

Molecular imaging of inflammation

Inflammation is

- caused by immune reactions (hypersensitivity reactions/autoimmune diseases), infections by pathogens (bacteria, viral, fungal, parasitic), toxins, chemical irritants, ionizing radiation, foreign bodies, burns, frostbite, trauma.....
- induced by mediators produced by damaged cells
- a protective response to
 - a) eliminate the initial cause of cell injury
 - b) remove necrotic cells and tissues
 - c) initiate the process of repair
- normally self limited but can also be harmful

Involved cells: Granulocytes (neutrophil, eosinophil, basophil), mast cells, monocytes/macrophages, lymphocytes (T and B), fibroblasts, and platelets

Involved proteins: Complement, coagulation, proteoglycans, kinines....

Classical signs of acute inflammation: Dolor (pain), Calor (heat), Rubor (redness), Tumor (swelling), Functio laesa (loss of function).

Vasodilatation and causes an increased blood flow resulting in redness (*rubor*) and increased heat (*calor*). The increased permeability of the blood vessels causes exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (*tumor*). Some of the released mediators such as bradykinin increase the sensitivity to pain (hyperalgesia, *dolor*).

Inflammation can be classified as either Acute or Chronic:

	Acute	Chronic
Onset:	fast (minutes or hours)	slow (days)
Cellular infiltrate:	predominantly neutrophils	monocytes/macrophages and lymphocytes
Tissue injury/damage:	normally mild and self-limited	often severe and progressive irreversible destruction

Acute inflammation:	Vascular:	vasodilatation, vascular permeability (accumulation of fluid/plasma in the extra-vascular tissues; early transudate (low protein content; exudates (high protein content)), endothelial activation, increased expression of adhesion molecules, chemotaxis; increased rolling/adhesion/transmigration of activated leukocytes – emigration to the extravascular tissues;
	Cellular:	recruitment and activation of neutrophils and leukocytes.

	Tissue:	edema; normally no severe tissue injury and scar formation
	Example:	acute contact hypersensitivity reaction of the skin (Nickel); toxic shock syndrome, acute appendicitis; bacterial broncho pneumonia
Chronic inflammation:	Vascular:	endothelial activation, angiogenesis , expression of adhesion molecules, chemotaxis; increased rolling/adhesion/ transmigration of activated leukocytes; leukocyte emigration
	Tissue:	persistent tissue injury, tissue destruction by inflammatory cells, repair with fibrosis/scar formation cancer induction possible
	Cellular:	mononuclear cell infiltrate monocytes/macrophages, T- and B- lymphocytes, plasma cells). Thus, the prolonged inflammation, leads to a shift in the type of cells present at the site of inflammation
	Example:	chronic contact hypersensitivity reaction of the skin (Nickel) Autoimmune diseases (rheumatoid arthritis, systemic lupus erythemtodes, multiple sclerosis) pulmonary silicosis skin ulcers

Imaging modalities for non invasive in vivo imaging of inflammation:

- Positron emission tomography (**PET**) – function, metabolism
- Single-Photon Emission Computed Tomography (**SPECT**) – function, metabolism
- Optical Imaging (**OI**) – function, metabolism
- Magnetic Resonance Imaging (**MRI**) – anatomy, (function, perfusion)
- Computed Tomography (**CT**) – anatomy, (perfusion)

The golden standard in nuclear medicine imaging of inflammation is the use of autologous radiolabeled leukocytes (^{111}In -oxine-SPECT)

Targets of non invasive *in vivo* PET imaging of inflammation:

- 1) Glucose metabolism: ^{18}F -Fluoro-2-deoxy-D-glucose (^{18}F FDG)-PET
- 2) Cell proliferation: ^{18}F 3'-Deoxy-3'-(18)F-fluorothymidine (^{18}F FLT)-PET
- 3) Angiogenesis: ^{18}F -Galacto-RGD-PET
- 4) Hypoxia: ^{18}F -Fluoromisonidazole (^{18}F FMISO)-PET
 ^{18}F -Fluoro-azomycinarabino-furanoside (^{18}F FAZA)-PET
 ^{64}Cu Diacetyl-bis(N^4 -methylthiosemicarbazone) (^{64}Cu -ATSM)-PET
- 5) Perfusion: ^{64}Cu -pyruvaldehyde-bis(N^4 -methylthiosemicarbazone) (^{64}Cu PTSM)-;
 ^{15}O - H_2O -PET

6) For cell tracking:

In vitro labeling of cells of interest (T-Lymphocytes):

- a) [^{64}Cu]-pyruvaldehyde-bis(N4-methylthiosemicarbazone) ([^{64}Cu]PTSM); PET
- b) [^{64}Cu]-DOTA labeled antibodies against cell membrane proteins
[^{89}Zr]-labeled antibodies against cell membrane proteins; PET

In vivo labeling of cells of interest (T-Lymphocytes)

- a) Injection of [^{64}Cu]-DOTA- or [^{89}Zr]-labeled antibodies against specific cell membrane proteins; PET
- b) Transduction of the cells of interest with a reporter gene such as herpes simplex thymidine kinase (TK) followed by injection of the TK-transduced T cells into mice and by application of a protein-specific (TK-specific) radiolabeled probe such as 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)-guanine (^{18}F -FHBG) or 2'-fluoro-2'-deoxy-5-iodo-1-beta-D-arabinofuranosyluracil ([^{18}F]FIAU); PET
- c) Use of transgenic mice with endogenous TK-expression in T cells followed by injection of ^{18}F -FHBG or [^{18}F]FIAU
- d) Adoptive transfer of endogenously TK-expressing T cells (derived from TK transgenic mice) into wild-type mice followed by [^{18}F]FIAU or [^{18}F]FIAU injections for PET investigations

Targets of non invasive *in vivo* **SPET** imaging of inflammation:

For cell tracking: [^{111}In]-oxine labeled Leukocytes for SPECT

Targets of non invasive *in vivo* **OI** imaging of inflammation:

- 1) Cathepsin B: Using Cathepsin B activatable fluorescence probes
- 2) Matrix-metalloproteinase (MMP): MMP activatable fluorescent probes
- 3) Angiogenesis using specifically $\alpha\text{V}\beta_3$ binding RGD peptides
- 3) Neutrophil elastase detecting OI fluorescent probe
- 4) Hypoxia detecting OI fluorescent probe
- 5) Apoptosis detecting – Annexin OI fluorescent probe
- 6) Bone destruction detecting OI fluorescent probe
- 7) NF κ B activation: Investigation of diseased NF κ B luciferase reporter mice which express luciferase under control of three NF κ B response elements (luciferin-injection for bioluminescence OI)
- 8) Reactive oxygen and nitrogen species (ROS/RNS): Using an specific luminescence probe (L-012)
- 9) Measurement of perfusion and vascular leakage using specific OI fluorescence probe
- 9) For cell tracking:

In vitro labeling of cells of interest with the fluorescence dye Cy5

In vivo labeling: Cells of interest (T-Lymphocytes) derived from luciferase
Transgenic mice were adoptively transferred into wild-type mice followed by luciferin injections for bioluminescence OI

Targets of non invasive *in vivo* **MRI** imaging of inflammation:

- 1) Determination of the edema and joint destruction
- 2) Investigation of perfusion and vascular leakage using contrast agents or by arterial spin labeling (ASL)
- 3) For cell tracking:

In vitro labeling of cells of interest (T cells) with superparamagnetic iron oxide particles (SPIOs)

Targets of non invasive *in vivo* CT imaging of inflammation:

- 1) Detection of bone destruction
- 2) Investigation of perfusion and vascular leakage using contrast agents