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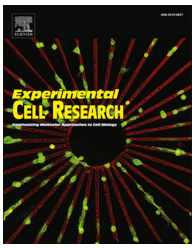

Research Article

Impact of bacteria and bacterial components on osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells

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ABSTRACT

Adult mesenchymal stem cells (MSC) are present in several tissues, e.g. bone marrow, heart muscle, brain and subcutaneous adipose tissue. In invasive infections MSC get in contact with bacteria and bacterial components. Not much is known about how bacterial pathogens interact with MSC and how contact to bacteria influences MSC viability and differentiation potential. In this study we investigated the impact of three different wound infection relevant bacteria, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, and the cell wall components lipopolysaccharide (LPS; Gram-negative bacteria) and lipoteichoic acid (LTA; Gram-positive bacteria) on viability, proliferation, and osteogenic as well as adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells (adMSC). We show that all three tested species were able to attach to and internalize into adMSC. The heat-inactivated Gram-negative *E. coli* as well as LPS were able to induce proliferation and osteogenic differentiation but reduce adipogenic differentiation of adMSC. Conspicuously, the heat-inactivated Gram-positive species showed the same effects on proliferation and adipogenic differentiation, while its cell wall component LTA exhibited no significant impact on adMSC. Therefore, our data demonstrate that osteogenic and adipogenic differentiation of adMSC is influenced in an oppositional fashion by bacterial antigens and that MSC-governed regeneration is not necessarily reduced under infectious conditions.

Introduction

Mesenchymal tissues contain a stem cell population that is responsible for its regeneration [26]. Best characterized to date are bone marrow-derived MSC (bmMSC). As bmMSC, adMSC are multipotent, i.e. they are able to differentiate into all types of cells belonging to the connective tissue, as e.g. adipocytes, osteoblasts, chondrocytes and myocytes [30,45]. As defined by the International Society for Cellular Therapy (ISCT) and the International Federation of Adipose Therapeutics and Sciences (IFATS), stem cells in the stromal vascular fraction of adipose tissue are CD31−,

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CD34+, CD45−, and CD235a− [1], assigning CD34 an important role for positive selection of stem cells. After plastic adherence, adMSC are CD31−, CD34−, CD36+, CD44+, CD45−, CD73+, CD90+, CD105+, and CD106−, thus expressing MSC markers common also to bone marrow-derived MSC, and have at least multipotent mesenchymal differentiation potential [7,20,44,45]. In contrast to bmMSC, adMSC can be isolated in large quantity and minimally invasive from the stromal vascular fraction of subcutaneous adipose tissue gained by liposuction, making up 7% of the cells found in liposuction-derived and collagenase-digested adipose tissue [36] and being present at a concentration of roughly 50,000 cells per ml tissue [22], which is 100-fold higher than that found for bmMSC [18].

Wound infections are common post-operative or post-traumatic complications. According to the German National Reference Centre for Surveillance of Nosocomial Infections, post-operative wound infections have an incidence of 1.6%. Almost half of these infections (46%) were superficial infections of the skin and subcutaneous tissues, but 30% of the infections involved deeper tissues such as muscles and fasciae, and up to 24% visceral cavities and organs [29]. The incidence of post-operative wound infections strongly depends on the type of surgery and the microflora of the respective operation site. Up to 9% of the patients undergoing open colon surgery suffer from post-operative wound infections, while the incidence of wound infections after joint replacements is between 0.5 and 2%.

Although wound infections are very common and therefore stem cells of the affected tissue get inevitably in contact with bacteria and bacterial components, the effects of this contact on stem cells are insufficiently characterized. Current studies showed that bmMSC and adMSC express Toll-like receptors (TLR) such as TLR2, 3, 4 and 7 involved in the recognition of bacterial structures [16,40]. Activation of the TLR influences the migratory activity of MSC, and continuous exposure of MSC to the bacterial cell wall component lipoteichoic acid (LTA) or lipopolysaccharides (LPS, endotoxin) leads to a reduction of the expression of TLR2 and TLR4. LPS additionally increases osteogenic differentiation in adMSC [16]. However, the influence of whole bacteria and bacterial components on differentiation of MSC is only incompletely characterized to date.

Therefore, we investigated the impact of heat-inactivated Gram-positive and Gram-negative wound infection bacteria as well as of the bacterial cell wall components LPS and LTA on viability, proliferation, and differentiation (osteogenic/adipogenic) of adipose-derived mesenchymal stem cells. For that purpose we chose two Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and one Gram-negative (*Escherichia coli*) species. *S. pyogenes* is associated with a variety of infections of the skin and mucosal membranes as well as wound infections, necrotizing fasciitis or sepsis [5,9]. *S. aureus* is the most important causative agent of wound infections (21% of the cases in Germany 2006–2010) and *E. coli* caused about 15% of the wound infections between 2006 and 2010 in Germany [29]. All three strains were obtained from wound or deep tissue infections and are therefore of high clinical relevance.

Material and methods

Unless otherwise indicated, all plastic ware was purchased from Greiner Bio-One (Frickenhausen, Germany), whereas all reagents were obtained from Sigma-Aldrich (Taufkirchen, Germany).

Bacterial strains and culture conditions

The *E. coli* strain used in this study has been freshly isolated at the Rostock University Medical Center from a patient with a wound infection. The *S. aureus* strain (*spa* type t331) derives from an endoprosthetic hip infection. The *S. pyogenes* M14 strain JS95 is an isolate from a patient with necrotizing fasciitis and was kindly provided by Emanuel Hanski (Hebrew University, Jerusalem, Israel). *E. coli* standard cultivation was in LB medium at 37 °C under constant shaking in ambient air conditions. *S. pyogenes* was grown in Todd Hewitt broth supplemented with 0.5% yeast extract (THY medium) under a 5% CO_2/20% O_2 atmosphere without shaking.

S. aureus cultures were grown in Brain Heart Infusion (BHI) medium at 37 °C under constant shaking in ambient air conditions.

Stem cell isolation, cultivation, and stimulation

adMSC were isolated from adipose tissue obtained from healthy patients having undergone tumescence-based liposuction. adMSC were isolated from adipose tissue via CD34-specific magnetic beads. Isolation of adMSC and their cultivation followed the procedure described previously [32]. Absence of contaminating monocytes/macrophages and endothelial cells was confirmed by flow cytometry, proving the absence of CD14+/CD68+ and CD31+ cells, respectively. After passage three, adMSC were seeded into experimentation with a density of 20,000 cells per cm^2. The differentiation media used were US (unstimulated control cultures), OS (osteogenic differentiation stimulating medium), AS (adipogenic differentiation stimulating medium). The basal US medium was high-glucose Dulbecco's Modified Eagle Medium (DMEM), supplemented with GlutaMAX-I, 1% penicillin/streptomycin (100 U/ml and 100 µg/ml, respectively, both Life Technologies GmbH, Darmstadt, Germany), and 10% fetal calf serum (FCS, PAN Biotech GmbH, Aidenbach, Germany). OS medium was basal medium supplemented with 10 mM β-glycerophosphate, 250 mg/l ascorbic acid and 1 µM dexamethasone. AS medium was basal medium supplemented with 1 µM dexamethasone, 500 µM 3-isobutyl-1-methylxanthine, 200 µM indomethacin, and 10 µM insulin. Renewal of medium was every second to third day. At confluence, culture with distinct stimulation media began, termed day zero, and took up to 35 d.

Adherence and internalization

Adherence and internalization of bacteria to adMSC was determined with the above-mentioned *E. coli*, *S. pyogenes* and *S. aureus* strains by an antibiotic protection assay as described previously [8]. Briefly, for the antibiotic protection assay early stationary phase bacteria were suspended in DMEM supplemented with 10% fetal calf serum to an optical density (OD; at 600 nm) of 0.05. Six wells of adMSC grown to sub-confluence in 24-well plates were infected with each 1 ml of the bacterial suspension. After 2 h, the eukaryotic cells were washed with phosphate-buffered saline (PBS). The cells from three wells were lysed with distilled water, and the number of bacteria contained in the lysate was assessed by viable counts. In the other three wells the eukaryotic cells were exposed to culture medium supplemented with penicillin (50 U/ml) and streptomycin (50 µg/ml) for the Gram-positive species or with ampicillin (100 µg/ml) for *E. coli* for another 2 h. Then these cells were washed and lysed, and the bacterial numbers were

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