The Initial Inflammatory Phase of Bone Healing is Influenced by the Fixation Stability

INTRODUCTION:
Bone healing involves a complex cascade of events influenced by biological and mechanical factors. During the initial phase of fracture healing an inflammatory reaction takes place in the hematoma formed immediately after trauma. Previously we have shown that immune cell subpopulations in the fracture hematoma (FH) differ during the first hours (1h-4h) of the healing process and in comparison to a soft tissue hematoma (STH) [1]. The aim of this study was to investigate the influence of the fixation stability on the inflammatory phase of bone healing. It was hypothesized that the cell subpopulations in the FH would be influenced by the fixation stability. Furthermore we expected a differential cytokine expression pattern in the periosteum adjacent to the osteotomy gap due to the fixator stability.

METHODS:
A double, mid-shaft osteotomy of the tibia (2 cm gap) was performed in female sheep and stabilized with a rigid (RF) (n=6) or rotationally instable (IF) (n=5) external fixator [2]. Hematoma samples from the osteotomy gap and the samples from the periosteum adjacent to the gap were harvested 60h postoperatively in both groups. As reference peripheral blood was taken preoperatively (B0) and periosteum of the intact tibia during surgery (P0). Cells from the hematoma were prepared for FACS-analysis and labeled with fluorescence coupled antibodies. FACS-analysis was performed with a BD FACS-Calibur system and the FlowJo software. Percentages were normalized to B0 for each animal. Total RNA was isolated from the periosteum using trizol. For statistical evaluation qRT-PCR was performed using GAPDH as housekeeping gene. To analyze the expression of selected genes, qRT-PCR was performed using GAPDH as housekeeping gene. For statistical evaluation the Mann-Whitney U test was used. Percentages were normalized to B0 for each animal.

RESULTS:
FACS-analysis demonstrated a lower leukocyte percentage in the FH of both groups 60h postoperatively compared to preoperatively taken blood (B0) (RF, p=0.031; IF, p=0.063), while a notably decrease in the macrophage percentage compared to BO was only seen in the RF group (p=0.063). The IF group showed a higher macrophage (p=0.056, Fig. 1) percentage compared to B0, while the B cell percentage in the RF group compared to B0 was significantly lower (p=0.016). The helper cell percentage was slightly higher in the RF (104 [100-115]) compared to the IF (90 [64-115]) group. Compared to B0, there was a higher Treg percentage in the IF group (p=0.063) and a significantly higher percentage in the RF group (p=0.016, Fig. 1).

In contrast, the percentage of cytotoxic T cells in the RF was lower compared to IF group (p=0.095) and decreased in relation to B0 (p=0.063).

DISCUSSION:
This study is the first to show that already within the first hours after tibial osteotomy, mechanical stabilization has a significant influence on the cellular composition of the fracture hematoma and thus determines the inflammatory phase. In the rigid fixation group, the decrease of leukocytes and macrophages in the FH at 60h compared to preoperatively taken blood was more pronounced than in the instable fixation group. Furthermore, a significant increase in regulatory T cells at 60h compared to blood was only seen in the rigid fixation group. These results might indicate the gradual decline of inflammation in the rigid fixation group and thus suggest that there might be a prolonged inflammatory phase in the instable fixation group.

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