International Immunopharmacology 57 (2018) 112–120

Contents lists available at ScienceDirect

International Immunopharmacology

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

Regulation of fetal hemoglobin expression during hematopoietic stem cell development and its importance in bone metabolism and osteoporosis

O. Kos[a], C. Alexander[b], K. Brandenburg[b], Z. Chen[a], A. Heini[c], D. Heumann[c], I. Khatri[a], J.P. Mach[d], E.T. Rietschel[e], A. Tersikh[f], A.J. Ulmer[b], T. Waelli[c], K. Yu[a], U. Zähringer[b], R.M. Gorczynski[a,*]

[a] Institute of Medical Sciences, University of Toronto, Toronto, Canada
[b] Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Borstel, Germany
[c] Clinique La Prairie, Clarens-Montreux, Switzerland
[d] Department of Biochemistry, University of Lausanne, Switzerland
[e] Alsterblick 14, D-22397 Hamburg, Germany
[f] Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California, USA

ARTICLE INFO

Keywords:
REDOX environment
Fetal hemoglobins
Inflammatory cytokines
Osteoclast/osteoblast differentiation in vitro

ABSTRACT

We have shown that an altered tissue redox environment in mice lacking either murine beta Hemoglobin major ($Hgb\beta_{ma}KO$) or minor ($Hgb\beta_{mi}KO$) regulates inflammation. The REDOX environment in marrow stem cell niches also control differentiation pathways. We investigated osteoclastogenesis (OC)/osteoblastogenesis (OB), in bone cultures derived from untreated or FSLE-treated WT, $Hgb\beta_{ma}KO$ or $Hgb\beta_{mi}KO$ mice.

Marrow mesenchymal cells from 10d pre-cultures were incubated on an osteogenic matrix for 21d prior to analysis of inflammatory cytokine release into culture supernatants, and relative OC:OB using (TRAP:BSP, RANKL:OPG) mRNA expression ratios and TRAP or Von Kossa staining. Cells from WT and $Hgb\beta_{ma}KO$ mice show decreased IL-1β,TNFα and IL-6 production and enhanced osteoblastogenesis with altered mRNA expression ratios and increased bone nodules (Von Kossa staining) in vitro after in vivo stimulation of mRNA expression of fetal Hgb genes (Hgbε and $Hgb\beta_{mi}$) by a fetal liver extract (FSLE). Marrow from $Hgb\beta_{mi}KO$ showed enhanced cytokine release and preferential enhanced osteoclastogenesis relative to similar cells from WT or $Hgb\beta_{ma}KO$ mice, with no increased osteoblastogenesis after mouse treatment with FSLE. Pre-treatment of WT or $Hgb\beta_{ma}KO$, but not $Hgb\beta_{mi}KO$ mice, with other molecules (rapamycin; hydroxyurea) which increase expression of fetal Hgb genes also augmented osteoblastogenesis and decreased cytokine production in cells differentiating in vitro. Infusion of rabbit anti- Hgbε or anti- $Hgb\beta_{mi}$, but not anti-Hgbα or anti- $Hgb\beta_{ma}$ into WT mice from day 13 gestation for 3 weeks led to attenuated osteoblastogenesis in cultured cells. We conclude that increased fetal hemoglobin expression, or use of agents which improve fetal hemoglobin expression, increases osteoblast bone differentiation in association with decreased inflammatory cytokine release.

1. Introduction

A transmembrane molecule, receptor activator of nuclear factor-kappaB ligand (RANKL) expressed on osteoblasts and bone marrow stromal cells [1] is a known ligand for RANK, a receptor which regulates osteoclast (OC) differentiation, activation and survival. RANK:-RANKL interaction induces osteoclastogenesis [2]. Osteoprotogerin (OPG) produced by osteoblasts (OB) and marrow stromal cells, lacks a transmembrane domain, and is a known decoy receptor for RANKL [3,4]. Altered expression of RANKL/OPG in both animal models and humans has been shown to regulate bone development [2–5]. It has been known for some time that inflammatory and anti-inflammatory cytokines, including TNFα, IL-1β, IL-6 and TGFβ [6–9], regulate osteoclastogenesis, with altered RANKL:OPG ratios implicated in these changes [10,11]. We ourselves have reported on in vitro culture model in which bone stem cells differentiate towards osteoclasts or osteoblasts as monitored by mRNA expression of RANKL, OPG, tartrate resistant acid phosphatase (TRAP) and bone sialoprotein (BSP) [12]-see also Fig. 1 in the study below. When such cultures were incubated under conditions of simulated microgravity (neutral buoyancy cultures) both

Abbreviations: TRAP, Tartrate resistant acid phosphatase; BSP, bone sialoprotein; RANKL, receptor activator of nuclear factor-kappaB ligand; OPG, osteoprotogerin; OC, osteoclast; OB, osteoblast; LPS, lipopolysacharide; MPLA, monophosphoryl Lipid A; F(A)SLE, fetal (adult) sheep liver extract; 5HMF, 5-hydroxymethyl furfural; PRR, pattern recognition receptors; GSSH, glutathione; NAc, N-acetyl cysteine; HgbF, fetal hemoglobin; DSS, dextran sodium sulfate

* Corresponding author at: 429 Drewry Avenue, Willowdale, Ontario M2R2K6, Canada.
E-mail address: reg.gorczynski@utoronto.ca (R.M. Gorczynski).

https://doi.org/10.1016/j.intimp.2018.02.010
Received 28 December 2017; Received in revised form 7 February 2018; Accepted 13 February 2018

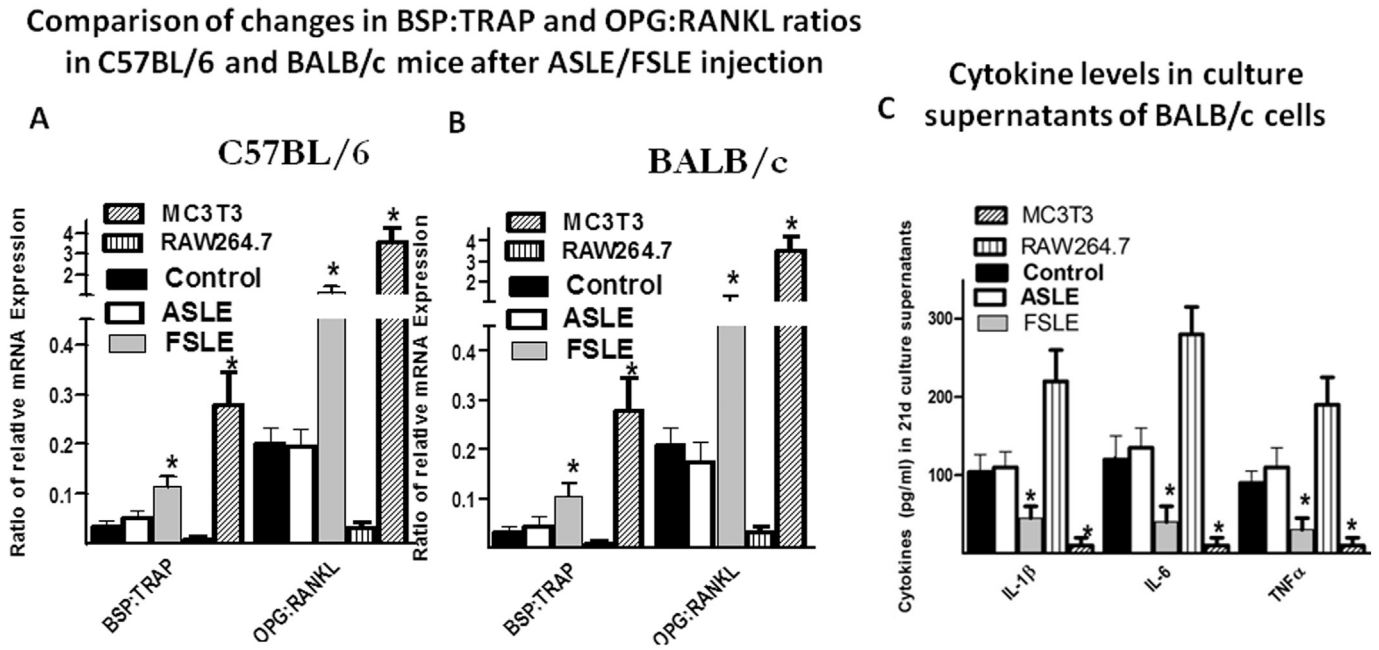

Fig. 1. Relative mRNA expression of markers of bone differentiation (Fig. 1A/1B) and cytokine production (Fig. 1C) at 21d in marrow cultures derived from C57BL/6 or BALB/c mice treated in vivo (×4 injections) with FSLE or ASLE (see text and Materials and Methods for more details). All groups used 5 mice, assayed individually, and results are pooled from 2 such studies. Data from pooled triplicate cultures of RAW264.7 and MC3T3 cell lines, used as markers of osteoclasts or osteoblasts respectively, are also shown. * indicates $p < 0.05$ compared with control or ASLE treated mice.

altered RANKL:OPG ratios and enhanced production of TNFα, IL-1β and IL-6 was seen [12], consistent with the enhanced osteoclastogenesis seen in such scenarios.

In independent studies we have also previously reported that injections of a crude fetal liver extract, FSLE, could regulate cytokine production from lymphocytes of treated mice, with major components of the biological activity in FSLE including LPS/monophosphoryl Lipid A (MPLA), the major intracellular tripeptide anti-oxidant glutathione (GSH), and fetal hemoglobin (HbF) or the purified fetal ovine γ globin chain (Hbγ) [13]. Others have also established that free adult hemoglobin enhances the production of tumor necrosis factor-α (TNFα) induced by LPS in human mononuclear cells [14], and amplify the rate of mortality by free LPS in rats [15,16]. Unlike in humans in mice there is no clear-cut fetal hemoglobin chain corresponding to Hgbγ, whose expression curtails rapidly following embryonic life, although a (Hgbε) chain is expressed only transiently in utero. Two forms of Hgbβ are known to persist in adulthood in mice, although expression of the fetal predominant chain ($Hgb\beta_{mi}$- > 70% Hgbβ at birth) is surpassed by the adult predominant chain in adults ($Hgb\beta_{ma}$- > 70% at 8 weeks) [17]. More recently we showed that FSLE could attenuate inflammation and inflammatory cytokine production in a murine model of inflammatory bowel disease (IBD) which follows administration of Dextran sodium sulfate (DSS) [18,19]. In addition, we observed that mice lacking $Hgb\beta_{mi}$ ($Hgb\beta_{mi}$KO) were exquisitely sensitive to IBD induced by DSS, with increased inflammatory cytokine production relative to WT controls. Furthermore, while amelioration of inflammatory cytokine production occurred in WT and $Hgb\beta_{ma}$KO mice following treatment with FSLE, this same treatment had no effect on IBD in $Hgb\beta_{mi}$KO mice. 5-hydroxymethyl furfural (5HMF), hydroxyurea or rapamycin, all of which were shown independently to augment mRNA expression of the gene encoding $Hgb\beta_{mi,}$ produced similar effects to FSLE. It is known that induction of fetal hemoglobin, with a superior oxygen carrying capacity/delivery to hypoxic tissues, can attenuate inflammation and oxidative stress via the NRF2 antioxidant signaling pathway, which may represent an important mechanism by which FSLE contributes to altered inflammatory cytokine production [20]. At a more general level we speculated injection of FSLE might affect local REDOX environments, including that in stem cell precursors in isolated niches, which in turn could alter differentiation within those niches independent of indirect effects mediated through altered inflammatory cytokine production [21–23].

In the studies described below we have investigated the ability of FSLE, and other molecules implicated in altering REDOX environments, on expression of fetal Hgb chains in cultured mouse bone marrow stem cells, on release of inflammatory cytokines from cultured cells, and on expression of mRNA markers of osteoclastogenesis/osteoblastogenesis. In addition, we have asked whether any effects on altered mRNAs associated with bone development were altered in the absence of genes encoding $Hgb\beta_{ma}$ (as seen in cells derived from $Hgb\beta_{ma}$ KO mice) or $Hgb\beta_{mi}$. We show that in the absence of $Hgb\beta_{mi}$. there is a differentiation bias towards osteoclastogenesis, which cannot be ameliorated by injection of FSLE.

2. Materials and methods

2.1. Mice

C57BL/6 and BALB/c mice of either sex were used throughout. Wild-type BALB/c mice were purchased from the Jackson laboratories (Bar Harbor, ME). Homozygous mice lacking either murine beta Hemoglobin major ($Hgb\beta_{ma}$KO) or minor ($Hgb\beta_{mi}$KO), both on a C57BL/6 background, were generated as described previously [19]. These mice were fertile, developed normally, and had no overt gross pathology [19]. WT control BL/6 mice were obtained from crossing heterozygote KO mice, and these littermate controls were used throughout. Lymphohematopoietic cell subsets in KO animals showed no obvious differences from their WT controls. All mice were housed five per cage under specific pathogen-free conditions, allowed standard diet and water ad libitum, and used at 6–8 wk. of age.

Animal experimentation was performed following guidelines of an accredited animal care committee (protocol no. AUP.1.18 at the University Health Network, Toronto, Canada) [18].