogenesis data from the TREM-2- and DAP12-deficient NHD patients, and also the DAP12−/− mice. Based on the significant defect in osteoclastogenesis observed both in-vitro and in-vivo in DAP12-deficient mice, it is likely that there are other DAP12-associated receptors (in addition to TREM-2) that are operative in murine osteoclastogenesis (Figure 2). Moreover, it appears that while human TREM-2 activates osteoclastogenesis, mouse TREM-2 has an inhibitory role in the formation of osteoclasts. It is possible that, at least in our culture conditions, human TREM-2 is engaged by high avidity/affinity ligands that trigger activation, while mouse TREM-2 binds low affinity ligands that induce inhibition (Figure 2).

Thus, the role of TREM-2 in osteoclastogenesis remains unresolved, with human studies indicating that TREM-2 is required for the normal differentiation and function of osteoclasts, and mouse studies giving conflicting results as to the relationship between TREM-2, DAP12 and osteoclastogenesis. No doubt, understanding these opposing functions of TREM-2 will depend on the identification of TREM-2 ligands and their expression in bone.

7. TREM-2 LIGAND(S)

The ligand for TREM-2 is yet unknown, but several candidates have been proposed. A study by Daws et al. postulated a role for TREM-2 as a pattern recognition receptor specific for polyanionic microbial products. Soluble TREM-2 bound to bacteria, and bacteria bound to TREM-2 expressing cells. Additionally, these authors identified a putative endogenous ligand expressed by immortalized astrocytoma cell lines (Daws, Sullam, Niemi, et al. 2003). However, the specific molecules mediating these interactions have yet to be identified, and this will be critical to the further studies of TREM-2.

Studies by Hamerman et al. demonstrated that a putative TREM-2 ligand is expressed on bone-marrow derived macrophages (Hamerman, Jarjoura, Humphrey, et al. 2006). These studies extended from the observation that siRNA abrogation of TREM-2 expression by bone-marrow derived macrophages augmented inflammatory cytokine production, suggesting that bone marrow-derived macrophages express a TREM-2 ligand that engages TREM-2 and triggers an inhibitory signal. Hamerman et al. found that soluble TREM-2 receptor bound to bone-marrow derived macrophages and that this binding could be specifically blocked with a TREM-2 antibody. These data are promising; however, further studies will be required to identify the specific binding partner of TREM-2 expressed by macrophages.

Recent studies by Takegahara et al. suggest that TREM-2 ligand may be part of a multimeric complex. These author generated mice genetically deficient in PlexinA1, a molecule originally characterized for its role in axon guidance and cardiac morphogenesis (Takegahara, Takamatsu, Toyofuku, et al. 2006). They found that PlexinA1-deficient mice have a significant increase in bone mass as compared to wild type mice, and that this effect was likely mediated by interactions between PlexinA1 and a previously identified ligand, Semaphorin6D (Sema6D). Because Plexins often function in concert with a co-receptor, Takegahara et al. screened for interactions between the Plexin/Semaphorin system and potential candidate receptors that had previously been shown to act in bone homeostasis. This approach led to the identification of a receptor complex containing TREM-2, PlexinA1 and DAP12. Additional studies demonstrated that activation of PlexinA1 by Sema6D could be blocked by decreasing TREM-2 expression with siRNA. These data suggest that TREM-2 may function as part of a multimeric receptor complex, in which TREM-2 may bind Sema6D or, more likely, a second ligand associated with Sema6D. These results suggest that recognition of a distinct ligand by TREM-2 may require the presence of both a co-receptor such as PlexinA1 and its ligand, Sema6D.

8. CONCLUSION

The role of the TREM-2/DAP12 complex in normal bone modeling and osteoclast function is evidenced by a) the observation that genetic defects of human TREM-2 and DAP12 result in bone cysts b) the expression of TREM-2/DAP12 in pre-osteoclasts; c) the inability of human TREM-2- and DAP12-deficient pre-osteoclasts to develop into mature bone resorptive osteoclasts. In mouse, DAP12-deficiency impairs osteoclastogenesis and results in a mild osteopetrosis but no bone cysts were observed, suggesting that osteoclastogenesis may not be the only function controlled by TREM-2/DAP12, at least in human. Surprisingly, TREM-2-deficiency in mouse led to accelerated osteoclastogenesis in vitro without osteopetrosis or bone cysts in vivo, revealing an unexpected inhibitory function of mouse TREM-2. It is possible that in murine bone marrow-derived cultures TREM-2 is engaged by low avidity/affinity ligands that elicit an abortive DAP12 signaling. A complete understanding of the discrepancies between TREM-2 functions in human and mouse has been hampered by the failure to determine the specific ligands for the TREM-2, but recent data describing a multi-subunit receptor complex that includes Plexin A1, TREM-2 and Sema6D provide exciting insights into potential TREM-2 ligands. Ligand identification and the study of TREM-2-deficient mice will reveal the mechanisms involved in TREM-2-mediated bone modeling.

9. ACKNOWLEDGMENTS

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