Biochemical and Biophysical Research Communications 464 (2015) 1145–1150

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Selective inhibition of TNFR1 reduces osteoclast numbers and is differentiated from anti-TNF in a LPS-driven model of inflammatory bone loss

A.I. Espirito Santo [a, 1], A. Ersek [a, 1], A. Freidin [a], M. Feldmann [a, 2], A.A. Stoop [b, 3], N.J. Horwood [a, *]

[a] Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Roosevelt Drive, Headington, Oxford, OX3 7FY, United Kingdom
[b] GlaxoSmithKline, Biopharm R&D, Stevenage, UK

ARTICLE INFO

Article history:
Received 13 July 2015
Accepted 20 July 2015
Available online 22 July 2015

Keywords:
TNF receptor 1
p55
Lipopolysaccharide
Calvaria
Bone

ABSTRACT

The treatment of autoimmune disorders has been revolutionised by the introduction of biologics such as anti-tumour necrosis factor (anti-TNF). Although in rheumatoid arthritis patients a bone sparing effect of anti-TNF has been shown, the mechanism is not fully understood. Anti-TNF molecules block tumour necrosis factor (TNF) and prevent signalling via both TNF receptor 1 (TNFR1; p55) and TNF receptor 2 (TNFR2; p75). However, signalling via TNFR2 is reported to have protective effects in a number of cell and organ systems. Hence we set out to investigate if pharmacological inhibition of TNFR1 had differential effects compared to pan-TNF inhibition in both an in vitro cell-based model of human osteoclast activity and an in vivo mouse model of lipopolysaccharide (LPS)-induced osteolysis. For the in vitro experiments the anti-human TNFR1 domain antibody (dAb) DMS5541 was used, whereas for the in vivo mouse experiments the anti-mouse TNFR1 dAb DMS5540 was used. We show that selective blocking of TNFR1 signalling reduced osteoclast formation in the presence of TNF. Subcutaneous LPS injection over the calvaria leads to the development of osteolytic lesions within days due to inflammation driven osteoclast formation. In this model, murine TNFR2 genetically fused with mouse IgG1 Fc domain (mTNFR2.Fc), an anti-TNF, did not protect from bone loss in contrast to anti-TNFR1, which significantly reduced lesion development, inflammatory infiltrate, and osteoclast number and size. These results support further exploring the use of TNFR1-selective inhibition in inflammatory bone loss disorders such as osteomyelitis and peri-prosthetic aseptic loosening.

1. Introduction

The relationship between inflammation and bone loss has long been recognised in arthritis and chronic inflammatory disorders where reducing inflammation has a protective effect on bone [1,2]. By dampening inflammation there is a reduction in osteoclast number and activity whilst inhibitory effects of pro-inflammatory cytokines on osteoblast function are removed leading to an overall improvement in bone mass. Of the many cytokines implicated in this process, tumour necrosis factor (TNF) has been shown to potently stimulate osteoclast numbers by both increasing the pool of osteoclast precursors and by stimulating osteoblasts and T cells to produce more of the osteoclast differentiation factor, receptor activator of NF-kB ligand (RANKL). It has been reported that TNF does not induce osteoclastogenesis alone but does it in the presence of low amounts of RANKL, which is sufficient together with TNF to promote osteoclastogenesis [3]. TNF signals via two receptors, TNF receptor 1 (TNFR1; p55) and TNF receptor 2 (TNFR2; p75). Osteoclasts precursors express both TNF receptors, TNFR1 and TNFR2 and in vivo experiments using TNFR1 our TNFR2 knockouts have shown that signalling through only TNFR1 enhances

* Corresponding author.
E-mail address: nicole.horwood@kennedy.ox.ac.uk (N.J. Horwood).
[1] These authors contributed equally to this manuscript.
[2] Professor Sir Marc Feldmann has received consulting fees from GlaxoSmithKline (more than $10,000).
[3] Dr. Stoop owns stock or stock options in GlaxoSmithKline and is listed as an inventor on patents for a tumour necrosis factor receptor type I antagonist, which are assigned to GlaxoSmithKline.

http://dx.doi.org/10.1016/j.bbrc.2015.07.094
0006-291X/

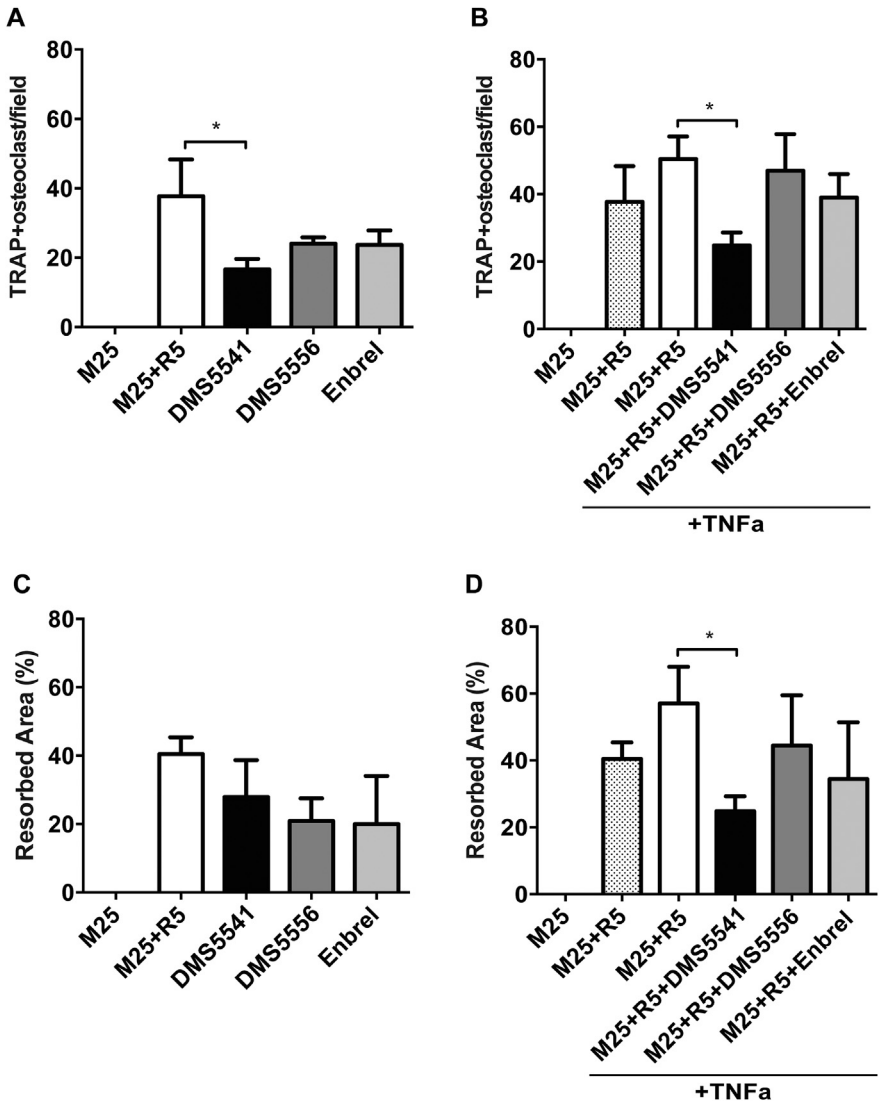

Fig. 1. Osteoclast formation is reduced by anti-TNFR1 whilst osteoblast formation is unaffected. Human monocytes were separated via elutriation and plated at 1×10^6/ml with M-CSF 25 ng/ml for 2 days. Adherent cells were then counted and placed into 96 well plates at 1×10^5 cells/well with M-CSF at 25 ng/ml and RANKL at 5 ng/ml. After 7 days, cells were fixed and stained for TRAP ($n = 3$). DMS5541 and DMS5556 were used at 100 nM, Enbrel at 10 nM. A) TRAP positive osteoclast number per field in the presence of dAbs. B) TRAP positive osteoclast number per field in the presence of dAbs and TNFα at 10 pg/ml. Monocytes were prepared as above and plated onto hydroxyapatite coated 96 well plates at 1×10^5 cells/well with M-CSF at 25 ng/ml and RANKL at 5 ng/ml. After 7 days, cells were removed and clear areas quantified ($n = 2$). C) Resorption area in the presence of dAbs (%). D) Resorption area in the presence of dAbs and TNFα at 10 pg/ml.

osteoclastogenesis whereas signalling through only TNFR2 supresses osteoclastogenesis [4].

Effects on osteoblasts are less clear as both positive and negative effects have been shown depending upon the concentrations and cell type/stage used [5]. Thus whilst a reduction in high TNF levels may be protective for bone, it is possible that lower amounts are required to maintain normal bone homoeostasis.

One of the most revolutionary treatments for rheumatoid arthritis (RA) and other autoimmune disorders in the last decade has been the use of anti-TNF [6,7]. Following on from initial studies measuring cytokine production by rheumatoid synovial tissue in vitro [8] and the spontaneous development of arthritis in mice overexpressing human TNF [9], TNF was the first cytokine to be fully validated as an effective therapeutic target for RA.

There are several different anti-TNF biologics including the monoclonal antibodies (mAb) Infliximab (Remicade) a chimeric mAb and adalimumab (Humira) a fully humanised mAb whilst etanercept (Enbrel) is a TNF receptor 2 (TNFR2; p75) – Fc fusion protein [10]. In a variety of systems TNFR1 is reported to be the major receptor that induces pro-inflammatory signalling and gene expression in response to TNF [11]. Experiments in mice have suggested neuro-, cardio- and osteoprotective and anti-inflammatory roles for TNFR2; so is blocking all TNF signalling the most effective and safest way to target inflammation? TNFR2 deficient mice challenged with lipopolysaccharide (LPS) showed a significant reduction in bone mineral density compared with wild type (WT) controls and TNFR1 deficient mice, supporting the importance of intact TNFR2 signalling for the maintenance of bone mass [12]. To investigate if further improvements in osteo-protection could be accomplished by selectively inhibiting only TNFR1, while sparing TNF-TNFR2 signalling, we used a series of dAbs against either human or mouse TNFR1. The use of pharmacological agents, instead of genetic knock-outs, could also increase the possible translational relevance to clinical intervention of our finding. Therefore, we choose to investigate the effects of selective TNFR1 inhibition on both human osteoclast numbers and activity as