Role of IL-17A signalling in psoriasis and associated bone loss

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ABSTRACT

Inflammation is a physiological reaction to tissue injury, pathogen invasion and a natural response to various stress stimuli. Innate and adaptive immune cells are activated and recruited to the site of inflammation to suppress or promote inflammation. The recruitment and activation of immune cells is modulated by cytokines and chemokines, which are regulated by transcription factors, such as AP-1 (Fos/Jun), NF-κB, NFATs and STATs. Moreover, it is now appreciated that chronic inflammation can lead to systemic effects affecting the whole organism by mechanisms which are not well understood.

Here we review our recent data obtained from the analyses of psoriasis patient samples as well as from AP-1 (Fos/Jun)-dependent, genetically engineered mouse models. The deletion of two AP-1 factors JunB and c-Jun in an inducible manner in adult mice, specifically in Keratin-5 expressing tissues, leads to a psoriasis-like disease. Importantly, the epidermal proteome of the mutant mice is comparable to psoriasis patient samples. Our analyses revealed that the activation of S100A8/A9-dependent C3 complement as well as a miR-21-dependent TIMP-3/TACE pathway leading to TNF-α shedding, are causally involved in disease development.

Epidermal deletion of only JunB in mice leads to chronic skin inflammation with increased levels of pro-inflammatory cytokines and multi-organ involvement. Our recent findings show that chronic skin inflammation induces bone loss through systemic elevated IL-17A signalling. This novel mechanism involves inhibition of osteoblast-mediated bone formation by reduced Wnt signalling with no effect on RANKL-dependent osteoclastic bone resorption. These data have important translational implications; blocking of IL-17A signalling, which is already approved for the treatment of psoriasis, should also be considered to prevent the adverse skeletal consequences of chronic inflammatory diseases.

Introduction

The AP-1 transcription factor complex is composed of homo- and heterodimeric complexes consisting of members of the Jun, Fos, ATF (activating transcription factor) and MAF (musculoaponeurotic fibrosarcoma) protein families. AP-1 transcription factors can be activated by growth factors, cytokines, chemokines, hormones and multiple environmental stresses (1). AP-1 activity can be regulated at the transcriptional, post-transcriptional level, by microRNAs or by interactions with other transcription factors and modulators. Importantly, different AP-1 dimers are expressed in a cell- and stage-dependent manner and AP-1 activity can promote or inhibit processes such as inflammation strictly depending on the cellular context (1-3). Genetically engineered mouse models (GEMMs) carrying specific genetic alterations of AP-1 genes have provided novel insights into their functions, in particular in bone, liver and skin biology. Recently, several reports demonstrated that AP-1 factors have important roles in common human diseases such as psoriasis, fibrosis and cancer.

Psoriasis is a chronic disabling skin disease that affects approximately 2% of the population. Persistent plaques of inflamed and scaly skin characterise the prototypic form of plaque psoriasis. It has been demonstrated that both the innate and adaptive immune systems play important roles in the patho-physiology of psoriasis. Several mouse models of psoriasis, targeting either immune cells or keratinocytes have been developed, constituting powerful tools to dissect the underlying molecular mechanisms that trigger specific aspects of this disease (4, 5). Psoriasis is a heterogeneous chronic inflammatory disease often associated with co-morbidities, which affect the cardio-vascular system, neurologic parameters, metabolism but also bone loss. Here we will provide a brief sum-

mary of what we learned from a mouse model for psoriasis and will discuss recent data linking chronic skin inflammation to bone loss.

The Jun/AP-1-dependent mouse model for psoriasis

Epidermal deletion of both JunB and c-Jun (*DKO**) in an inducible manner in adult mice leads to a psoriasis-like disease within 2 weeks (4, 6). The acute phenotype of *DKO** mice shares many hallmarks observed in psoriasis patients, for instance inflammation of joints, hyper- and parakeratosis of the epidermis as well as epidermal infiltration of T cells and neutrophils (6).

To validate the AP-1-dependent GEMM for psoriasis, we compared the signalling pathways altered in the *DKO** skin and in lesional skin from psoriasis patients. The epidermal proteome of the mutant mice was found to have high similarity to the one from psoriasis patient samples (7). In both the mouse model and in psoriasis patient-derived material, we identified S100A8/A9-dependent C3/CFB complement activation, as well as a *miR-21*-dependent TIMP-3/TACE pathway leading to TNF-α shedding (7, 8).

S100A8/A9 proteins were the top upregulated proteins in psoriatic epidermis together with the alternative complement pathway C3-CFB. Since the secretion of S100A8/A9 and C3 by keratinocytes induces the production of pro-inflammatory cytokines like IL-17, MCP-1 and RANTES, we propose that induction of C3 by S100A8-S100A9 leads to "primed" keratinocytes and subsequently to uncontrolled immune cell activation, angiogenesis, hyperproliferation of keratinocytes and finally to the chronic systemic inflammation that characterises psoriasis (7).

Altered functions of miRNAs are currently proposed to be important for the development of many diseases. Several miRNAs were described to be dysregulated in psoriasis, but their causal contribution to disease development has not been demonstrated. In the *DKO** mouse model, epithelial deletion of JunB and c-Jun leads to a down-regulation of TIMP-3, followed by an up-regulation of TACE activity resulting in dramatically increased soluble TNF-α levels (9). Interestingly, miR-21, a validated onco-miR that targets TIMP-3, was also upregulated in psoriasis. We have recently shown that *miR-21* expression is increased in epidermal lesions of patients with psoriasis, as well as in the Jun/AP-1 psoriasis-like mouse model. Importantly, *miR-21* upregulation led to reduced epidermal TIMP-3 expression, activation of TACE/ADAM17 and increased TNF-α levels (8). Mechanistically, using patient-derived skin samples and the Jun/AP-1 GEMM of psoriasis, we demonstrated that increased *miR-21* levels are the result of impaired transcriptional activity of Jun/AP-1 and/or JunB down-regulation, as reported for psoriasis. We further show that inhibition of IL-6 and thus activation of STAT-3 by JunB induces the expression of *miR-21* and a psoriasis-like phenotype. Inhibition of *miR-21* by locked nucleic acid (LNA)-modified anti-*miR-21* compounds ameliorated disease pathology in patient-derived psoriatic skin xenotransplants (PDX) and in the Jun/AP-1 psoriasis-like GEMM. Importantly, the beneficial outcome of targeting *miR-21* was identical to anti-TNF-α (Enbrel) treatment. The therapeutic benefit of restoring TIMP-3 expression or targeting TACE was also demonstrated in the Jun/AP-1 psoriasis-like GEMM. Therefore, modulating *miR-21* and the downstream targets TIMP-3 or TACE may be a potential therapeutic strategy for treating psoriasis (8).

Role of IL-17A signalling in psoriasis

IL-17A, a cytokine originally described to be an exclusive product of Th17 cells, has gained increasing attention in the pathology of psoriasis in the last decade. Recently, two IL-17 blocking agents secukinumab and ixekizumab have been approved for the treatment of moderate-severe psoriasis (10-12). We have observed increased IL-17A and IL-23 expression in our Jun/AP-1 psoriasis-like mouse model (*DKO**) (13) (and unpublished data). Furthermore, S100A8/A9 proteins, also upregulated in the *DKO** skin and in human psoriasis, have been linked to the biology of this cytokine (14). It is now clear that psoriasis is not merely a skin disease but more a systemic inflammatory disease with several co-morbidities (15-17). Neither the molecular mechanisms underlying these co-morbidities nor the role of IL-17 and related cytokines in these pathologies are well understood. We recently described a new co-morbidity of psoriasis, systemic bone loss and mechanistically linked it to increased IL-17A signalling, which will be discussed below.

Role of IL-17A in the cross-talk between skin inflammation and bone loss

IL-17A plays a central role in inflammatory bone diseases and Secukinumab has been approved for psoriatic arthritis as well as ankylosing spondylitis (18). Rheumatoid arthritis (RA), the inflammatory bone disease with a rich macrophage infiltrate in the joint tissue, causes local bone destruction mostly by the paracrine actions of TNF-α and IL-6 (19). It has been described that in the context of RA, IL-17A leads to RANKL production by mesenchymal cells (synovial cells and osteoblasts) in the joint to activate the osteoclasts for bone degradation (20, 21).

The effect of IL-17A on bone in the context of chronic systemic inflammation, however, was not previously addressed. We recently reported the effects of chronic skin inflammation, such as psoriasis, on bone, and the associated mechanisms leading to bone loss (22). Psoriasis patients with no joint involvement, but with increased serum IL-17A levels, were subjected to X-treme CT analysis. We observed a significant decrease in bone parameters, such as trabecular bone mineral density and bone volume together with decreases in bone formation biomarkers, such as P1NP and Osteocalcin. Surprisingly, no changes in biomarkers for bone degradation, such as CTX, neither in the osteoclastogenic cytokine RANKL were observed. These results indicate that psoriasis patients are in a low-bone formation state with no concurrent increase in bone degradation. Serum IL-17A levels inversely correlate with serum P1NP as well as

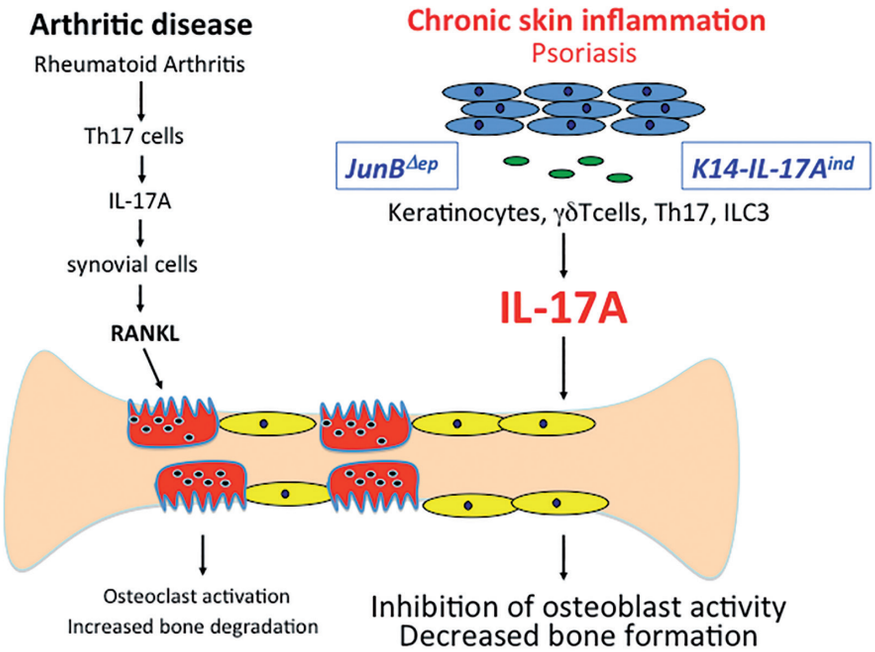

Fig. 1. Chronic skin inflammation can cause context-dependent inflammatory bone loss.

bone volume and bone mineral density. Importantly, when patients were stratified using serum IL-17A levels, the differences in bone volume were even more evident (22). These results indicate that inflammation originating from the inflamed skin and distant from the local bone tissue leads to bone loss via distinct mechanisms, namely decreased bone formation.

Studies using GEMMs for chronic skin inflammation with co-morbidities

To determine whether IL-17A expression from inflammatory skin was sufficient to lead to bone loss, mice with IL-17A expression in Keratin-5 expressing tissues, such as the skin keratinocytes, were employed (*K14-IL-17A^{ind}*) (23). These mice develop psoriasis-like disease within 3 months of age with infiltration of Th17 cells, γδT-cells and group 3 innate lymphoid cells (ILC3) (22, 23). Detailed analysis of the bone parameters using state-of-the art technology, such as histomorphometry, microCT and qBEI imaging, showed decreased trabecular and cortical bone volume, with decreased bone formation rates. Mineral apposition rates, measuring the activity of each osteoblast, were also decreased (22).

AP-1 transcription factors are gatekeepers of many distinct functions of the skin, including inflammation (2, 3, 24). As described above deletion of c-Jun and JunB in keratinocytes in adult mice, leads to a psoriasis-like phenotype in 2 weeks. To analyse the effects of chronic skin inflammation on bone, we exploited a GEMM previously described in our lab that involves the deletion of JunB/AP-1 in Keratin-5 expressing tissues (*JunB$^{\Delta ep}$*) leading to massive, systemic skin inflammation with multi-organ involvement (25, 26). IL-17A levels are upregulated in the serum of these mice, and the major source of IL-17A was found to be the skin (22). FACS analysis identified γδT-cells and ILC3s as the IL-17A producing cells in the skin of *JunB$^{\Delta ep}$* mice. Interestingly, Th17 cells are not detectable in the skin of these mice, which is in line with recent reports showing that Th17 cells might not be the most relevant IL-17A producing cells in psoriasis (27-29). Our future studies will address the role of T-cell-derived IL-17A in skin inflammation and bone loss.

JunB$^{\Delta ep}$ mice have a bone phenotype similar to the *K14-IL-17A^{ind}* mice, with decreased bone volume and decreased bone formation rates (22). It has been well established that during RA, where osteoclast activation leads to local bone tissue damage in the joint, IL-17A stimulates the synovial cells and osteoblasts to produce RANKL. We evaluated the RANKL levels in both mouse models with chronic skin inflammation, and surprisingly, did not find any increase in RANKL expression (Uluckan *et al*., unpublished data). This also is in line with the fact that CTX, a biomarker for bone resorption, as well as osteoclast numbers, are not increased in neither of the two models. Interestingly, IL-17A inhibits the mineralisation capacity of osteoblasts *in vitro*, with decreased expression of osteoblast markers, such as osterix and osteocalcin (22). The same effect was observed on the terminally differentiated osteocytes, where expression levels of Dmp1, a gene necessary for osteocyte function, are dramatically reduced upon IL-17A stimulation. RNAseq analysis of osteoblasts treated with IL-17A showed decreased Wnt signalling. The reduction in Wnt target gene expression was observed *in vivo* in the bones of both mouse models with chronic skin inflammation. Importantly, inhibition of IL-17A signalling via administration of a blocking antibody rescued the bone formation rates as well as Wnt target gene expression. These results show that in the context of chronic skin inflammation, systemic IL-17A leads to bone loss by reduced bone formation, a completely different mechanism when compared to local inflammatory bone disease such as RA (22) (Fig. 1).

Gravallese *et al*. also recently reported that IL-17A leads to decreased osteoblast differentiation and function through inhibition of Wnt signalling. Interestingly, the authors also show increased periostal bone formation in the absence of IL-17A in a serum transfer arthritis model (30).

Sclerostin, a potent inhibitor of Wnt signalling and a target for therapeutic applications for osteoporosis patients, was not altered in the GEMMs with chronic systemic skin inflammation (22). Wehmeyer *et al*. reported in an elegant study that sclerostin inhibition leads to opposite effects for inflammatory bone loss compared to osteoporosis. The authors showed that

inhibition of sclerostin promotes TNF-α-dependent joint destruction (31). These results suggest that signalling during inflammatory diseases can be extremely complex depending on the context, the nature and the source of inflammation. Thus caution should be taken when applying findings from one model into different disease settings. Overall, these results further suggest that IL-17A blockade, recently approved for the treatment of moderate-severe psoriasis, can have beneficial effects on the bones of these patients.

Conclusions

In arthritic diseases such as rheumatoid arthritis, Th17 cells present in the joint synovium, together with other inflammatory mediators, stimulate synovial cells and osteoblasts to produce RANKL, which in turn activates osteoclasts to degrade bone. In the context of chronic skin inflammation, such as psoriasis, modelled by GEMMs, IL-17A produced from different cell types such as keratinocytes, Th17, ILC3, γδT-cells, reaches the bone via the circulation and inhibits Wnt signalling in osteoblasts and osteocytes, thereby reducing bone formation rates.

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