T and B cell participate in bone repair by infiltrating the fracture callus in a two-wave fashion

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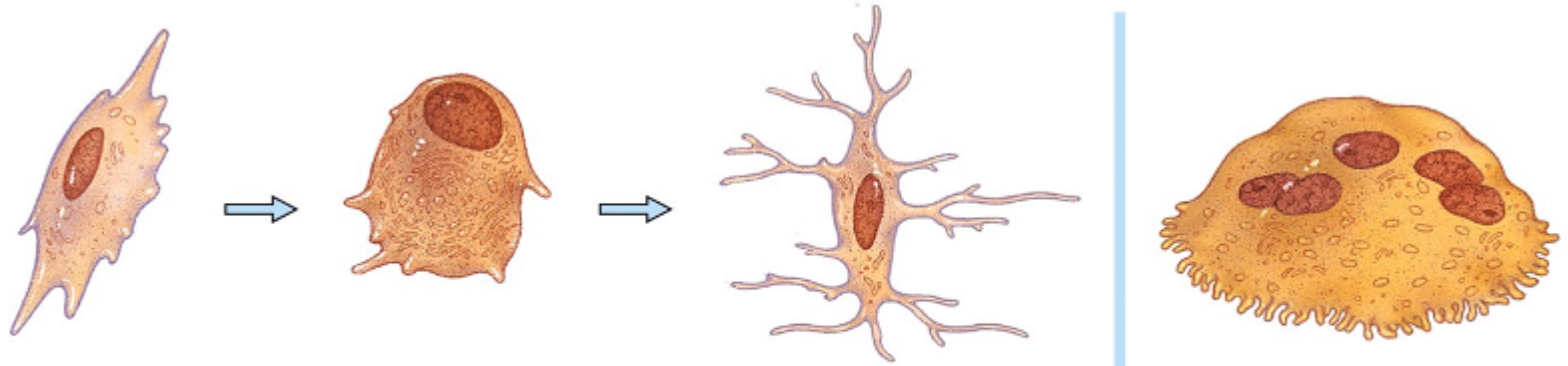
Introduction

- Bone:
  - scarless self-regeneration
  - 10-20% delayed healing or non-union

- Osteoblasts and Osteoclasts



https://www.easynotecards.com/notecard_set/7473

- Bone repair

Initial phase

- Fracture hematoma formation
- Inflammatory reaction triggering initiation of repair
- High expression of angiogenic factors

Soft callus formation

- About 7 days after fracture in mice
- Anti-inflammatory signaling
- Chondrocytes differentiate

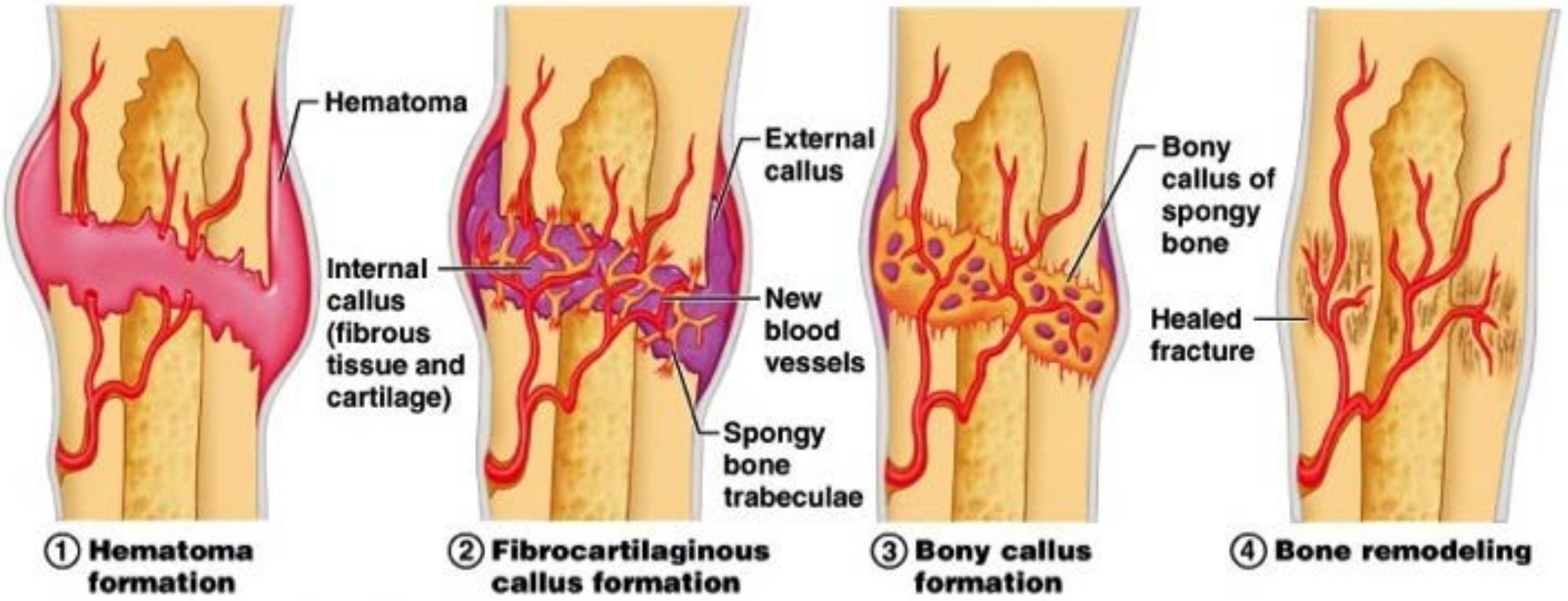
Woven bone (hard callus) formation

- About 14 days after fracture in mice
- High expression of angiogenic factors

Remodelling

- Starting about 21 days after fracture in mice

- Fracture healing and callus formation

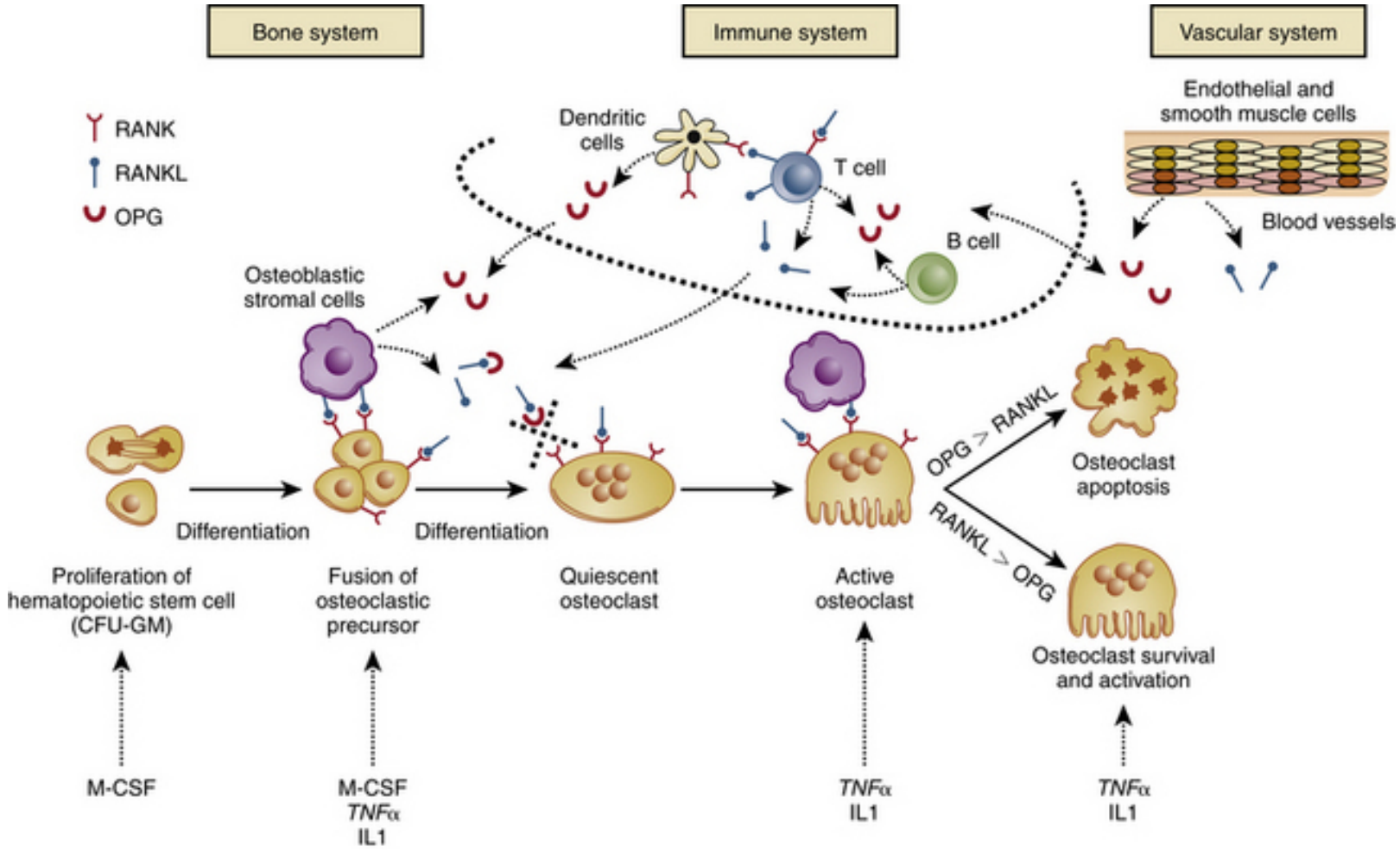


https://www.orthobullets.com/basic-science/9009/fracture-healing

- B- and T-cell interactions with skeletal cell system
  - Activated T-cells and osteoclasts: RANKL -> Osteoclastogenesis

  Direct activation of osteoclasts via TNF-α
    - -> bone resorption
    - BUT: CD4+ T-cells promote Osteoprotegerin (OPG) secretion by T-cells and **wild regulatory T-cells** support development and healing
  - B-cells and osteoblasts: OPG receptor -> blocks RANK-RANKL ligation -> inhibits octeoclastogenesis
    - ->bone apposition
    - B-cell deficient mice are osteoporotic (Li et al., 2007)

- Osteoprotegerin: decoy receptor for RANKL -> neutralizes its function in osteoclastogenesis



https://clinicalgate.com/bone-modeling-and-remodeling/

Methods

- 65 Wild type mice
- 61 mice with unilateral closed fracture (4 control mice)
  - Opening knee joint with patella dislocation
  - To produce the fracture they placed a pin into bone marrow cavity and used a three point bending machine

- Sacrification after 3, 7, 14, 21 and 28 post fracture
  - N = 11 per time point
  - Contralateral bones analyzed 1, 2, 3, 7, 14 and 21 days after surgery N = 6
  - Control group without intervention at t=0 (N = 4)

- Flow cytometry
  - Of flushed out bone marrow
  - Staining of CD3, CD4, CD8 and LIVE/DEAD stain
- Histological analysis
  - Femurs with sourround muscle
- Immunofluorescence analyses
  - Fractures bones with sourrounding muscle tissue
  - Staining with antobodies for B220, IgM, IgD, IgG1, IgG2ab, CD3e, CD4, Laminin, cathepsin K, osteocalcin and OPG (osteoprotegerin)
  - For quantitative analysis: 14d and 21d bones stained for T- and B-cells were gritted for counting
- Gene expression
  - Callus tissue arround intramedullary fixating pin

Results

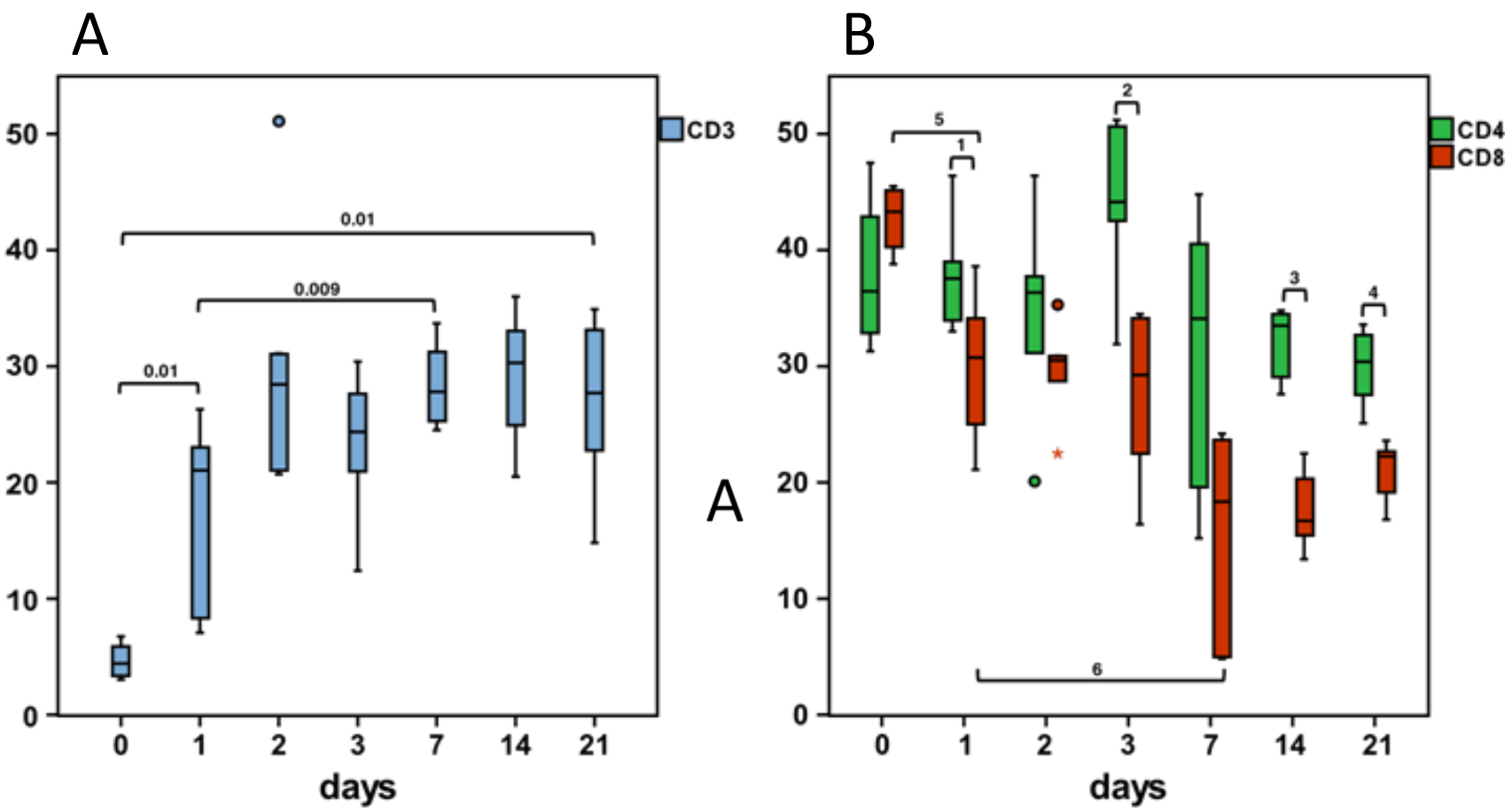
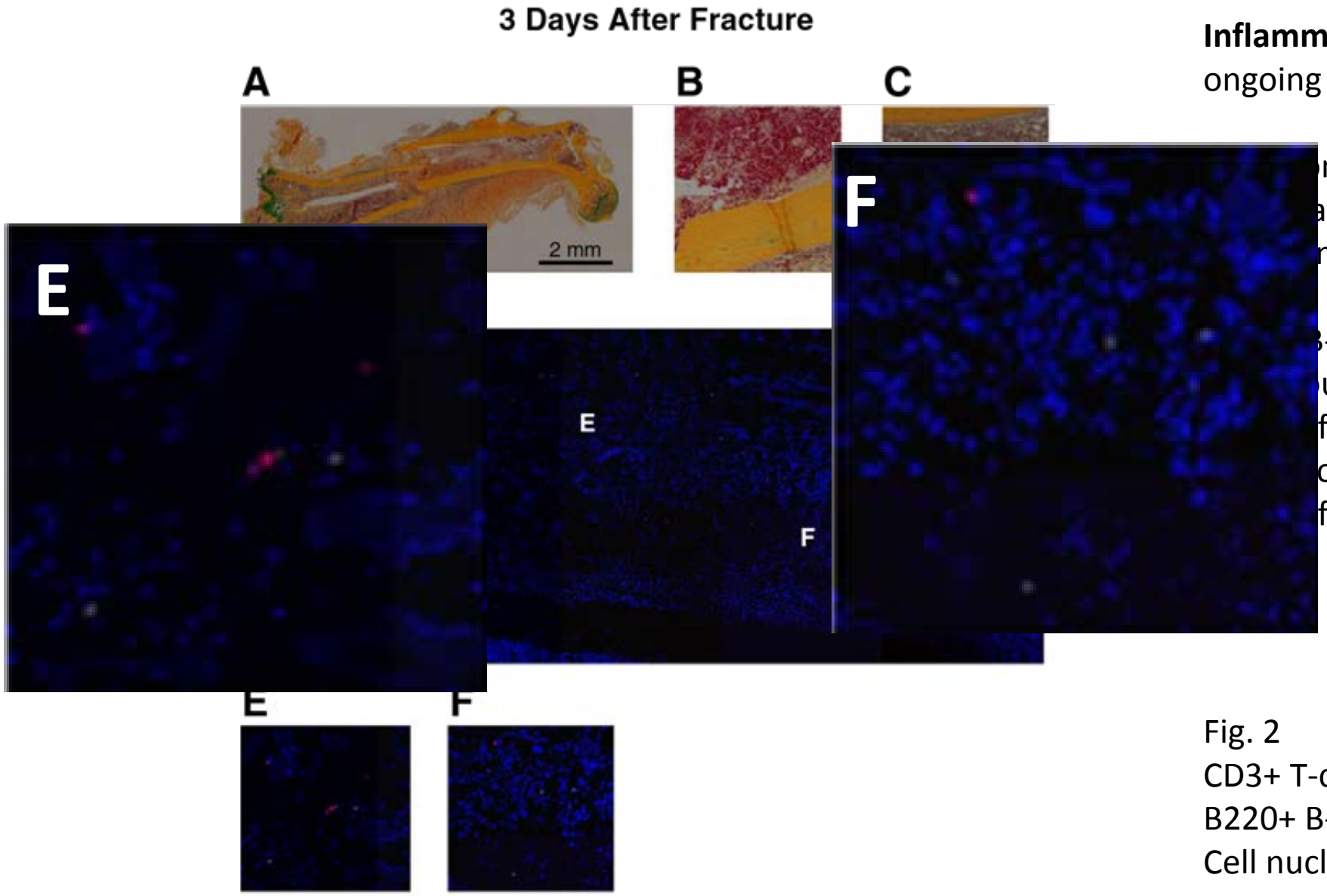


Fig. 1: Flow cytometry

A: comparison of CD3+ T-cells in **contralateral** bone marrow at different time points

B: Comparison of CD4+ and CD8+ T-cells in contralateral bone marrow at different time points



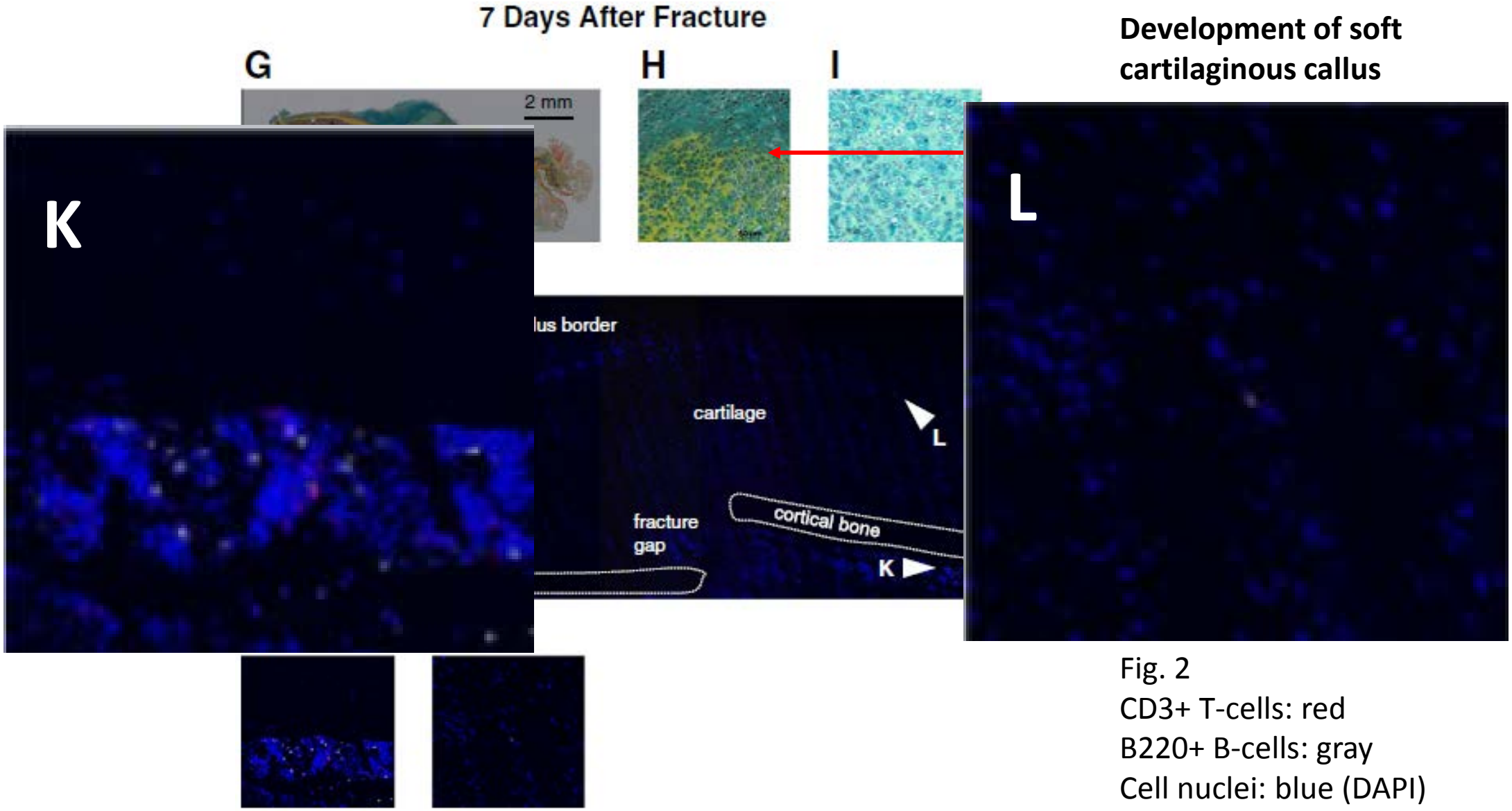
Inflammatory phase still ongoing

ma undergoes
ation evolving into
matous tissue

-cells are visible
ut the complete area
fractured bone - also
ounding muscle and
fracture gap

Fig. 2
CD3+ T-cells: red
B220+ B-cells: gray
Cell nuclei: blue (DAPI)

Development of soft cartilaginous callus



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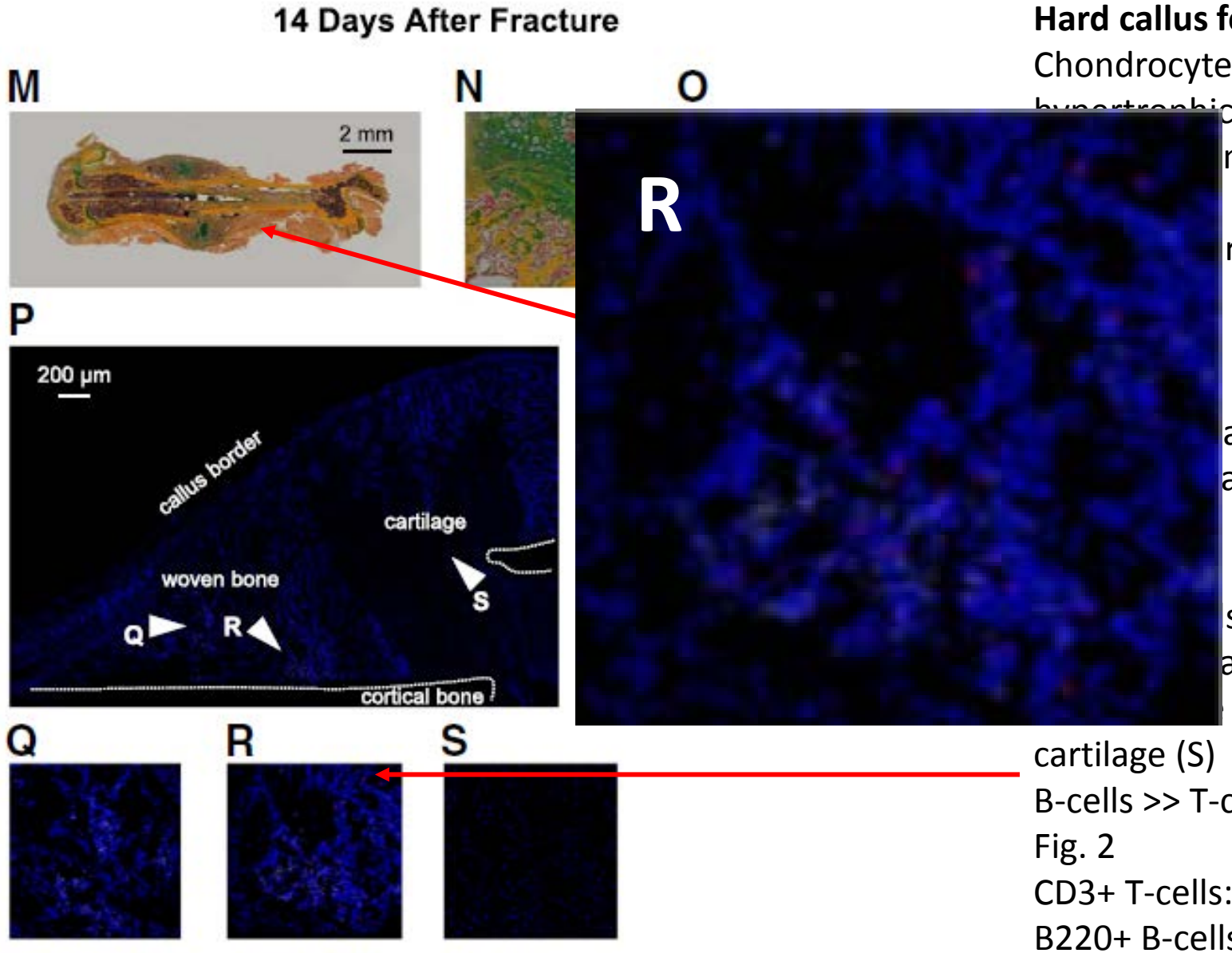


Hard callus formation

Chondrocytes become
hypertrophic synthesizing
matrix

rtilage is
and woven bone

ates from
and growing to

s reappear in large
areas nearby
but not in
cartilage (S)

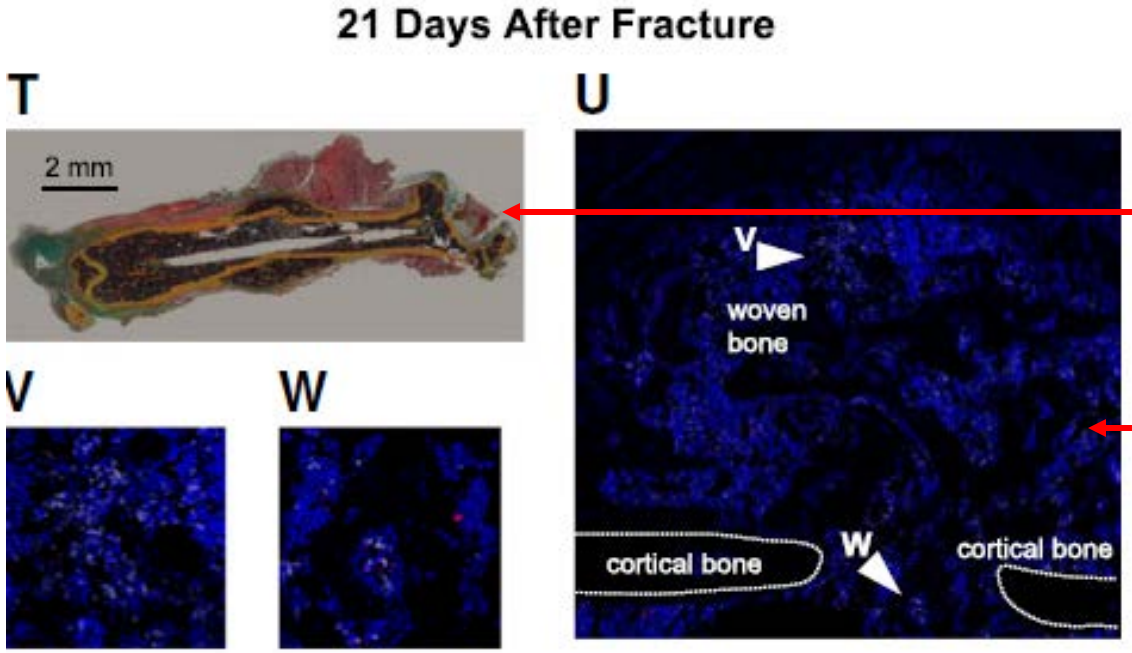
B-cells >> T-cells

Fig. 2

CD3+ T-cells: red

B220+ B-cells: gray

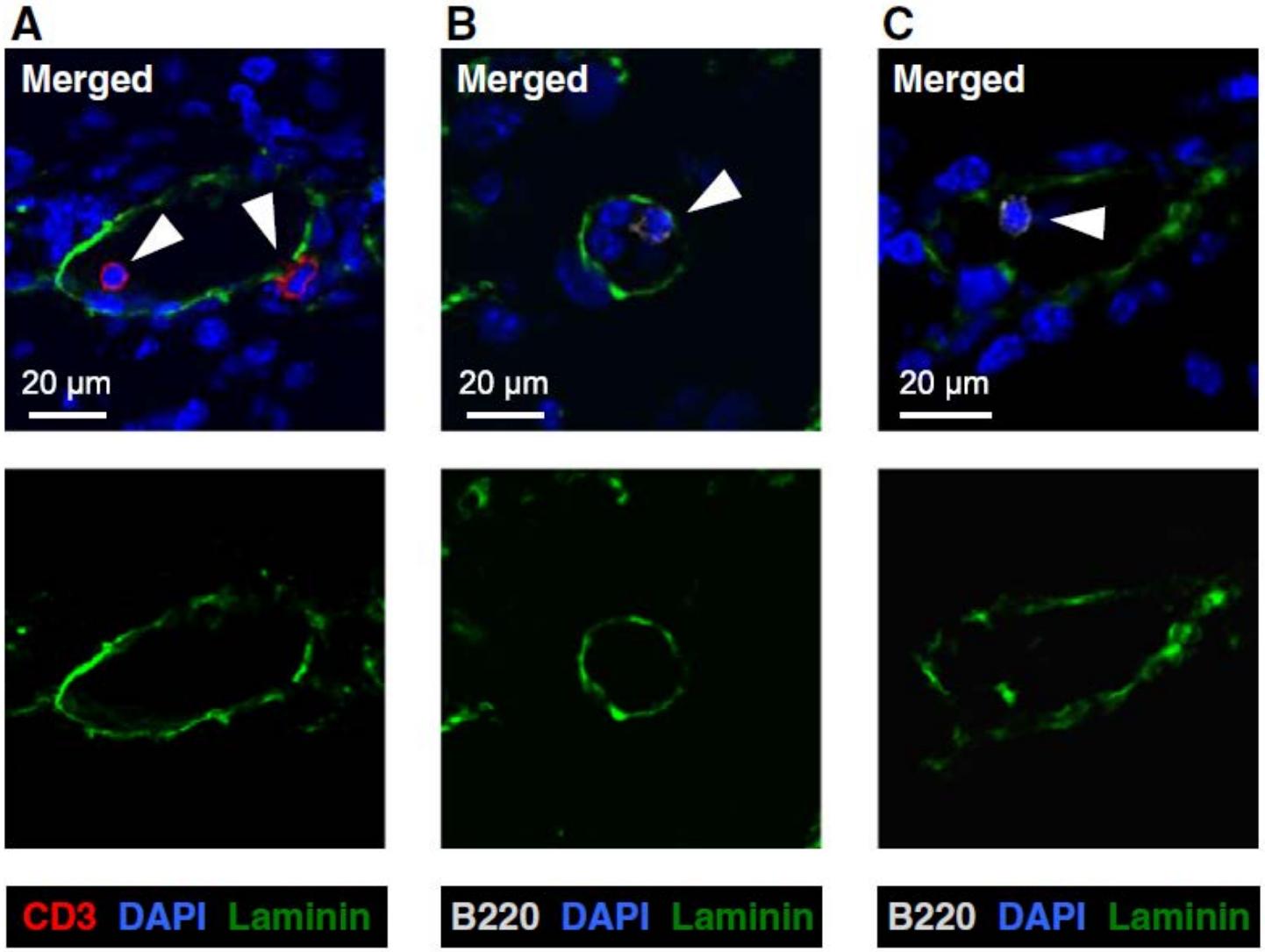
Cell nuclei: blue (DAPI)



Remodelling phase
No cartilage remaining
Bone marrow between new woven bone

High amount of B- and T-cells in callus
B-cells >> T-cells

Completion remodeling process
Bone regained pre-fracture form
CD3+ T-cells: red
B220+ B-cells: gray
Cell nuclei: blue (DAPI)



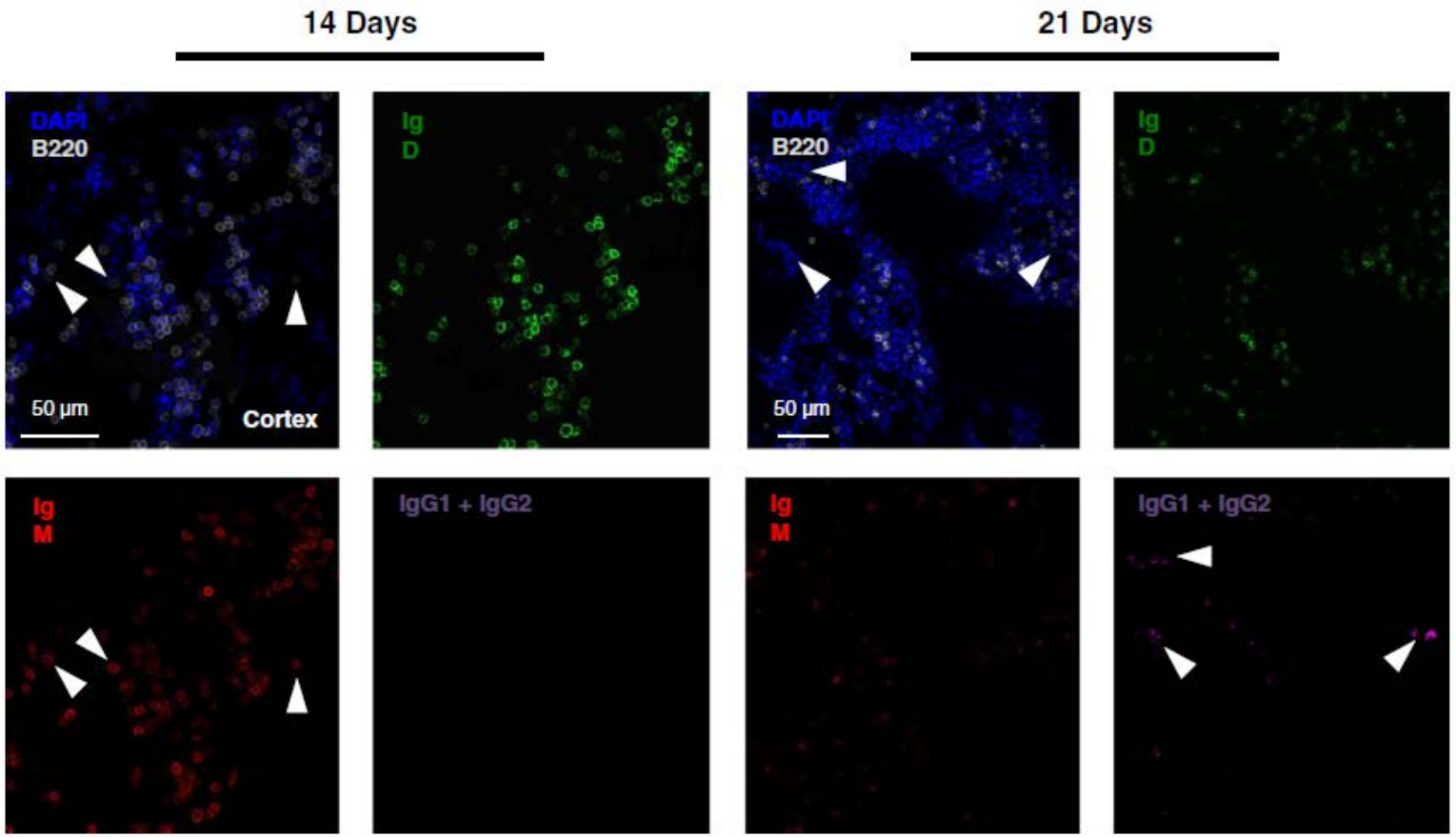
14 days after fracture
2nd wave of lymphocites

Colocation of T-cells and vasculature-associated protein laminin (A)

T- and B-cells intraluminal

-> strongly suggesting Infiltration of 2nd wave of lymphocytes via inner vasculature of callus

Fig. 3



Functional state of B-cells in callus

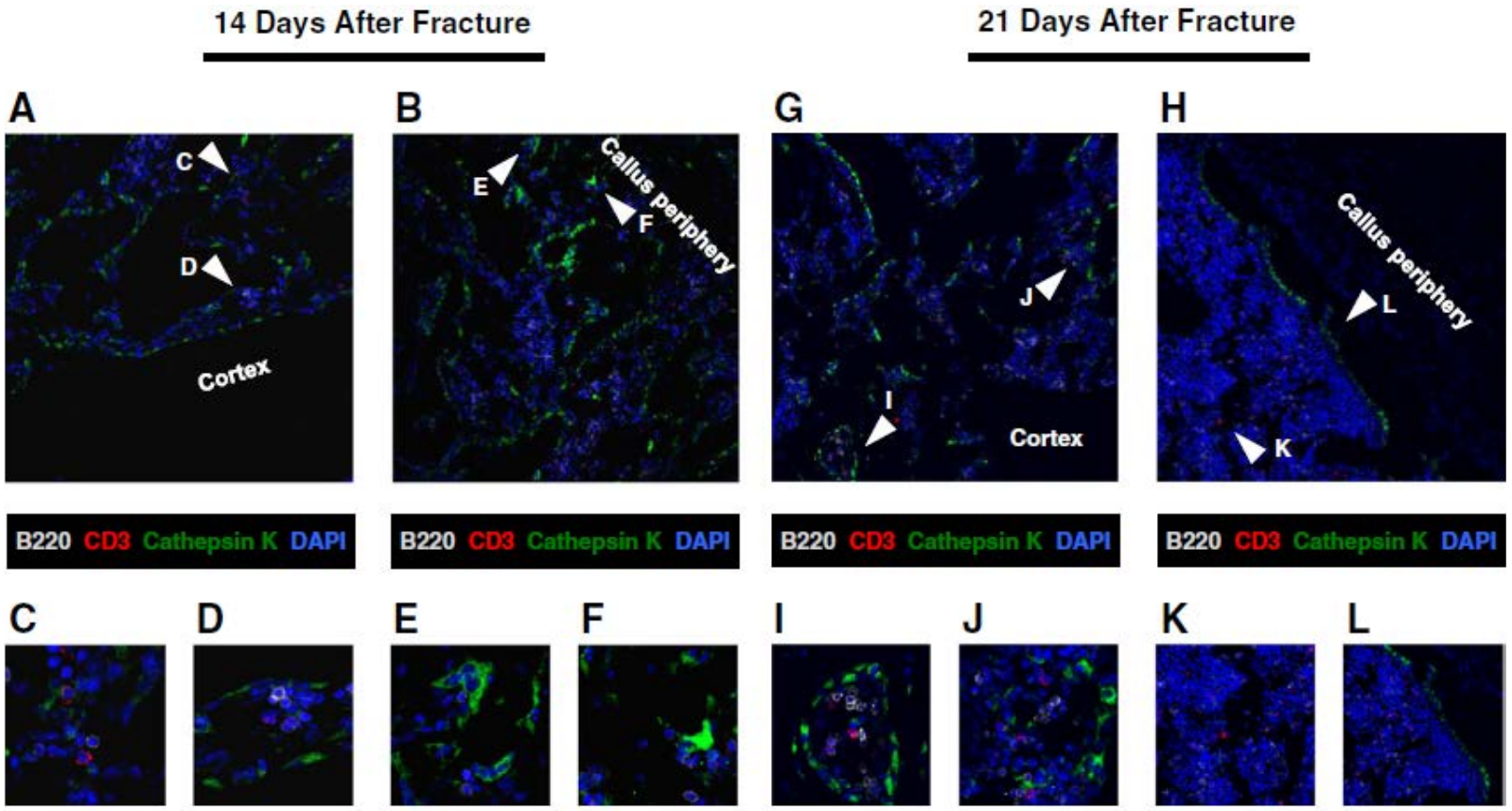
14 days:
majority: naive B220+ IgM+ IgD+
Fewer: B220+ IgM+ IgD-

- memory or immature (arrows)

21 days:
Naive B220+ IgM+ IgD+ AND
B220- IgG1 and 2+ (arrows)

- plasma cells
- Most potent OPG secretion

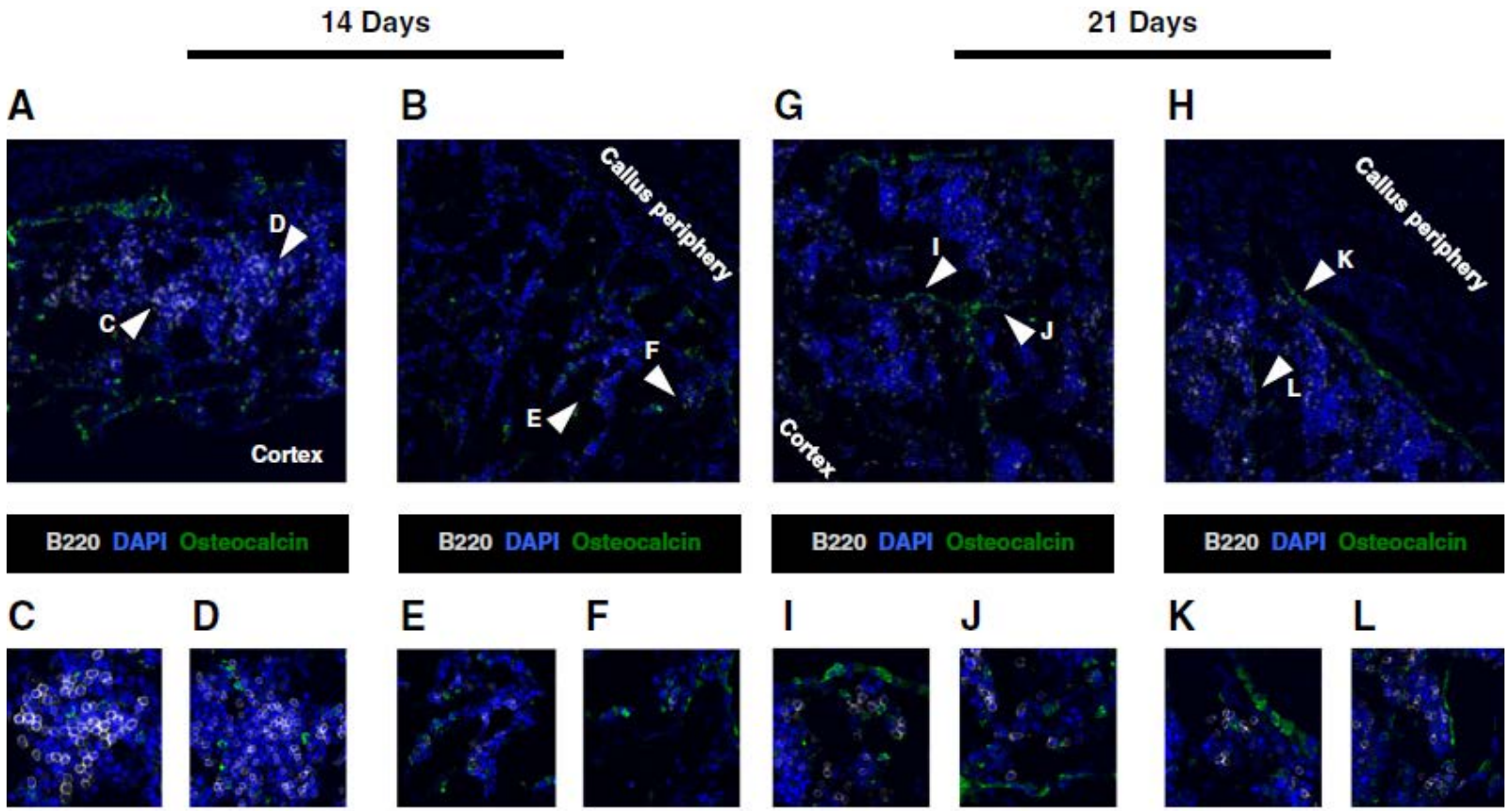
Fig. 4
B220+ gray
IgD green
IgM red
IgG1 and 2 purple



Cell-cell contact interaction of osteoclasts and lymphocytes

14 and 21 days:
Cathepsin K secreting osteoclasts (green)
Several Lymphocytes directly contacting osteoclasts

Fig. 5
CD3+ red
B220+ gray
Cathepsin K (Osteoclasts) green



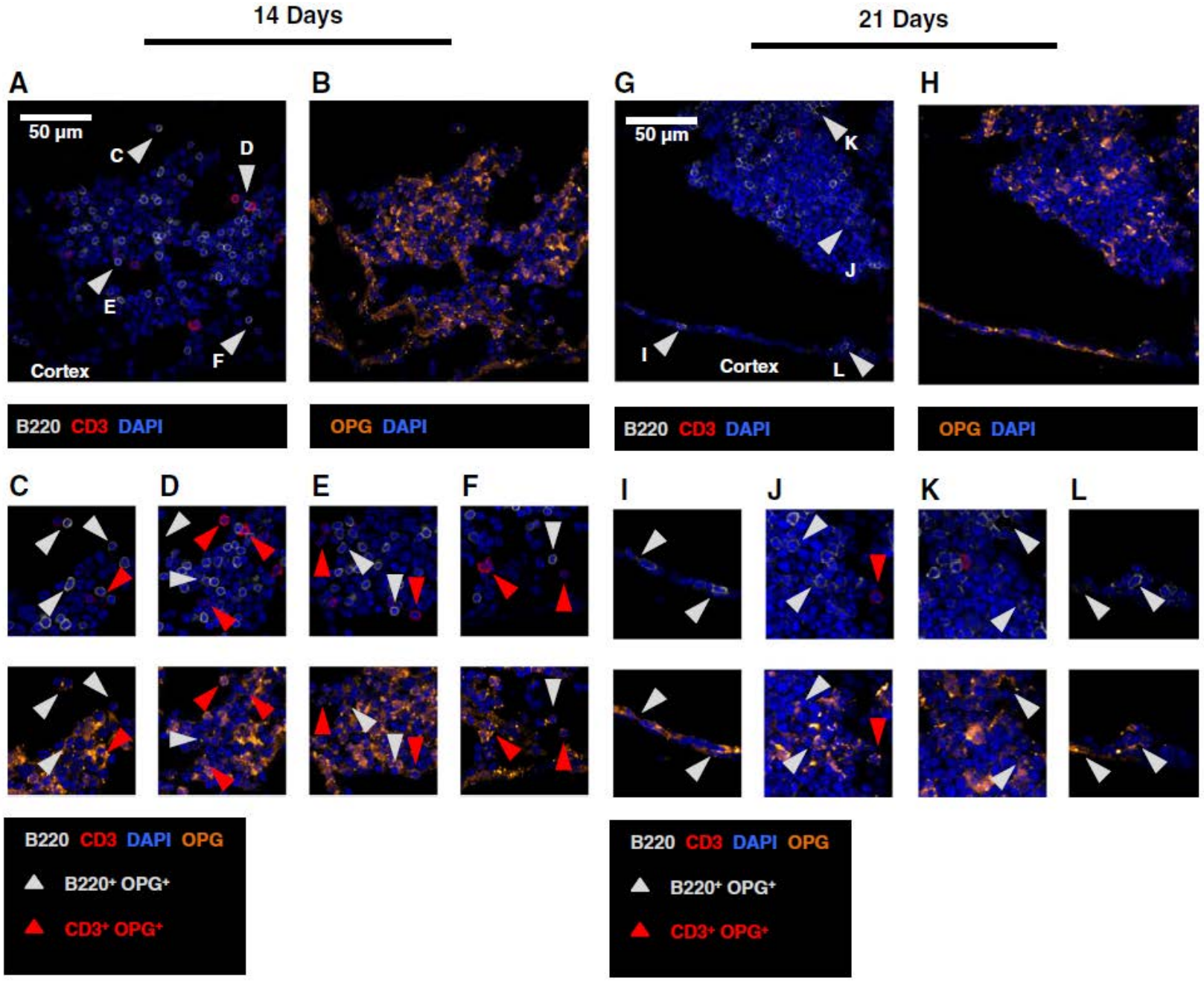
Cell-cell contact interaction of osteoblasts and lymphocytes

14 and 21 days:
Osteocalcin positive osteoblasts (green)

- Round shaped precurser cells
- Paslisade shape activated oseoblasts

B-cells have cell-cell contact with both types

Fig. 6
B220+ gray
Osteocalcin (Oseoblasts) green
Cell nucleii blue



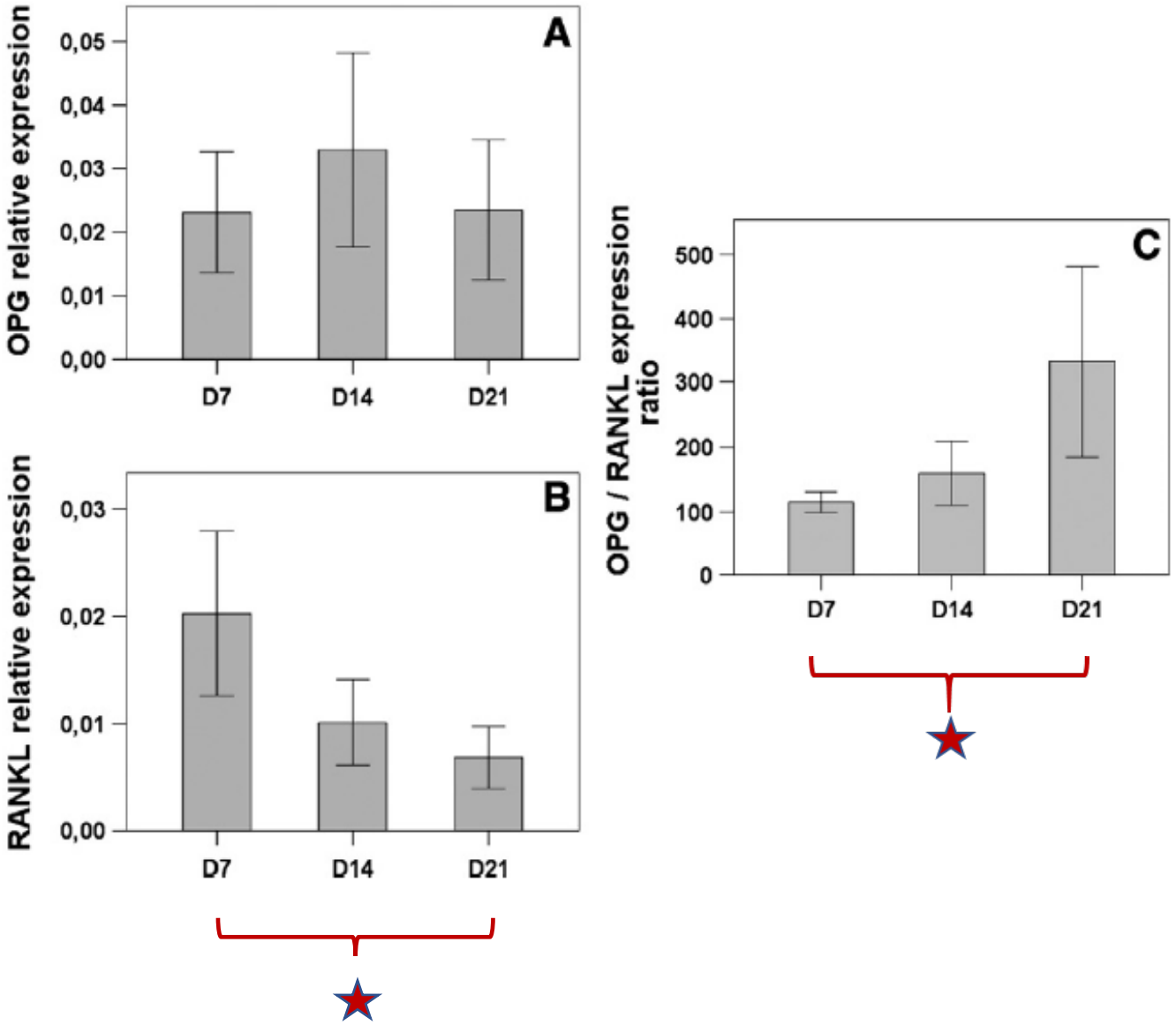
Cells producing OPG

14 and 21 days:
B-cells produced OPG

Also expressed by CD3+, B220- cells – probably osteoblasts

T-cells produced OPG

Fig. 7



Balance change of OPG and RANKL

A: RANKL

B: OPG

C: OPG/RANKL ratio

- Increasing shift towards OPG

Fig. 8

- Summary
  - Lymphocytes enter the fracture callus in two migratory waves
  - In first wave T- and B-cells are equaly distributed across the callus
  - Second wave of T- and B cells
    - accesses the callus probably via ist inner vessels
    - Higher amount of B- than T-cells
    - OPG secreting capacity progressively increases at further stages of fracture healing
    - B- and T-cells also stay in remodelling phase
    - Direct cell-cell contact of lymphocytes and osteoclasts and osteoblasts

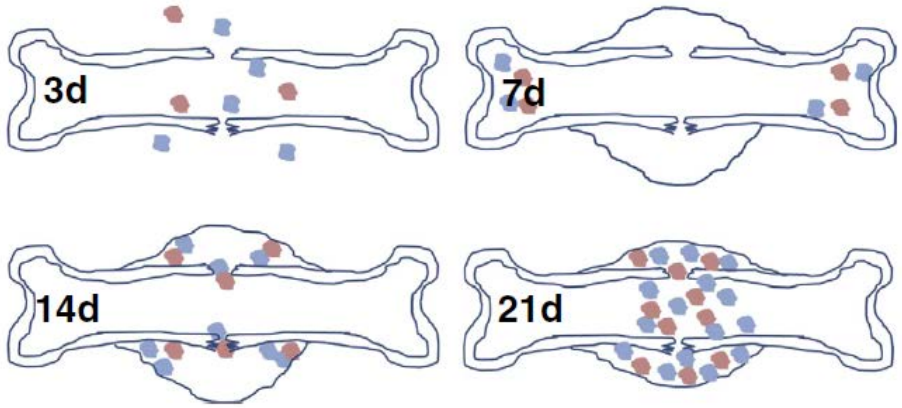


Fig.9
T-cells (red)
B-cells (blue)

Discussion

- Bone injury alters bone marrow composition not only in the fracture bone but systematically (Fig.1)
    - Physiologically higher percentage of CD8+ than CD4+ T-cells in bone marrow (Di Rosa and Pabst, 2005)
    - Reversed in injury situation

- T- and B-cells might directly affect the bone healing process
  - Through OPG and TNF-α
    - Mice with reduced or ablated TNF-α expression showed impaired healing (Aizawa et al., 2001)
    - OPG deficient mice show osteoporosis due to increased osteoclast activity (Kong et al., 1999)
    - Lymph node activation correlates with healing (Szczesny et al., 2007)
  - Until remodelling phase
  - Bone draining lymph nodes are affected by fracture (Szczesny et al.,2007)

- Further questions
  - Can also cell-cell contacts between lymphocytes, osteoblasts and osteoclasts lead to further differentiation of B- and T-cells?
  - Possibilities of using immune cells to further improve bone healing
    - Regulatory T-cells combined with bone marrow stroma cells enhance healing (Liu et al., 2011)
    - Cytokines as possible treatment (Mountziaris et al., 2011)

    > Newly discovered role of B-cells – is stimulation helpful for bone regeneration?

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