

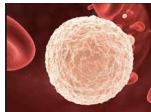
Inflammatory Cytokines: What Role Do They Play In Fracture Healing

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Disclosures

- Consulting
 - Invibio
 - RTI Biologics
- Institutional training and research support
 - Depuy Synthes
 - Stryker

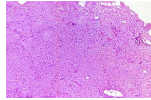
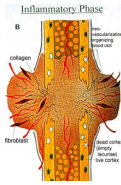
Leukocytes



- Play a pivotal role in host defense
- Also essential for fracture healing
- Fracture healing is initiated and directed by a physiological inflammatory response

Inflammation

- A critical and necessary initial phase
- WBCs infiltrate the fracture hematoma and produce several growth and differentiation factors that regulate fracture healing



Prolonged Inflammation

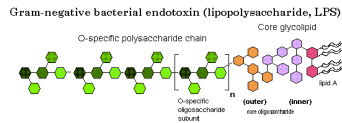
- Systemic
 - Severe trauma
 - Chronic inflammatory states
- Local
 - Infection
- Catabolic effects on fracture healing

Systemic inflammatory response syndrome (SIRS)

- An often noted complication associated with severe trauma
- Initiated by trauma which triggers an increase in pro-inflammatory cytokines
 - $\text{TNF } \alpha$
 - IL-2
 - Others

Lipopolysaccharide

- Trauma results in translocation of lipopolysaccharide (LPS) endotoxins from the gut



Lipopolysaccharide

- IM nail stabilized femoral rat osteotomy model
- Randomized to 3 groups
 - LPS systemically (IP)
 - LPS locally (at fracture site)
 - Control



Reikerås O et al. Acta Orthopaedica. 2005; 76: 749-753.

Lipopolysaccharide

- Mechanical stability of fracture evaluated at 6 weeks
- Bone mineral content and bone mineral density assessed by DEXA

Reikerås O et al. Acta Orthopaedica. 2005; 76: 749-753.

Results

- LPS induced a hypertrophic and immature callus

	Control group	LPS intraperitoneally	LPS locally	P-value (ANOVA)
Callus area	50 (5.5)	70 (5.4)	66 (2.5)	0.02
BMD	203 (16)	124 (9.8)	156 (11)	0.001
BMC	59 (4.0)	35 (3.9)	47 (3.7)	0.001

- Intra-peritoneal LPS group showed reduced mechanical strength

	Control group	LPS intraperitoneally	LPS locally	P-value (ANOVA)
Bending moment	8.8 (2.1)	3.1 (0.5)	11 (2.5)	0.02
Rigidity	1.9 (0.3)	1.0 (0.2)	2.2 (0.5)	0.06
Energy	24 (7.2)	6.8 (2.0)	34 (10)	0.04

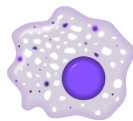
Conclusion

- Intra-peritoneal LPS reflects the clinical situation with fracture and endotoxinemia
- Endotoxinemia may impair clinical fracture healing

Reikerås O et al. Acta Orthopaedica. 2005; 76: 749-753.

Macrophage activation

- Rat femoral osteotomy model
- Macrophages activated by semisoluble aminated glucan
 - Intra-peritoneal
 - Locally
 - Control
- Fracture healing studied at 4, 8 and 12 weeks



Grundnes O, Reikerås O. J Orthop Sci. 2000; 5: 243-247.

Local Macrophage Activation

- Immature and hypertrophic callus

Table 1. Callus area (mm²) of rat femoral osteotomies after local (L) and intraperitoneal (IP) administration of semipurified antigenic glucan, and in control (C) rats 4, 8, and 12 weeks after osteotomy

Weeks	Local	Control	Intraperitoneal
4	69.1 61.4-95.8	55.4 40.4-61.6	73.9 70.1-80.8
<i>P</i> (L vs C) (C vs IP)	0.03	0.789	0.027
<i>P</i> (L vs IP)	76.5 71.3-96.0	53.8 41.8-73.1	58.8 23.9-43.4
<i>P</i> (L vs C) (C vs IP)	0.03	<0.001	0.027
<i>P</i> (L vs IP)	78.8 51.4-86.6	36.2 24.9-41.4	32.1 22.5-46.1
<i>P</i> (L vs C) (C vs IP)	<0.001	<0.001	0.040

- Reduced biomechanical properties

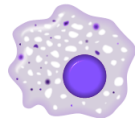
Table 2. Bending moment (Nm $\times 10^{-3}$) of rat femoral osteotomies after local (L) and intraperitoneal (IP) administration of semipurified antigenic glucan and in control (C) rats 4, 8, and 12 weeks after osteotomy

Weeks	Local	Control	Intraperitoneal
4	1.02 0.57-1.31	1.76 0.75-2.88	1.39 0.93-1.85
<i>P</i> (L vs C) (C vs IP)	1.47 1.04-5.15	6.51 1.08-7.95	6.93 6.72-9.24
<i>P</i> (L vs IP)	0.008	0.001	0.178
12	4.52 2.21-9.47	8.52 7.76-10.4	6.68 5.28-12.1
<i>P</i>	<0.001	<0.001	<0.001

Grundnes O, Reikerås O. J Orthop Sci. 2000; 5: 243-247.

Conclusion

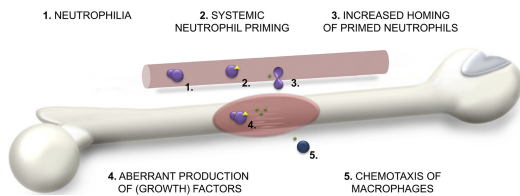
- Local macrophage activation during the initial phase of bone repair impairs fracture healing



Grundnes O, Reikerås O. J Orthop Sci. 2000; 5: 243-247.

Hypothesis

- Systemic inflammation impairs fracture healing by inappropriate homing of primed neutrophils towards the fracture hematoma



Bastian O, et al. J Leukoc Biol. 2011; 89: 669-673.

Fracture Healing in Absence of Adaptive Immune System

- Lymphocytes are main effector cells of the adaptive immune system
- Closed femur fracture model
 - Recombinant activating gene 1 (RAG1^{-/-}) knockout mice lacking adaptive immune system
 - Wild type mice

Toben D et al. J Bone Miner Res. 2011; 26: 113 - 124.

Fracture Healing in Absence of Adaptive Immune System

- RAG1^{-/-} animals had
 - Accelerated tissue resorption with higher osteoclast numbers at day 7
 - Enhanced biomechanical properties at 14 and 21 days
 - Greater callus mineralization and remodelling

Toben D et al. J Bone Miner Res. 2011; 26: 113 - 124.

Immunomodulation

- Rat femur osteomyelitis model
- Fracture fixation results in immunosuppression (decreased IL-12) at days 6, 10, and 12 compared to nonfractured controls
- Experimental group treated with IL-12 (daily IP 200 ng)



Lindsey BA et al. J Orthop Res. 2010; 28: 43 - 47.

Immunomodulation

- A decrease in IL-12 is thought to be clinically important because it is the gatekeeper for innate immunity
- It may be the key to normalizing function of the immune system against infection in trauma

Lindsey BA et al. J Orthop Res. 2010; 28: 43 - 47.

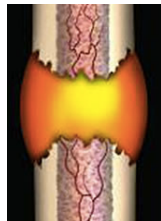
Immunomodulation

- IL-12 treatment group had significantly higher macrophage activation levels and total platelet counts at day 21
- Bacterial qualitative growth scores were significantly lower at day 10
- However overall infection rate was not changed

Lindsey BA et al. J Orthop Res. 2010; 28: 43 - 47.

Summary

- Inflammation plays a key role in the initial phase of fracture healing
- Excessive inflammatory response can lead to impaired fracture repair



Summary

- “Too much of a good thing can be wonderful”
Mae West
- “Too much of a good thing (inflammation) can impair fracture healing.”



Thank You
