ETH zürich

D-BIOL



Tissue Repair Part II

Prof. Dr. Joel Zindel
Assistant Professor (TT) for Tissue Repair
April 14th 2026

Notes for this lecture



Central concepts — exam-relevant



How we know: We look at the experimental data behind central paradigms. These slides are epistemologic in nature—**how we know what we know**—and should not be memorized for the exam.



Nerd factor: Historical or medical perspective or fun facts (slides with black background are from our own laboratory). Not to be committed to memory—although sometimes the most random facts stick best.

Agenda – Tissue Repair

This agenda organises key concepts by when they are discussed — not as strict biological boundaries.

LECTURE 1

Injury

seconds – minutes

- **Definitions:** homeostasis, injury, regeneration, repair, and failure modes.
- The molecular basis of injury and injury detection: DAMPs, PAMPs, and LAMPs.

LECTURE 2

Inflammation

min – hours – days

- Injury detection
- macrophage cloaking and threshold gating
- Coagulation and Complement
- Thromboinflammation

LECTURE 3

Granulation tissue

hours – days – weeks

- Model A: Macrophage-fibroblast circuits
- Model B: Stem cell compartments
- Fundamental repair processes

LECTURE 4

Remodelling

days – weeks – months

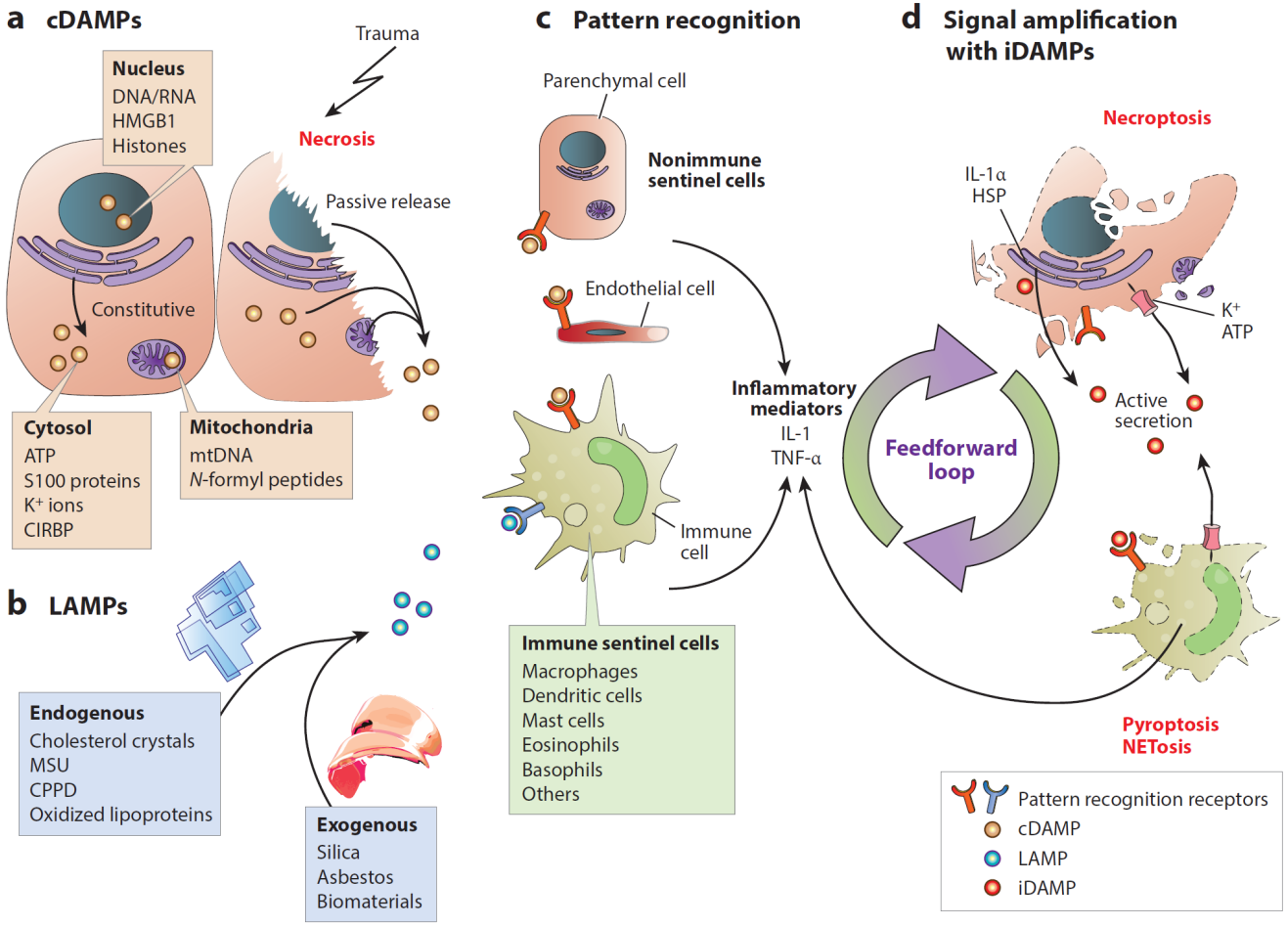
- MMP/TIMP balance, collagen I/III
- Myofibroblast resolution
- Fibrosis vs. regeneration

Phases are overlapping and tissue-dependent — complement, macrophages, and adaptive immunity span multiple lectures and are discussed in context.

Learning Objectives — Lecture 2: Inflammation

- Students can **explain** why **tissue-resident macrophages** are the **primary sensors** of sterile injury DAMPs — and why other tissue-resident immune cells (e.g. adaptive TRICs, NK/iNKT, ILCs, mast cells) are not, using the concepts of PRR repertoire breadth and network architecture.
- Students can **describe** the concept of **macrophage cloaking** as an operational definition of threshold gating — explain the experimental evidence from intravital microscopy, and articulate why the molecular basis of the threshold remains **incompletely defined**.
- Students can **explain** how the **coagulation** and **complement** cascades each **function** as **DAMP detection** systems — identifying the specific DAMP trigger for each pathway and explaining why this qualifies as DAMP logic.
- Students can **distinguish** between **immunothrombosis** and **thromboinflammation** — define each term, explain how coagulation, complement and cellular innate immunity converge into one integrated intravascular defense, and give examples of physiological output and failure modes.
- Students can explain why **resolution of inflammation is active and programmed** rather than passive decay.

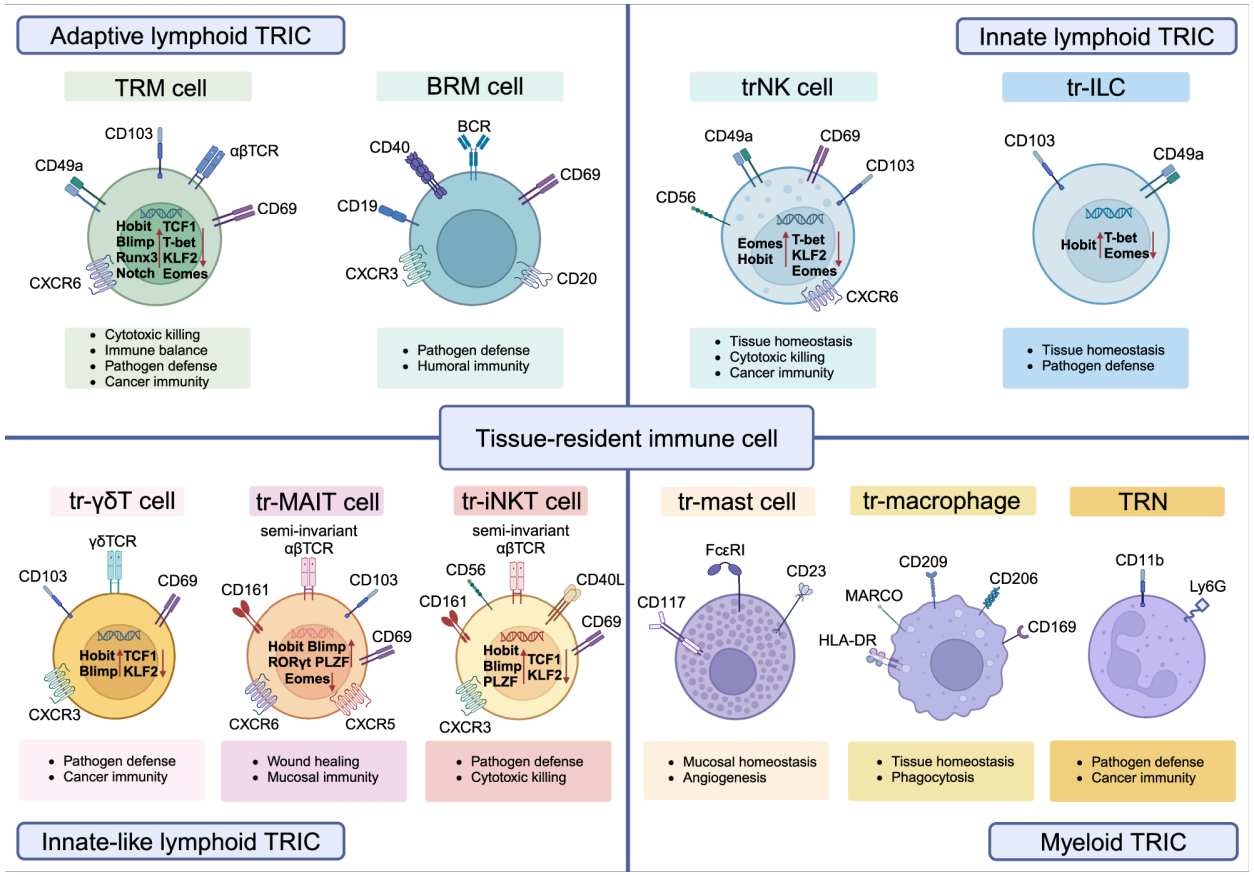
Recap: DAMPs



Adapted from Zindel & Kubes 2020 Annu Rev Pathol 2020

- **Sensing:** Immunostimulatory patterns are recognized by PRRs on both **nonimmune cells and specialized immune sentinels**. Ligation of PRRs leads to the secretion of proinflammatory cytokines (e.g., TNF-α and IL-1) as well as other inflammatory mediators.

Who detects DAMPs? A systematic look at tissue resident immune cells.



Li et al. Signal Transduction and Targeted Therapy

No DAMP sensors by design:

- T-cell (TRM), B-cell (BRM): adaptive immunity
- NK, iNKT, tr-γδT: killers, not sensors; limited PRR repertoire
- ILC: cytokine responders, not primary PRR-expressors

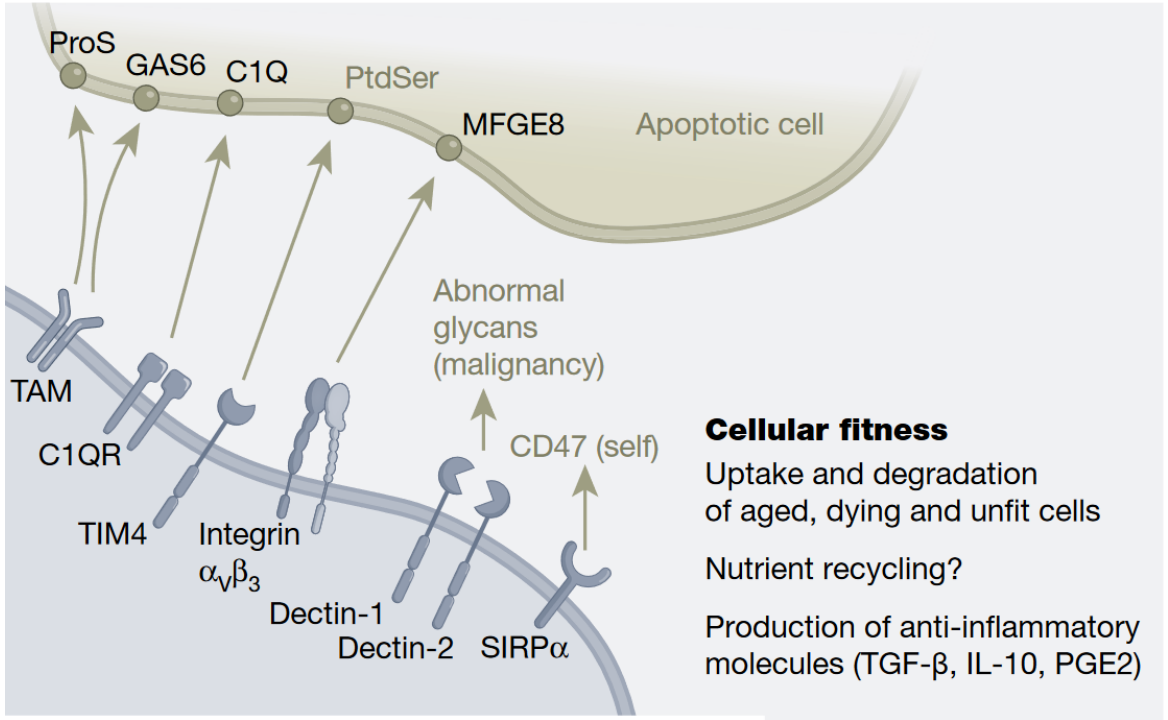
Partial DAMP sensors — context-dependent:

- MAIT: narrow DAMP PRR repertoire, can be activated TCR-independent, important for repair downstream of macrophages
- mast cell: narrow DAMP PRR repertoire, mostly excellent barrier alarm, FcεRI/complement-dominant;

Best sensors: <u>Tissue Resident Macrophages (TRM)</u>

- Gap-less, body-wide network
- Broadest PRR repertoire (TLRs, NLRP3, cGAS-STING, scavenger receptors)
- First responder in all sterile injury IVM models

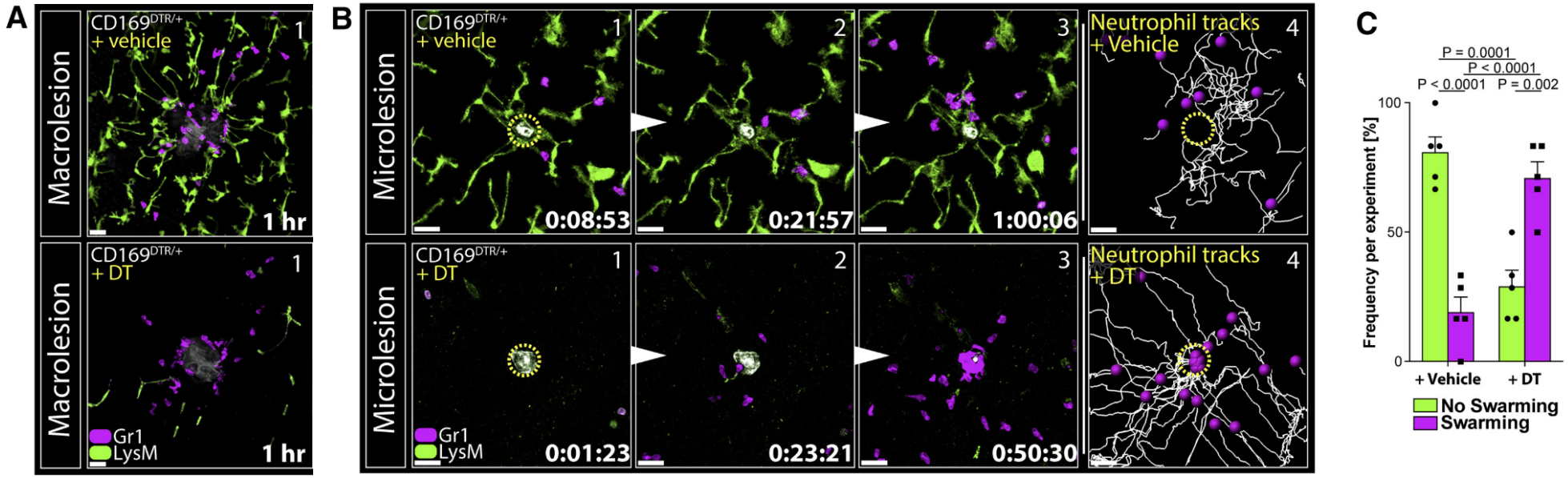
Recap: Efferocytosis



- Daily, billions of cells die in the tissue by undergoing apoptosis, yet apoptotic cells are rarely observed in homeostasis.
- Efferocytosis that efficiently clears apoptotic cells without causing inflammation.
- Efferocytosis is a well-defined molecular event:
  - Healthy cells express **CD47**, a «**don't eat me signal**» recognized by **SIRP1α** on phagocytes
  - Apoptotic cells express «**eat me signals**» the most important is **Phosphatidylserine** (PS) which is recognized by **TIM4** and many others (Figure)
- The process is anti-inflammatory characterized by the release of TGFb, IL-10, PGE2 and the inhibition of inflammatory cytokines such as TNF, IL1b, IL6

Injury and **Efferocytosis** both involve cell death and macrophages — yet one is silent and the other triggers full inflammation. What sets the threshold?

The threshold question

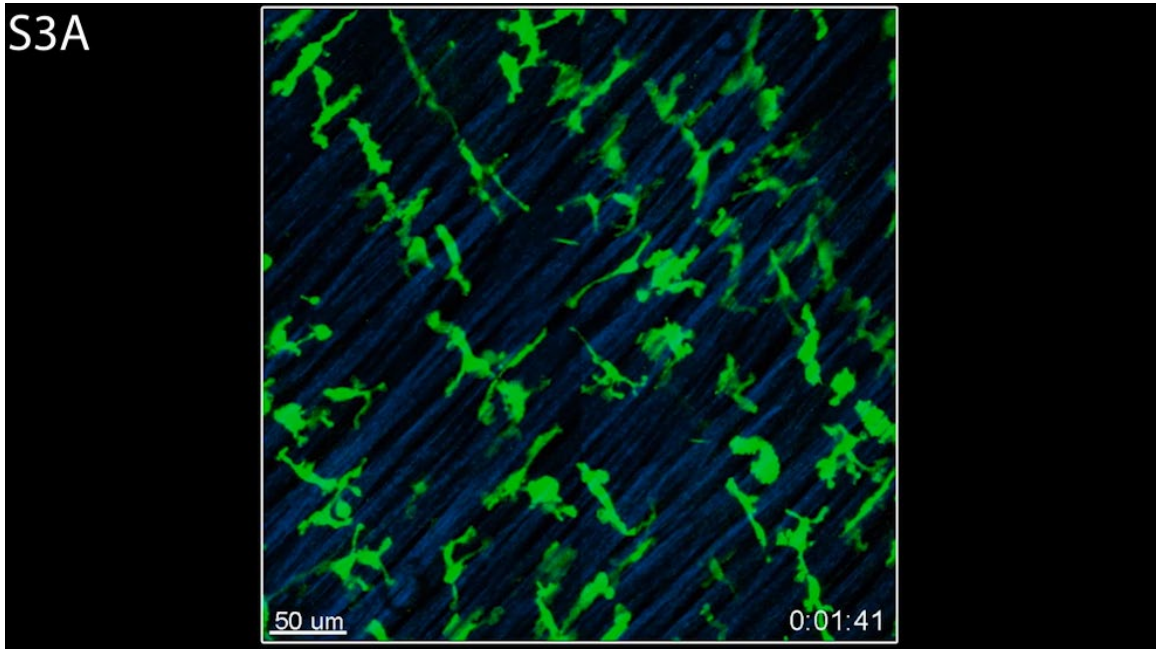


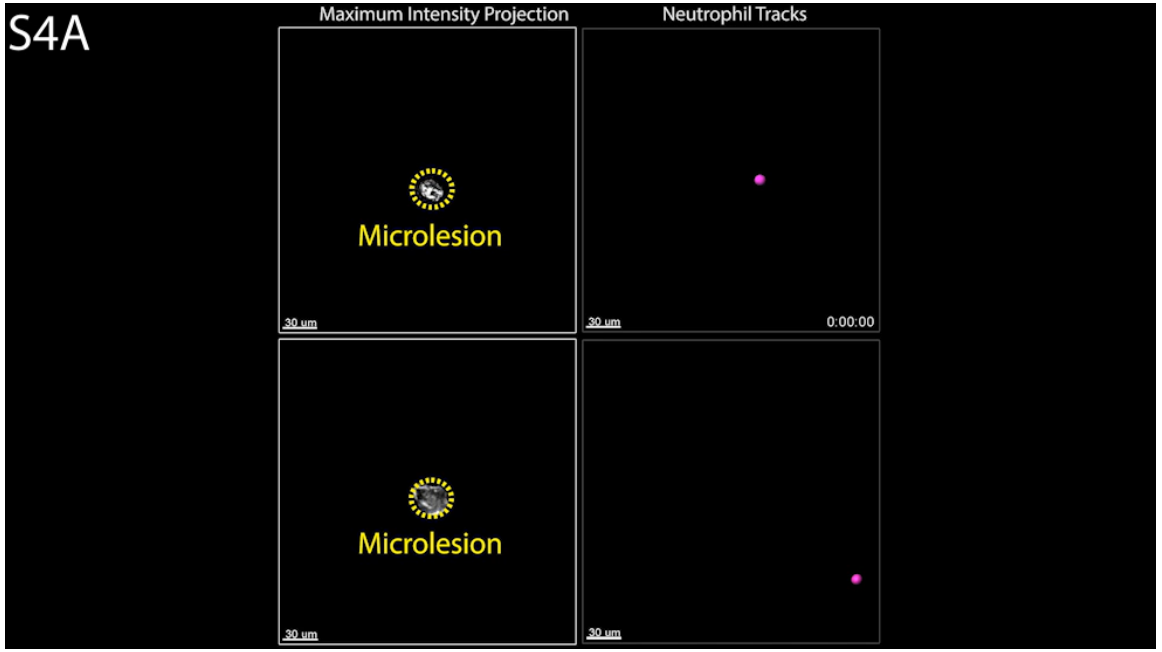
Uderhardt et al. Cell 2019 (Figure 3)

- **Neutrophil swarming** as **operational readout of threshold breach** — all-or-nothing
- (**A**) Tissue Resident Macrophages (TRMs) cloak microlesions within minutes via pseudopod extension — without moving their cell body. +DT = diphtheriae toxin depletion of macrophages.
- Cloaking is sequentially DAMP-driven: ATP (sensing) → RAGE ligands (convergence)
- Threshold is lesion-size and TRM-density dependent — not a fixed molecular switch
- (**B**) **Failure of cloaking** → collateral neutrophil damage → secondary monocyte containment
- **molecular basis of the threshold remains incompletely defined — an open and fundamental question**

The threshold question

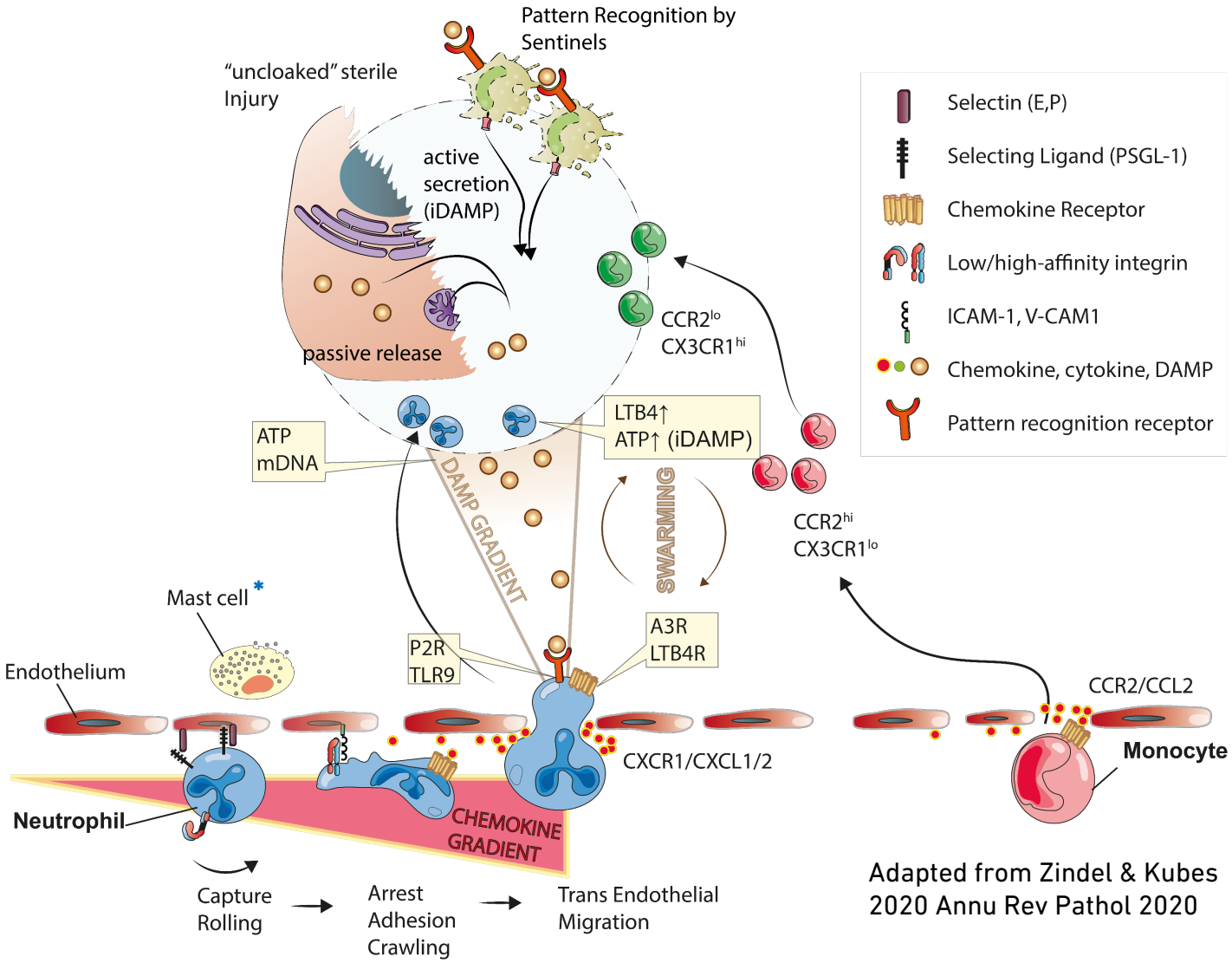
(Movies, content in previous slide explained or check out here!





Uderhardt et al. Cell 2019

Transendothelial **leukocyte recruitment** to sterile injuries



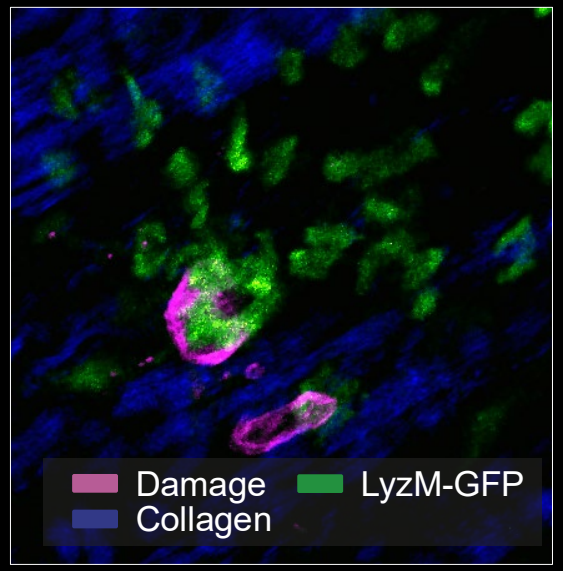
Adapted from Zindel & Kubes 2020 Annu Rev Pathol 2020

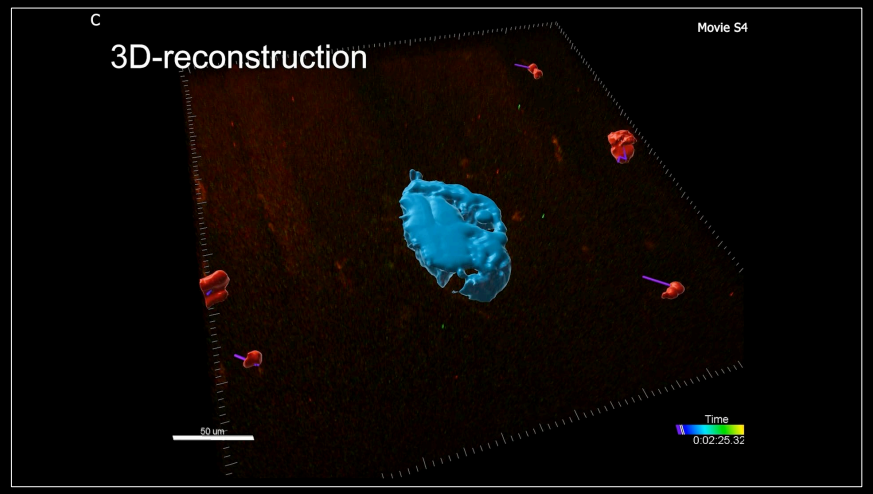
- **Cloaking failure → threshold breached**
- Sentinel-derived TNF/IL-1 endothelial activation: E/P-selectin upregulation, ICAM-1/VCAM-1 expression → chemokine presentation (CXCL1/2)
- **Neutrophils** first: transendothelial migration (same cascade as classical immunology)
- In the tissue: neutrophils follow the **DAMP gradients** (P2R, TLR9, A3R, LTB4R) → reach injury site
- First neutrophils undergo inflammatory cell death (e.g. NETosis) → iDAMP release (ATP, granule proteins) → feedforward amplification of swarming
- **Monocytes** second wave: $CCR2^{hi}/CX3CR1^{lo}$ recruited via CCL2 → will convert to macrophages (discussed next lecture)
- This figure is simplified — additional cells (mast cell degranulation, NK, ILC) contribute but are not shown.

***Nerd factor—Perivascular mast cells:** degranulation/TNF to activate endothelium and provide perivascular DAMP relay: bridging of tissue alarm to vascular activation.

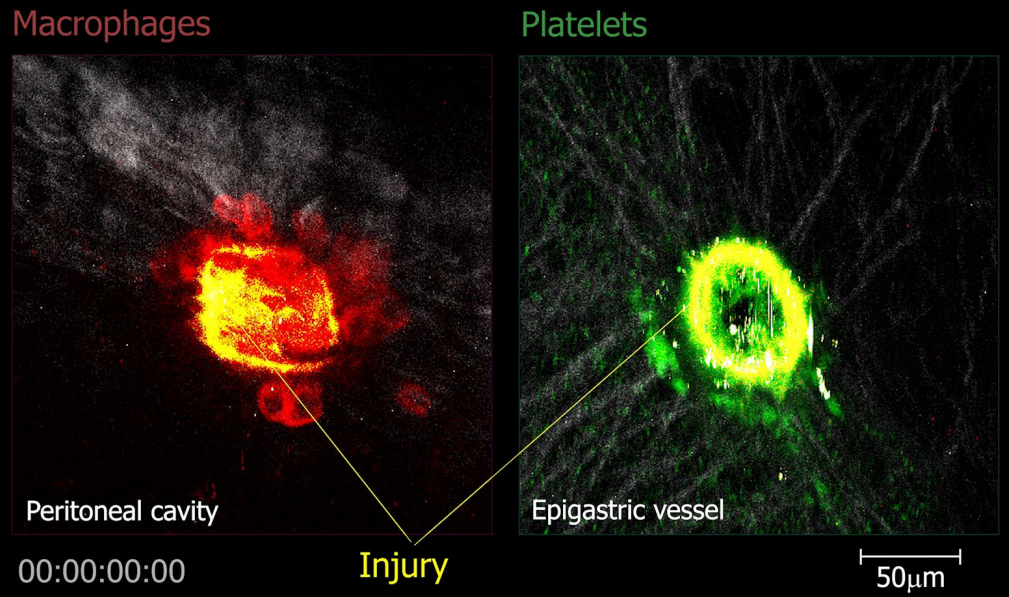
Unconventional immune cell recruitment

Canonical Immune cell migration

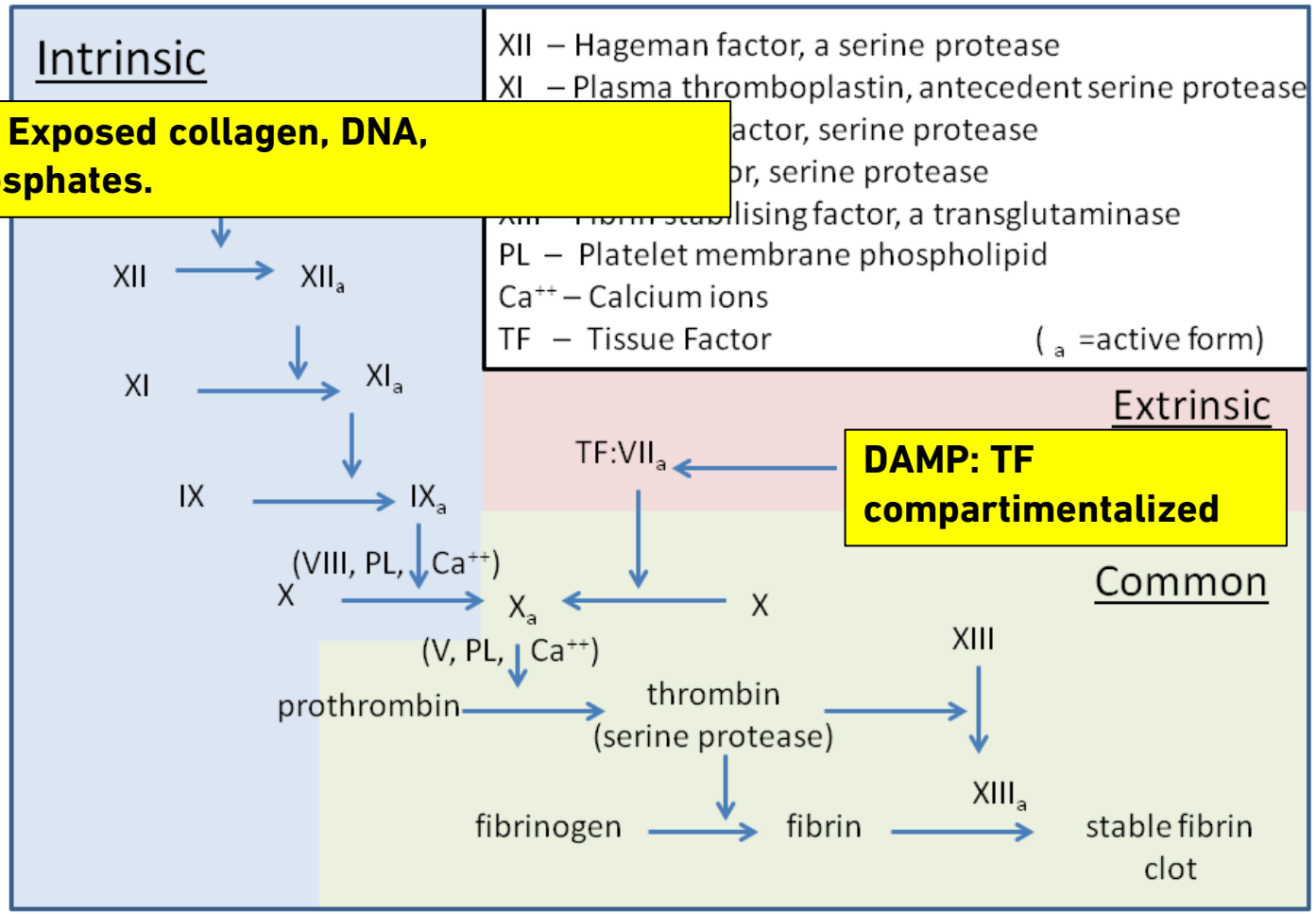




Peritoneal Macrophages migrate like platelets

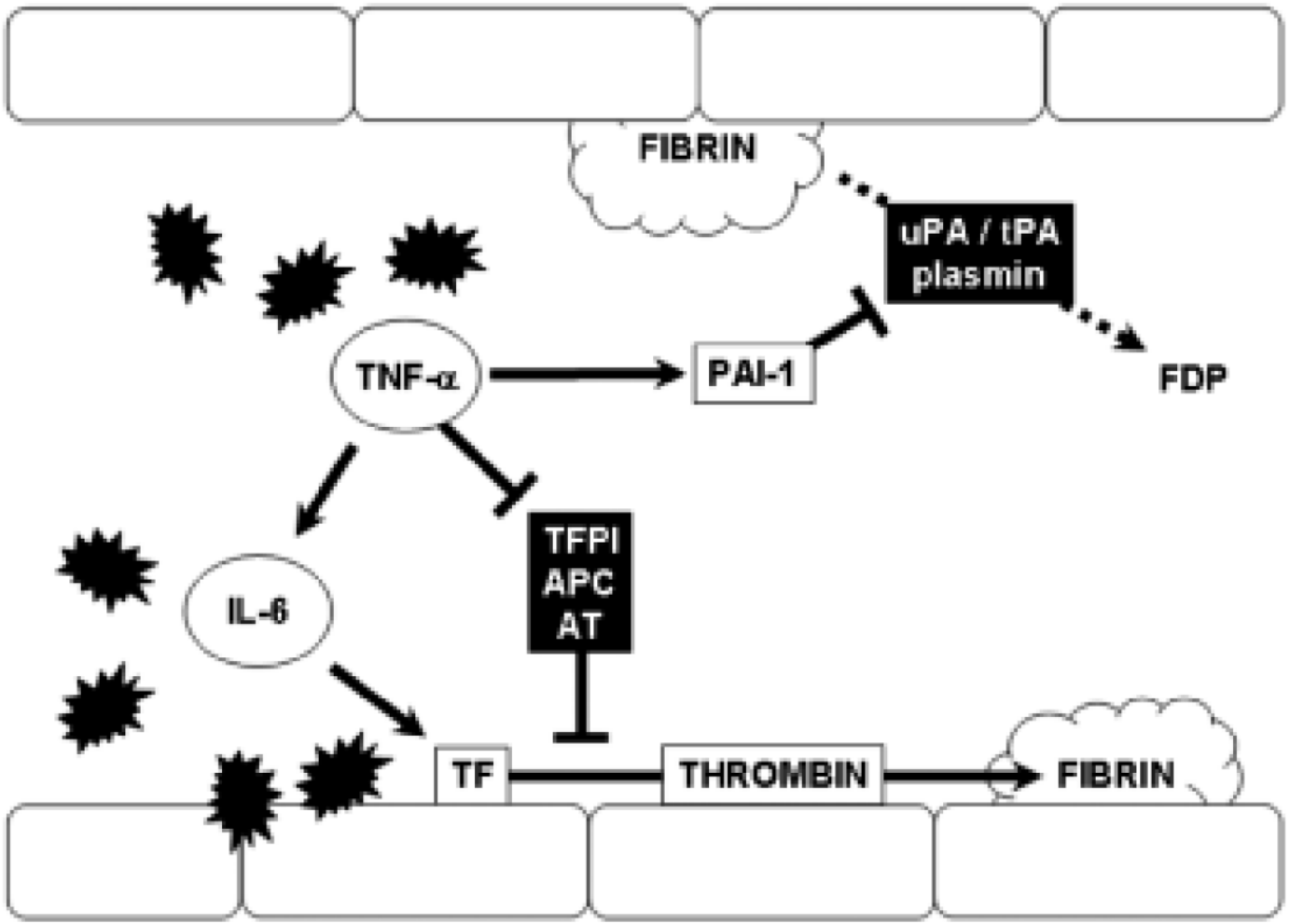


The **coagulation pathway** is a **DAMP detection** system



- Coagulation is **constitutively pre-positioned** — activates within seconds, parallel to cellular response
- **Extrinsic** pathway trigger = **DAMP** by compartmentalization — TF normally hidden behind intact endothelium on subendothelial fibroblasts/pericytes;
- **Intrinsic/contact pathway** trigger = cDAMPs directly — FXII activated by exposed collagen, extracellular DNA, polyphosphates from necrotic cells
- **Downstream link to inflammation**

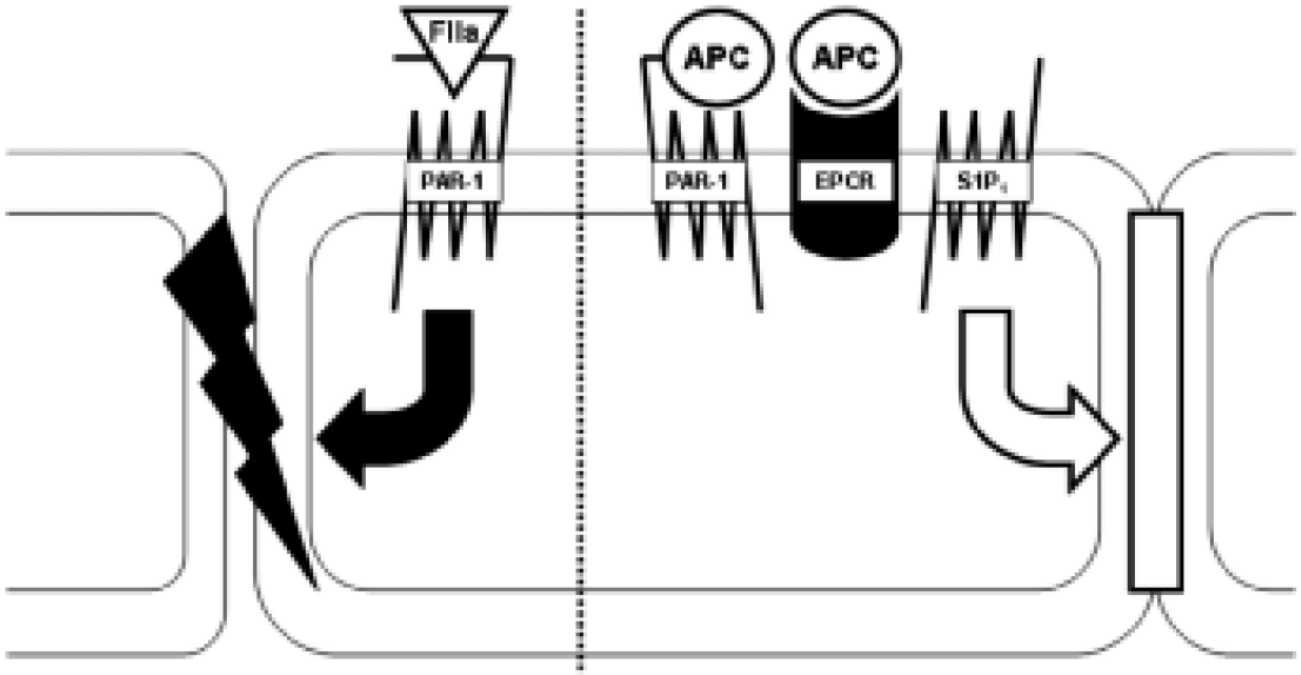
Inflammation → Coagulation



Choi et al, SWISS MED WKLY 2006;136:139–144

- **Proinflammatory cytokines** (IL-6, TNF-α) → TF upregulation on monocytes and microparticles from activated platelets/endothelium → TF:FVIIa → thrombin → fibrin (shown in mice, chimpanzees, and humans)
- Simultaneously, natural anticoagulants are downregulated: TNF-α reduces TFPI, APC, **antithrombin** → coagulation is no longer counterbalanced
- **Fibrinolysis** is **suppressed**: TNF-α/IL-1β → ↑PAI-1, ↓tPA production by endothelial cells → fibrin persists
- **Net result**: massive procoagulant shift — more fibrin generated, less inhibited, less cleared

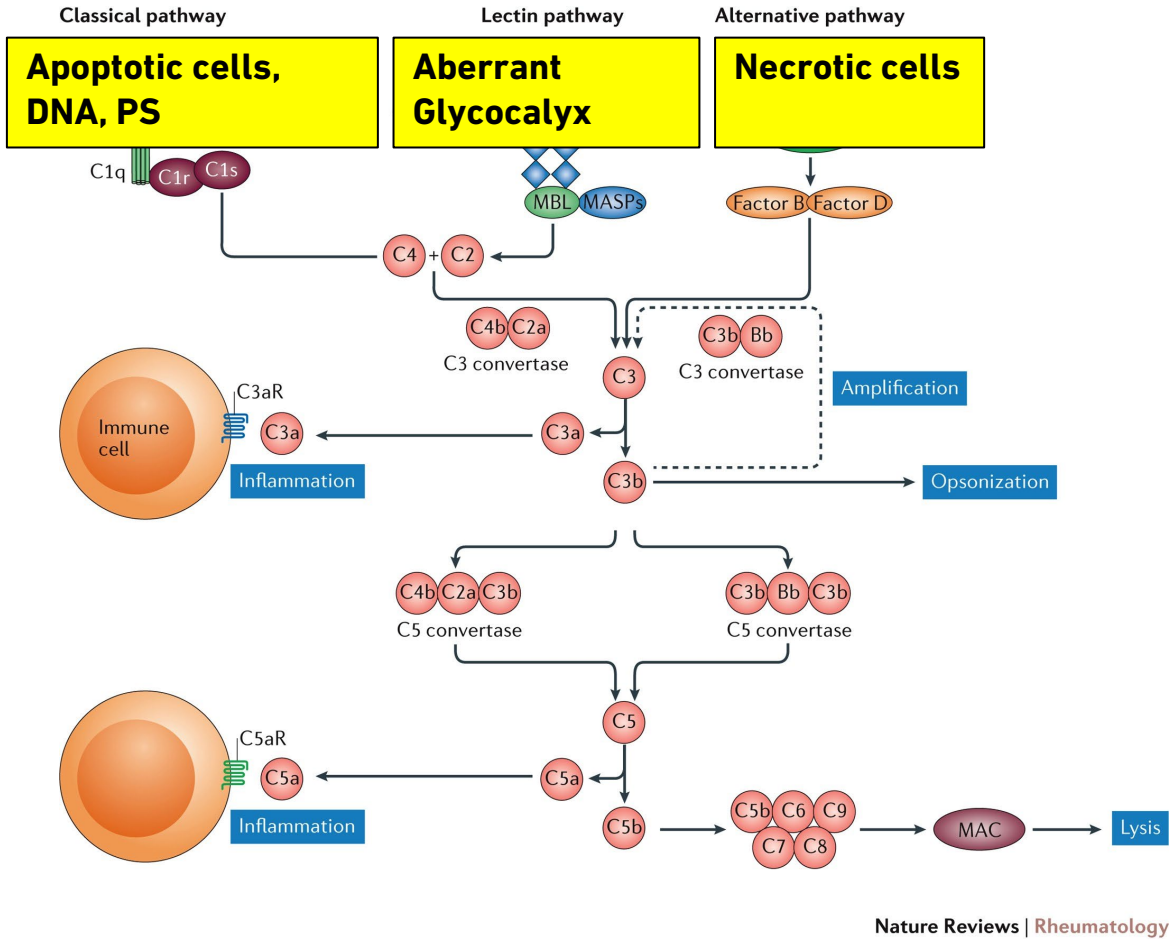
Coagulation → Inflammation



Choi et al, SWISS MED WKLY 2006;136:139–144

- Coagulation proteases activate **protease-activated receptors** (PAR-1 to -4) on mononuclear cells, endothelium, and others — translating coagulation signals into inflammatory output
- Thrombin → PAR-1 (best described): ↑IL-6, IL-8, MCP-1; NF-κB-dependent upregulation of adhesion molecules → leukocyte adhesion
- FXa/TF-FVIIa complex → PAR-2: pro-inflammatory effects in macrophages, neutrophil infiltration, ↑TNF-α and IL-1β
- Fibrin(ogen) and fibrin degradation products activate macrophages via TLR4. Molecular mechanism not fully resolved (Smiley et al. J Immunol 2001)
- Creates a self-amplifying feedforward loop: inflammation drives coagulation drives inflammation

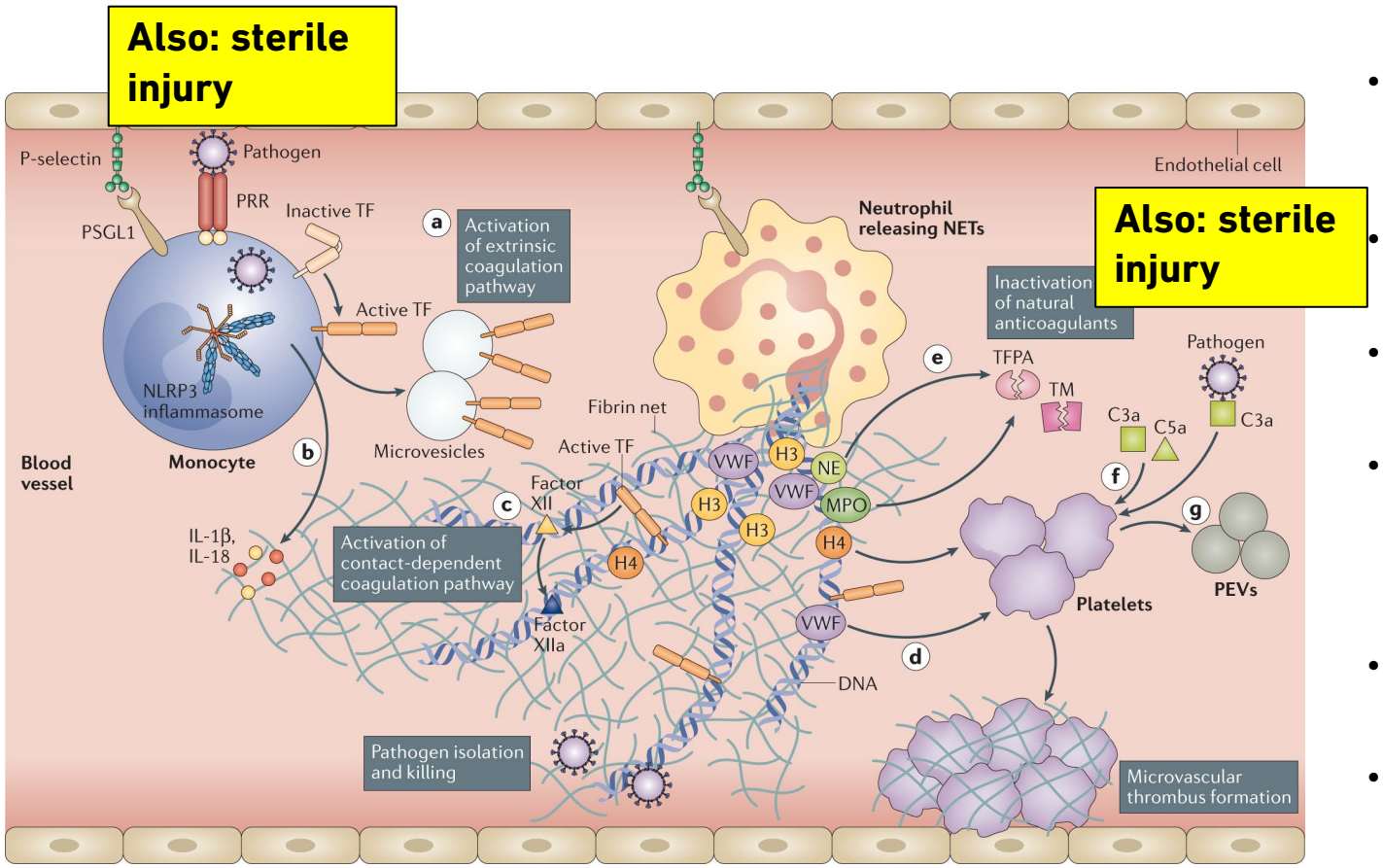
The **complement system:** a **DAMP detection and amplification** system



Trouw et al. Nature reviews rheumatology, 2017

- Complement is **constitutively expressed** in plasma, activates within seconds, parallel to coagulation and cellular response.
- Three pathways, all detect DAMPs — via different logic:
  - **Classical pathway**: C1q is a soluble PRR — directly binds apoptotic cells, exposed DNA and phosphatidylserine (PS) on damaged cells, independently of antibody (Nauta et al. Eur J Immunol 2002; Navratil et al. J Immunol 2001)
  - **Lectin pathway**: MBL and ficolins recognize aberrant glycocalyx patterns on necrotic and apoptotic cells in addition to microbial surfaces (Nauta et al. Eur J Immunol 2003)
  - **Alternative pathway**: healthy cells constitutively express CD55, CD59, CD46 → suppress C3 tick-over. Necrotic cells lose these regulators → complement proceeds unopposed → missing inhibitory signal = DAMP logic (Elward & Gasque J Biol Chem 2005)
- All three pathways converge at C3 → **link to inflammation**

Thromboinflammation*: convergence of three parallel systems



Bonaventura et al., Nat Rev Immunol 2021

***Terminology note:** Immunothrombosis (Engelmann & Massberg 2013) = the **physiological** process — thrombosis as an active tool of innate immunity. Thromboinflammation = the **pathological** deviation when immunothrombosis becomes uncontrolled. This slide shows both: the physiological machinery (figure) and its failure mode (red bullet).

- **Thromboinflammation*** = convergence of coagulation, complement and cellular immunity into one integrated process.
- **Trigger-agnostic machinery** — whether initiated by PAMPs or DAMPs, the molecular pathways are identical
- Monocytes → PRR activation → TF decryption → extrinsic pathway (path a) + NLRP3 → IL-1β/IL-18 (path b)
- Neutrophils → NETs → FXII activation (path c) → contact pathway; NETs bind VWF → platelet recruitment (path d); NE/MPO cleave TFPI/thrombomodulin → anticoagulant brake removed (path e)
- Platelets (Thrombocytes) → activated by C3a/C5a (path f) → release PEVs, polyphosphates → feedforward (path g)
- Physiological output fibrin-immune cell scaffold
  - pathogen/debris isolation and killing
  - **Required for repair**!
- **Failure modes:**
  - *Disseminated intravascular coagulation, e.g. COVID-19 coagulopathy*
  - *Aberrant repair*

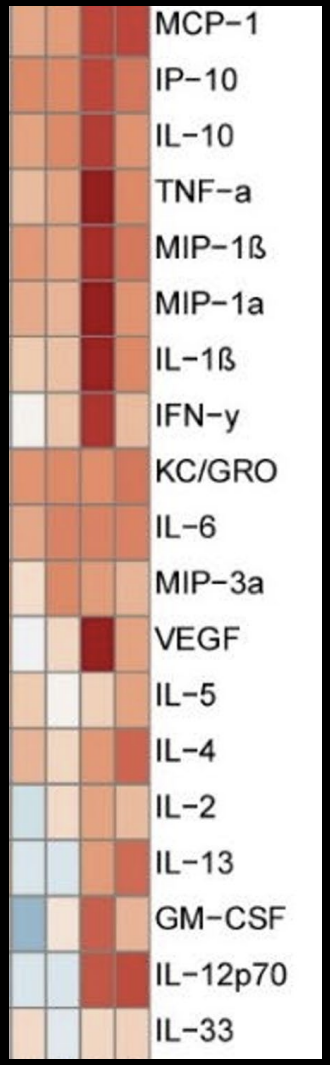
Thromboinflammation in the peritoneal cavity

Neutrophils (turbidity)

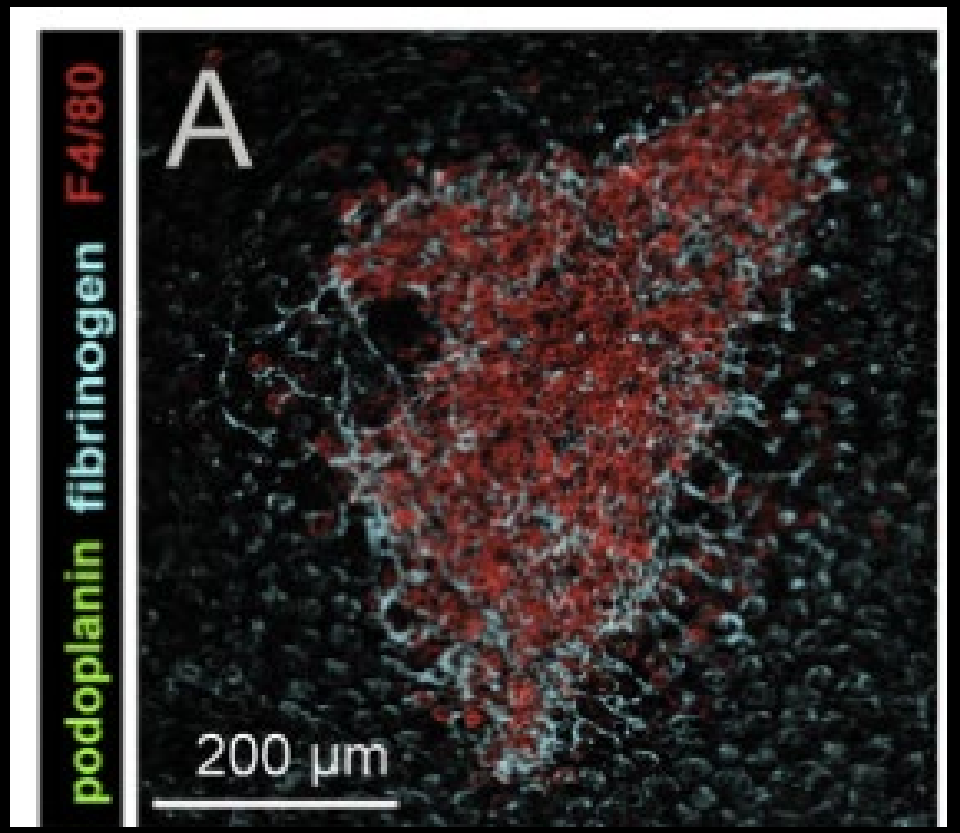


www.springermedizin.de

Cytokines

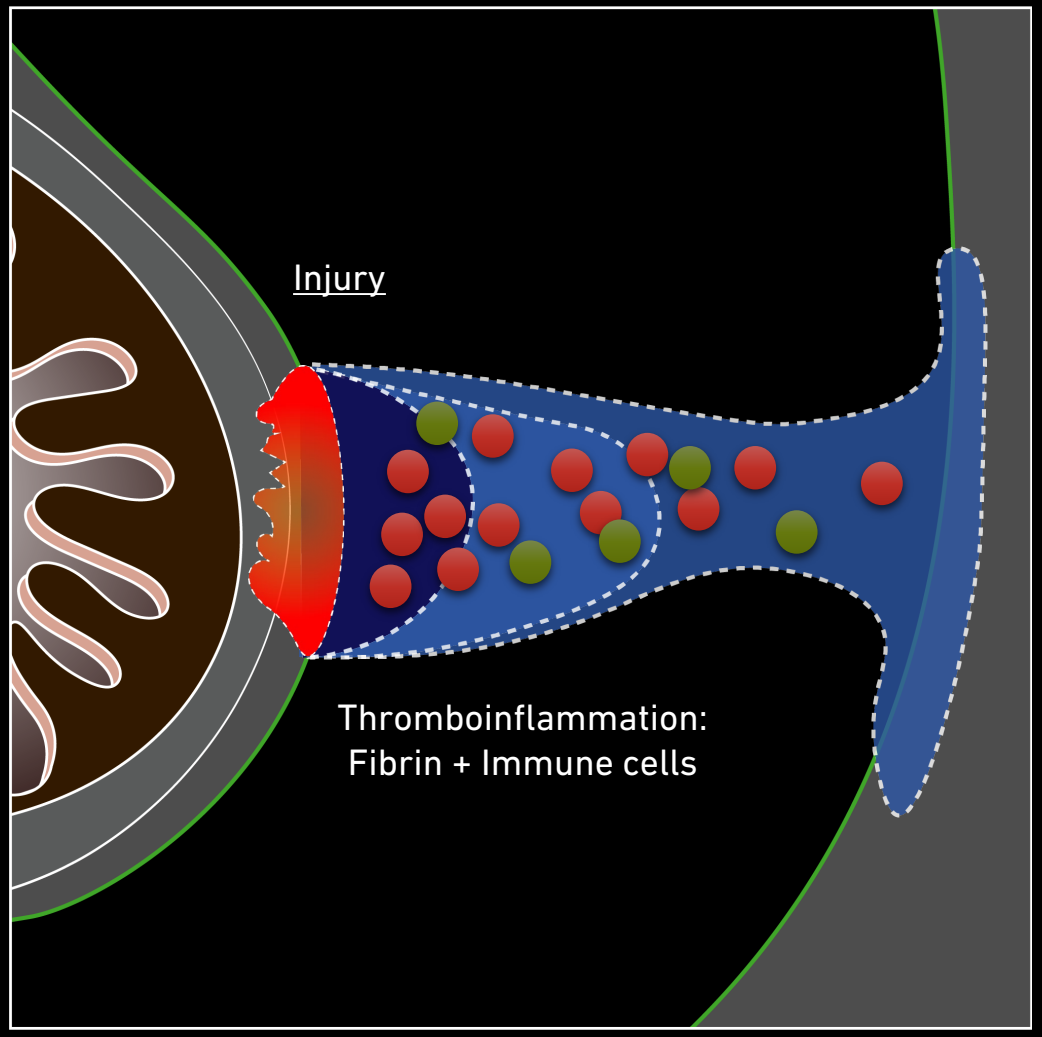


Macrophages, Fibrin, and later more immune cells

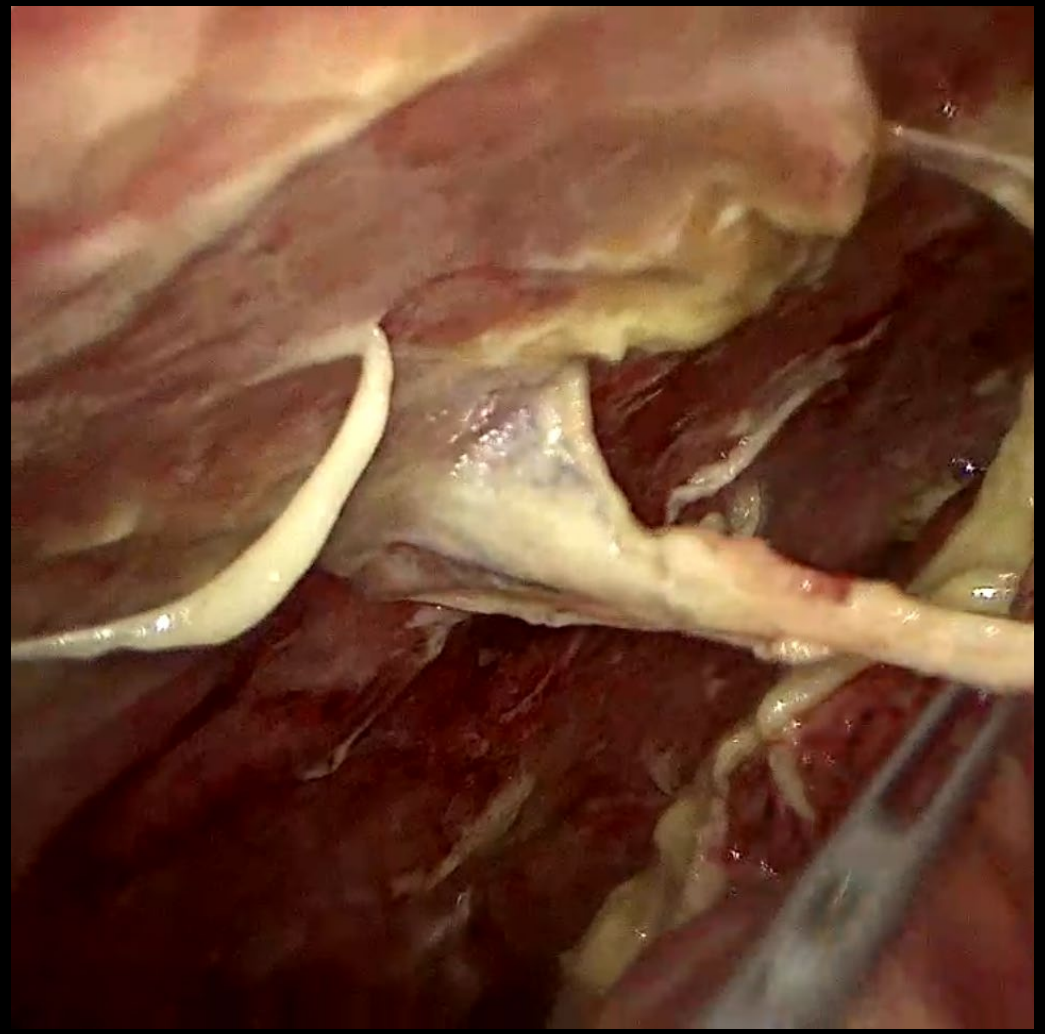


Vega Perez et al Immunity 2021

Thromboinflammation in the peritoneal cavity

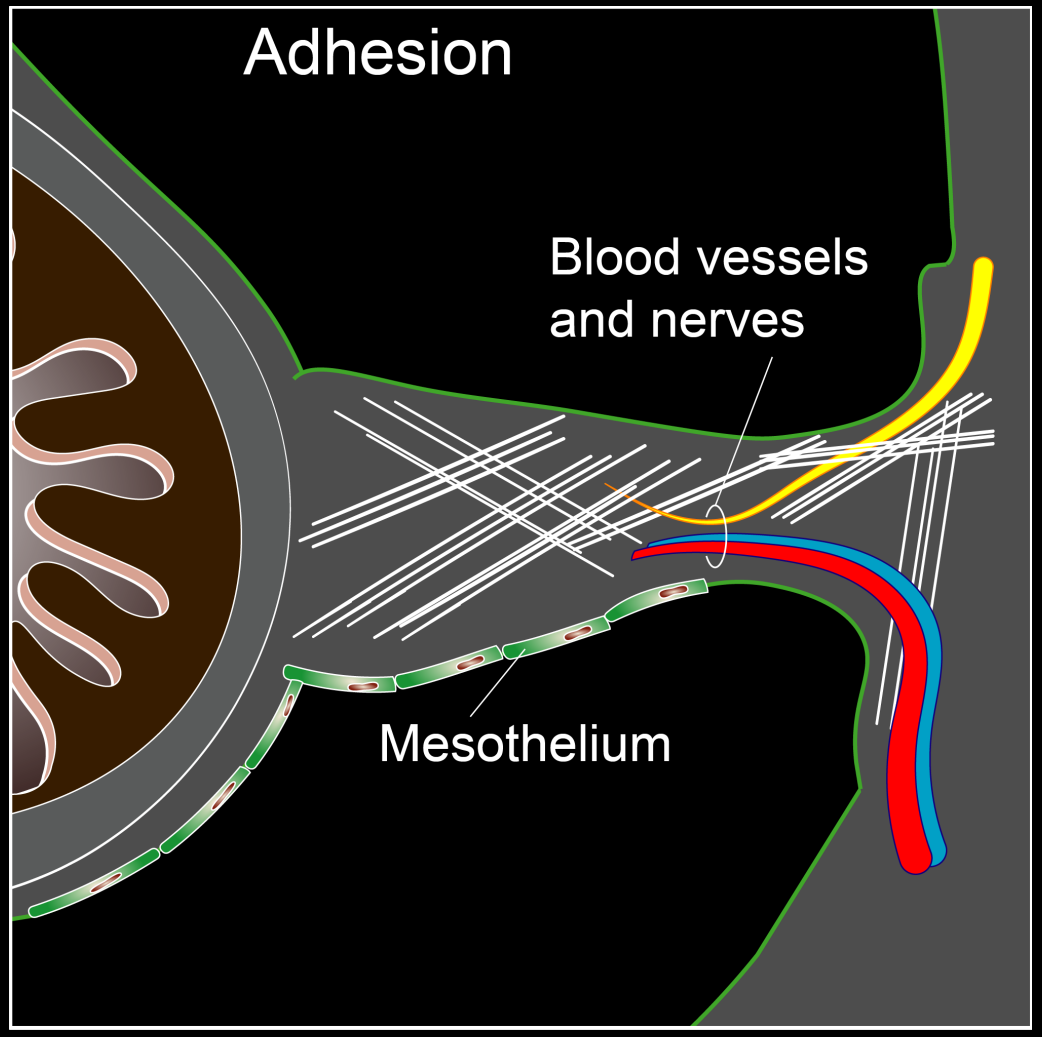


Fibrin



Hellebrekers 2011

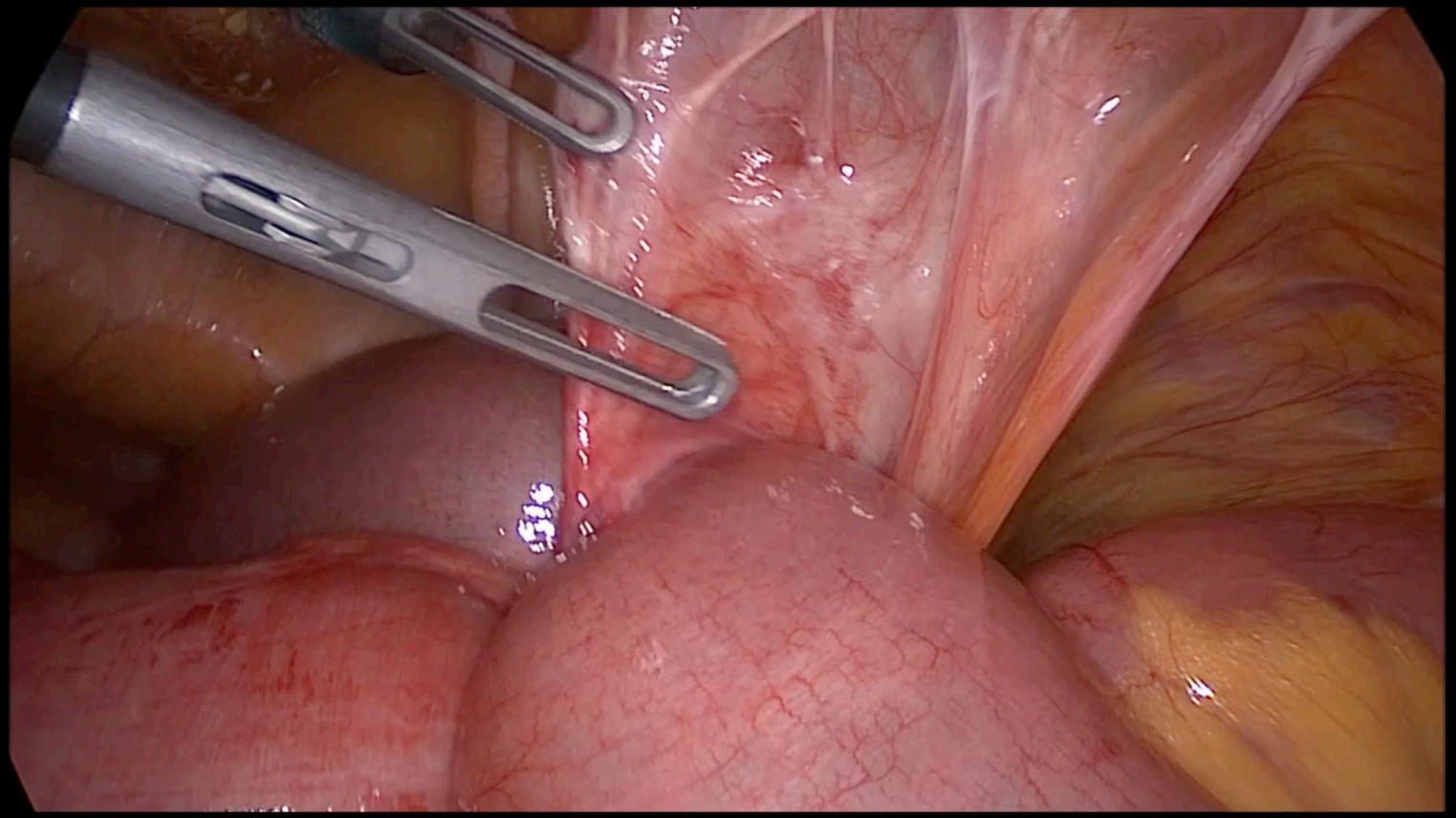
Fibrin Deposition and Scar Formation



Hellebrekers 2011

<u>Definition</u> Adhesion/ Maladaptive fibrotic scarring: Irreversible fibrotic scar tissue that connects two surfaces of the body that are usually separate.

Adhesions are a massive clinical problem



#1 Cause of bowel obstruction

#1 Cause of infertility

3.8% Re-Operations

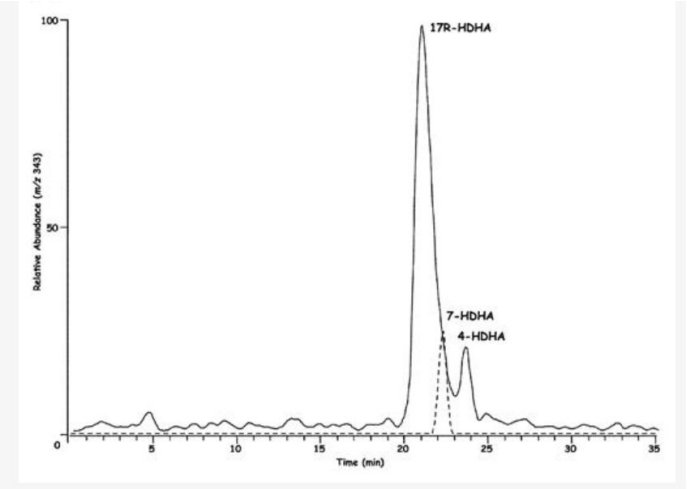
10% Re-Admissions

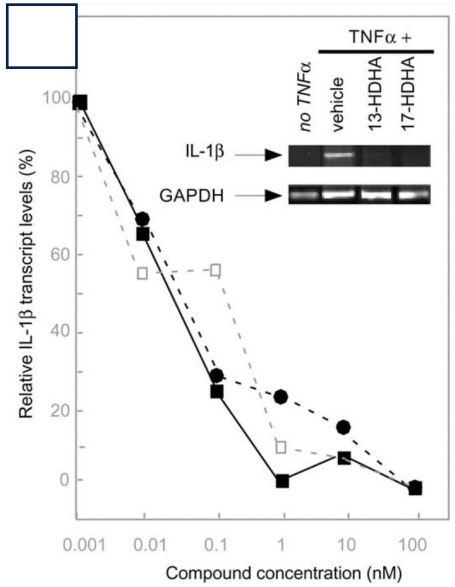
CHF More expensive than gastric or rectal cancer

No cure

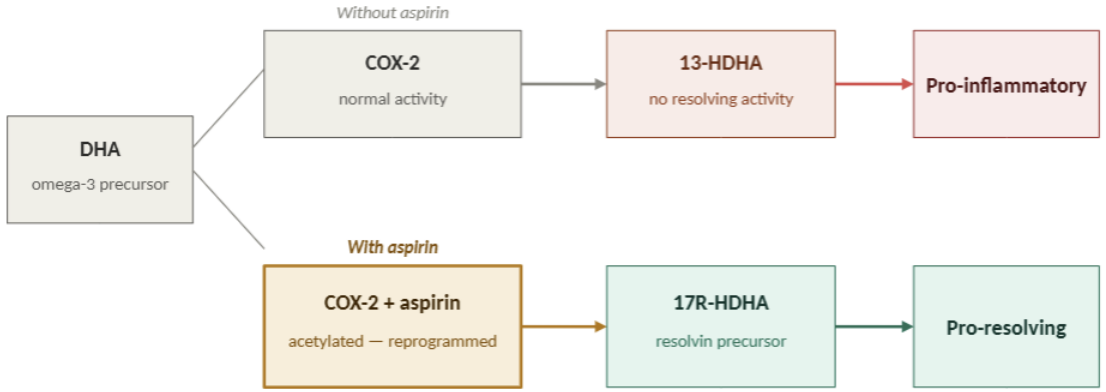
Zwicky 2021, Menzies 1990, Beck 2000, Barmparas 2010, Ellis 1999, Sulaiman 2001

Lipid mediator class switch: Discovery of resolvins





Serhan CN et al. JEM 2002



- What does the body actually produce during resolution? — used unbiased lipidomics of resolving exudates
- (**left panel**) During the resolution phase, aspirin-treated mice convert DHA (omega-3) → novel compounds: **17R-HDHA** and its derivatives (dominant peak in chromatogram)
- These compounds were **named resolvins** — resolution phase interaction products
- (**right panel**) Resolvins suppress pro-inflammatory cytokine production (IL-1β) at picomolar concentrations — IC50 ~50 pM.
- **Key conceptual insight**: aspirin reprograms COX-2 — same enzyme, same substrate (DHA), but acetylation switches the product from pro-inflammatory 13-HDHA → pro-resolving 17R-HDHA
- **Resolution** is therefore not passive decay — it is actively switched on by a new biosynthetic program

Resolution of Inflammation is active and programmed



Onset	Resolution	Failure modes
Minutes → hours	*Hours → days*	*If unresolved*
Pro-inflammatory signals Cytokines, chemokines	**Lipid mediator class switch** PGE_2/LTB_4 → lipoxins, resolvins, protectins (SPMs)	**Chronic inflammation** Resolution failure
Cell adhesion molecules E/P-selectin, ICAM-1	**NF-κB / TLR signalling off** Pro-inflammatory mRNA destabilized (TTP, ZC3H12A)	**Fibrosis** Excess fibrin/collagen deposition
Neutrophil recruitment Swarming, NETosis	**Neutrophil clearance** Apoptosis + efferocytosis by macrophages	**Adhesions** Maladaptive fibrotic scar — next slides
Anti-inflammatory brakes IL-10, TGF-β, cortisol	**Reverse migration** Neutrophils re-enter circulation → lung (transient) → bone marrow → apoptosis	**Thromboinflammation** DIC, MI, ARDS, COVID-19
Immunothrombosis Fibrin-immune cell scaffold — required for repair	**Macrophage reprogramming** Inflammatory → repair/efferocytic phenotype (→ Lecture 3)	
	Fibrinolysis moC/LPM-mediated — provisional scaffold removed	

Adapted from Fullerton & Gilroy, Nat Rev Drug Discov 2016

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ETH zürich

Prof. Dr. Joel Zindel
Assistenzprofessor
Joel.zindel@biol.ethz.ch

ETH Zürich
Institut für Molekulare Gesundheitswissenschaften (IMHS), D-BIOL
HPL F32.2
Otto-Stern-Weg 7
8098 Zürich