INTRODUCTION:
Transplantation of mesenchymal stem cells (MSC) from bone mar-
row may offer approaches for the therapy of critical bone defects. The
use of MSC in experimental bone fracture models commonly involves
induction and amplification of osteogenic cell differentiation e.g. by
addition of osteogenic supplements such as dexamethasone, ascorbic
acid, and β-glycerophosphate. In contrast, a physiological fracture
repair is triggered by the differentiation of local MSC, the migration of
additional MSC to sites of injury and local regulatory signals. Early
fracture repair occurs in an inflammatory microenvironment which is
characterized by invading leukocytes.
Soluble mediators from activated leukocytes such as cytokines and
lipid mediators are critically involved in the regulation of tissue repair. A
standard inflammation model is the stimulation of isolated peripheral
blood leukocytes with the non-lytic bacterial toxins (lipopolysaccharide,
LPS or toxic-shock syndrome toxin-1, TSST-1).
Thus, it was the purpose of the study to analyze the influence of
mediators released by bacterial toxin-activated leukocytes on the differ-
etiation of human mesenchymal stem cells in vitro.

METHODS:
Human mesenchymal stem cells (MSC) obtained from Lonza (Walk-
ersville Inc., MD, USA) were cultured in RPMI1640 (Invitrogen GmbH,
Karlsruhe, Germany) supplemented with L-glutamine (0.3 g l−1), sodium
bicarbonate (2.0 g l−1), and 10% fetal calf serum (FCS, Invitrogen) under
standard cell culture conditions (5% CO2 humidified atmosphere).
Leukocytes (peripheral blood mononuclear cells, PBMC) were isolated
by discontinuous double Ficoll-gradient separation from peripheral
blood of healthy volunteers. Subsequently, the cells were stimulated
for 24h under cell culture conditions with lipopolysaccharide (LPS, 5 μg),
toxic shock syndrome toxin-1 (TSST-1, 10-50 ng) or left non-stimulated.
After stimulation the cell supernatants (conditioned media) were cen-
trifuged for 2 min at 2000 g to remove cells and cell debris. Subsequently,
the cell culture media of subconfluent MSC (2nd-4th passage) in 6-well-
plates were replaced by 4 ml of each conditioned medium or control
media (supplemented RPMI, supplemented RPMI containing LPS or
TSST-1). The MSC were incubated with conditioned media or control
media up to 28 days. Osteogenic differentiation was microscopically
analyzed by Alizarin Red S staining. Cytokines (IL-1ra, IL-2, IL-6, IL-8)
in conditioned media were analyzed by ELISA (R&D Systems). Cell
epitopes (CD34, CD45, CD90, FSP, 5ε) were analyzed by flow
cytometry (FACS Calibur, Becton Dickinson). Fibroblast surface protein
(FSP) and alpha-smooth muscle actin (alpha-SMA) were analyzed
immunohistochemically.

RESULTS:
Both types of conditioned media obtained either by LPS- or by
TSST-1-stimulation of leukocytes led to a completely different MSC
morphology and differentiation route which was not observed in the
presence of control media. Leukocytes stimulated with LPS promoted
osteogenic differentiation of MSC accompanied by a pronounced bone
node formation (Fig.1).

In contrast, supernatants from TSST-1-activated leukocytes did neither
lead to bone nodule formation nor to osteogenic differentiation of MSC
but led to the occurrence of small spindle-like cells (Fig.2B). These cells
were found positive for CD90, CD34, CD45 and FSP (Fig.2D) and
increased their expression of TLR2 but not of TLR4. In addition, weak
intracellular alpha-SMA-immunoreactivity was measured.

Fig.2: Fluoromicrophotographs taken after culture of MSC in the pres-
ence of supplemented RPMI as control medium (A and C) or condi-
tioned medium obtained from TSST-1 (50 ng) -activated PBMC (B and
D). Cells were stained with calcein-AM to highlight cell morphology (A
and B). Expression of intracellular FSP (D) is shown (respective isotope
control, C). Cell nuclei were stained with DAPI (A,B,C,D).

The leukocyte-derived factor/factors which are responsible for the
differentiation of MSC in the used inflammation-model are still not
identified. The first analysis of distinct cytokines in the supernatants
of toxin-stimulated leukocytes revealed no significant differences in the
concentrations of IL-1ra, IL-6 or IL-8 after stimulation either with LPS
or TSST-1. In contrast, the release of IL-2 was significantly increased
from TSST-1-stimulated PBMC compared to LPS-stimulated PBMC.

DISCUSSION:
This study demonstrated the crucial role of leukocyte-derived factors
providing transplanted, invading or local MSC with differentiation
signals. PBMC stimulated either with LPS or TSST-1 release soluble
factor/factors which promote MSC differentiation either to the osteo-
genic lineage (LPS-stimulation) or to a alpha-SMA-positive cell type
(myofibroblast-like) which express also hematopoietic antigens such as
CD45 and CD34.

Further characterization of the leukocyte-driven differentiation of
MSC is useful for a possible direction of mesenchymal stem cells during
regenerative cell therapy and to elucidate the mechanisms of heterotopic
ossifications or pathological fibrosis.