Immunobiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Immunobiology

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

Influence of *Pasteurella multocida* Toxin on the differentiation of dendritic cells into osteoclasts

Sushmita Chakraborty[a], Bianca Kloos[a], Nina Roetz[a], Silke Schmidt[a], Tatjana Eigenbrod[a], Shigeki Kamitani[b], Katharina F. Kubatzky[a,*]

[a] Zentrum für Infektiologie, Medizinische Mikrobiologie und Hygiene, Universitätsklinikum Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany
[b] Graduate School of Comprehensive Rehabilitation, Osaka Prefecture University, Japan

ARTICLE INFO

Keywords:
Osteoclast
Osteoimmunology
Bacterial toxin
Immune evasion
Dendritic cells
G protein

ABSTRACT

Dendritic cells (DC) are antigen-presenting cells that connect the innate and adaptive immune system to ensure an efficient immune response during the course of an infection. Recently, DC came into the spotlight as a potential source of osteoclast progenitors, especially under (auto)inflammatory conditions. The virulence factor *Pasteurella multocida* Toxin (PMT) causes atrophic rhinitis in pigs, a disease characterised by a severe reduction of nasal bone. Our group and others have shown the potential of PMT in mediating differentiation of monocytes/macrophages into bone-resorbing osteoclasts. However, whether DC are target cells for PMT-induced osteoclast differentiation, is currently unknown. Using different murine DC model systems, we investigated the ability of PMT to induce osteoclast formation in DC. Similar to our previous observations in macrophages, PMT was endocytosed by DC and triggered intracellular deamidation of residue Q209 of the Gq alpha subunit. Still, PMT failed to induce prolonged secretion of osteoclastogenic cytokines and osteoclast formation; instead PMT-treated DC secreted interleukin-12 (IL-12), an inhibitor of osteoclastogenesis. In this study, we show that in comparison to bone marrow-derived macrophages, PMT induces maturation of DC through increased expression of the activation markers CD80 and CD86. As maturation of DC prevents their transdifferentiation into osteoclasts, we hypothesize that PMT, a potent osteoclastogenic toxin, fails to trigger osteoclastogenesis in DC due to its effect on DC maturation and IL-12 production.

1. Introduction

DC are important mediators of the immune system due to their ability to process and present antigens to T cells via the expression of MHC molecules on their cell surface. DC and macrophages develop from a common progenitor and it is thought that a so-called common DC progenitor can then further develop into either conventional DC (cDC) that migrate to specific tissues to finalise their differentiation or into plasmacytoid dendritic cells (pDC) that complete their differentiation in the bone marrow (Poltorak and Schraml, 2015). Mature DC are considered terminally differentiated, but in response to various growth and differentiation factors they can further differentiate, showing that these cells possess an unusually high degree of cellular plasticity (Wakkach et al., 2008). In addition to their pivotal role in the innate immune response and tolerance, a new aspect of DC functions came into the focus of research when it was found that myeloid DC share a common progenitor with osteoclasts (Miyamoto et al., 2001; Servet-Delprat et al., 2002). This prompted researchers to investigate whether under the right environmental conditions, DC might have the ability to transdifferentiate into osteoclasts.

Osteoclasts are considered to be haematopoietic, monocyte/macrophage-derived cells that differentiate in the bone through the action of two cytokines, M-CSF and RANKL, and have the unique ability to resorb bone material. Together with bone-building osteoblasts, they are responsible to maintain bone homeostasis. During inflammatory conditions, especially when chronic, bone loss can be observed due to increased osteoclast formation and activity. Under normal bone homeostasis, DC do not appear to contribute to bone remodelling as they are not located at the site of bone tissue but in lymphoid and non-lymphoid tissues (Laperine et al., 2016). In addition, DC-deficient mice do not show any skeletal abnormalities (McKenna et al., 2000). However, in inflammatory bone diseases such as rheumatoid arthritis or periodontal disease, both mature and immature DC have been observed at pathological sites like synovial and periodontal tissues (Highton et al., 2000; Laperine et al., 2016). As DC were not immediately recognised as important players in osteoclastogenesis, it was questioned whether these

* Corresponding author.
E-mail address: kubatzky@uni-heidelberg.de (K.F. Kubatzky).

http://dx.doi.org/10.1016/j.imbio.2017.09.001
Received 3 July 2017; Accepted 7 September 2017

cells simply promote macrophage differentiation into osteoclasts through the production of pro-inflammatory mediators or whether they are indeed able to actively participate in the process of osteoclastogenesis. Since DC express the necessary equipment to turn into osteoclasts, for example the M-CSFR (CD115), cathepsin K, tartrate-resistant acidic phosphatase (TRAP) and receptor activator of NF-κB ligand (RANKL), this was an intriguing possibility and therefore investigated (Boyce et al., 2006).

In a first attempt to study the potential of DC to transdifferentiate into osteoclasts, Rivollier et al. generated GM-CSF/IL-4-derived (G4) cDC from human blood monocytes (Rivollier et al., 2004). Much to their surprise, they observed that in the presence of M-CSF and RANKL, G4-DC (Rivollier et al., 2004) as well as DC derived through differentiation with Flt3L (Speziani et al., 2007) were actually more efficient and faster in fusion than blood monocytes, especially in the presence of pro-inflammatory cytokines. However, when the cDC displayed an activated phenotype, for example after LPS stimulation, they became unable to turn into osteoclasts (Rivollier et al., 2004; Speziani et al., 2007).

Since these initial reports could not rule out the possibility that the observed osteoclastic activity of DC was an interesting but unphysiological *in vitro* effect, the use of animal models such as calvaria-induced aseptic osteolysis subsequently proved that *in vivo* DC were indeed directed to the site of osteolysis (Maitra et al., 2010). In addition, clinical data support the finding that DC might play a relevant role in the pathophysiological context. It was shown that incubation of DC with the synovial fluid from arthritic patients increased the differentiation of these DC into osteoclasts, even in the absence of interacting cells such as T cells (Laperine et al., 2016; Rivollier et al., 2004).

Pasteurella multocida toxin (PMT) is well known for its strong impact on the bone system due to its direct action on osteoclasts and osteoblasts in porcine atrophic rhinitis (Horiguchi, 2012). For PMT-mediated osteoclast formation, both monocytes, as well as macrophages were shown to serve as target progenitors (Gwaltney et al., 1997; Kloos et al., 2015; Martineau-Doize et al., 1993). Recently, we could show that this occurs via a RANKL-independent mechanism that involves the production of pro-inflammatory cytokines as well as a persistent G protein activation (Chakraborty et al., 2017). Apart from this direct effect of PMT on osteoclast precursors, our group has shown alternate mechanisms of PMT in influencing osteoclastogenesis by modulating the expression of osteoclastogenic cytokines such as RANKL, TNF-α, IL-6 and IL-1β in B cells and by skewing the effector response of T helper (Th) cells towards osteoclast promoting Th17 cells (Hildebrand et al., 2011, 2015).

PMT is an AB toxin that is taken up by host cells through receptor-mediated endocytosis. The release of the active toxin into the cytosol allows the catalytic part to act as a deamidase on a specific glutamine residue within the alpha subunits of specific heterotrimeric G proteins, so that this glutamine is converted into glutamic acid (Babb et al., 2012). As a consequence, the Gα subunit is constitutively activated leading to numerous downstream signalling events. Previously, two initial studies investigated whether PMT might be able to interfere with the capacity of DC to activate the immune system (Bagley et al., 2005; Blocker et al., 2006). In their report the authors could show that the activation of the small GTPase RhoA, a typical signalling molecule downstream of PMT-induced G protein activation, induces pronounced changes in morphology and actin polymerization of peripheral blood monocytes treated with PMT (Blocker et al., 2006). This resulted in reduced cellular motility in response to chemokines or complement components suggesting that PMT decreases the ability of DC to travel to the site of infection and to trigger an adequate immune response (Blocker et al., 2006). Bagley et al. showed that treatment of human monocyte-derived DC as well as murine bone-marrow derived DC with high amounts of PMT resulted in the upregulation of activation markers needed for the interaction with T cells, which subsequently caused an increased stimulation of T lymphocytes (Bagley et al., 2005). Interestingly, despite this activating effect, PMT did not induce the production of certain pro-inflammatory cytokines in human DC and the maturation of the DC did not translate into the production of antibodies *in vivo*. Additionally, we recently showed that co-incubation of PMT with LPS strongly increases the LPS-mediated maturation of human monocytes, but that this simultaneous treatment strongly reduced the T cell-activating ability of LPS-treated monocytes through inhibition of IL-12 production by PMT, suggesting that there are cell type and model system-dependent differences (Hildebrand et al., 2012). However, none of these reports addressed a potential role of DC in osteoclast formation and in the present study we investigated whether PMT could trigger the formation of osteoclasts using different DC model systems. Our data show that PMT, despite its strong osteoclastic activity on monocytes/macrophages, does not induce the differentiation of DC into osteoclasts. In contrast to macrophages that readily form osteoclasts in the presence of PMT alone, neither G4 DC nor Flt3L-derived DC were able to form osteoclasts. Instead we found an increased expression of the activation markers CD80 and CD86 which is characteristic for DC maturation. In addition, we observed that PMT-treated DC secreted the osteoclastogenic cytokine TNF-α in high amounts, but produced only low amounts of the cytokines IL-1β and IL-6. PMT-induced TNF-α alone seemed to be insufficient to allow for osteoclast differentiation of DCs.

2. Materials and methods

2.1. Mice

Mice were maintained under SPF conditions in accordance with the German policies on animal welfare.

2.2. Reagents

RPMI 1640, foetal bovine serum (FBS), DPBS, Penicillin/Streptomycin and 2-Mercaptoethanol were purchased from Biochrom GmbH, PAA laboratories, PAN biotech, Merck and Sigma, respectively. The antibody against Gαq was obtained from Santa Cruz Biotechnology. An antibody that recognises the Q209E modification of $G\alpha_q$ was a gift of Prof. S. Kamitani (Osaka, Japan), a secondary HRP-linked antibody was obtained from Jackson ImmunoResearch (anti-rat IgG). Antibodies for FACS analysis were obtained from Biolegend (CD11c, CD45R, rat IgG2a-PE), BD Biosciences (CD11b, CD80, hamster IgG1-APC, hamster IgG2κ-PE) and eBioscience (CD86, Rat IgG2b-FITC). PCR primers were purchased from Apara (Denzlingen, Germany). Flag-Flt3L producing CHO flag Flk.2 Clone 5 cells were kindly provided by Prof. N.A. Nicola (WEHI Institute, Australia).

2.3. Differentiation of bone marrow cells

Bone marrow (BM) cells were isolated from the humerus, femur and tibia of female, app. 10 weeks old C57BL/6 mice. These cells were then used to generate bone marrow-derived dendritic cells (BMDC), or bone marrow-derived macrophages (BMDM). Using LGM3-cell conditioned medium as a source of granulocyte/macrophage colony stimulating factor and IL-4, BMDC were differentiated for seven days. Alternatively, cell differentiation was performed using purified Flag-Flt3L (Naik et al., 2010). Here, 4×10^6 cells were seeded in the presence of 2 µl recFlt3L. Every third day, 1 ml of fresh Flt3L-containing medium was added until the cells were differentiated on day 10. BMDM were differentiated using L929-cell conditioned medium (LCCM) as described previously (Chakraborty et al., 2017).

2.4. Production of recombinant PMT

Recombinant PMT was produced from BL21(DE3)pLysS bacteria and purified via an FPLC-GST column and subsequent thrombin cleavage (50 U) followed by gel filtration on a Sephacryl S200 HR column.