IMMUNOLOGY

INFLAMMATION AND REPAIR

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INFLAMMATION

Defenses

- The skin is the most difficult surface to penetrate.
- Free fatty acids in sebaceous glands act as detergents.
- Lactic acid in perspiration, acidic pH, and a relatively dry environment also hinder microbe survival

Defenses

- Mucous membranes may eliminate microbes through active propulsion (e.g., mucociliary escalator in the lung).
- Many surfaces are also protected by mucus secretion.
- Cationic peptides, lysozyme, lactoferrin, and secretory IgA are present and are anti-microbial.

Defenses

- Normal flora through use of nutrients and occupation of space can prevent pathogen colonization.
- Normal flora may also produce antimicrobial substances.
- Acidic pH in stomach, bladder, kidneys inactivate many microbes as do bile salts in the intestine.
- High urine flow also limits the establishment of infection.

Inflammation

- Inflammation is a protective response to rid the organism of both the initial cause of injury as well as addressing the consequences of the injury.
- The inflammatory response may be precipitated by:
- Toll like receptors (TLRs)
- CD14 receptor in plasma membrane binds lipopolysaccharide of bacterium, unmethylated CpG nucleotides, as well as other triggers such as double stranded RNA, are found on cell surfaces and endosomes;

Inflammation

- NOD-like cytosolic receptors (NLRs) also have microbial recognition domains
- Sense:
- Uric acid
- ATP
- The DNA binding protein, HMGB-1
- DNA in cytoplasm (tissue injury)
- Activate a multiprotein cytosolic complex (inflammasome), that induces production of IL-1
- IFα, leading to VEGF activation (hypoxia), leading to FLK1 activation (and axon repair)

Inflammation

- RIG-like cytosolic receptors (RIGRs) recognize viral RNA
- Immune mediation

Inflammation

- There is an initial vascular response (vasodilatation) as a result of histamine release (sentinel mast cells) and nitric oxide formation (endothelium).
- This leads to increased blood flow (hyperemia) in area of injury.
- Endothelial gaps develop with secretion of kinins leukotriene B, neuropeptide P_1, and VEGF.
- This leads to constriction of endothelial cells and widening of intracellular spaces, permitting diapedesis of blood cells to focus of injury.
- This occurs in the first 30 minutes of injury.

Inflammation

- IL-1, TNF, IFN-γ maintain contraction of endothelial cells.
- This begins at 6 hours and lasts for 24 hours.

Inflammation

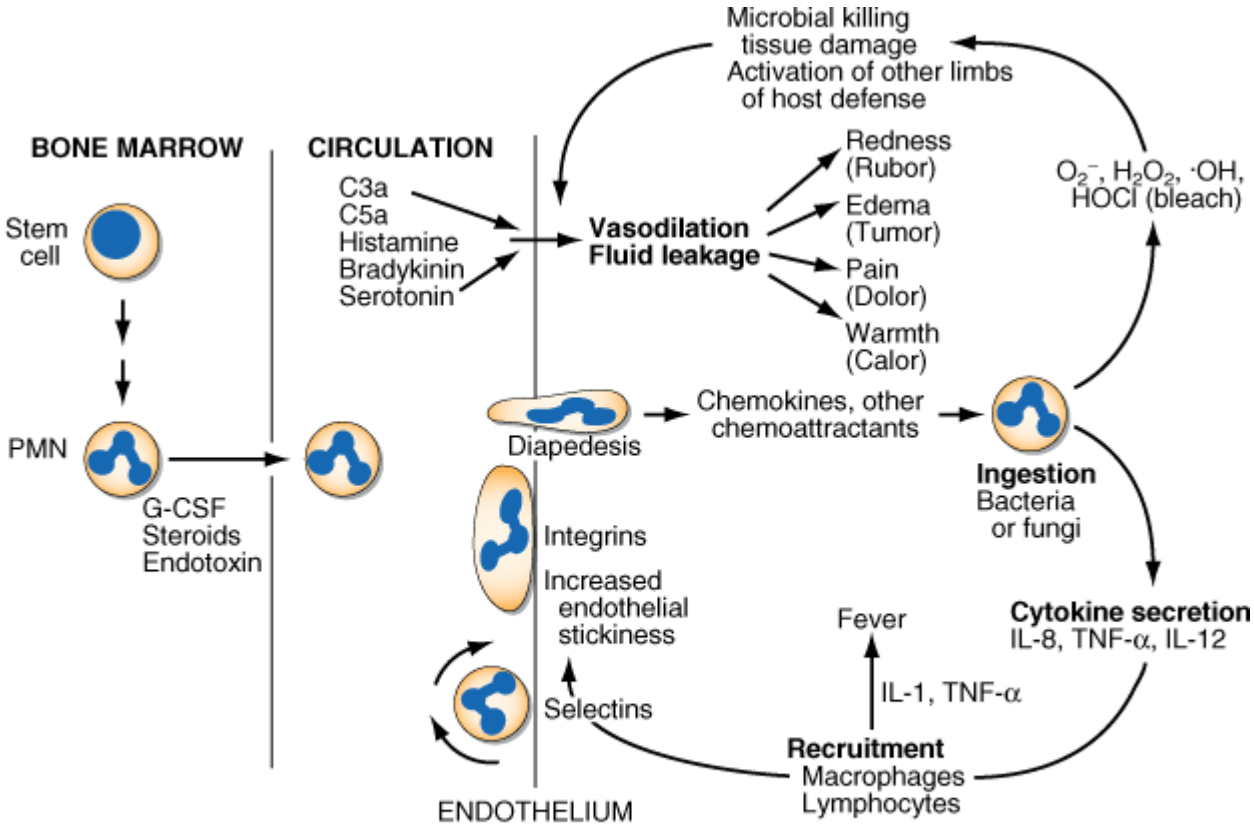

Fig. 61-1 Accessed 07/01/2010

Cellular differentiation

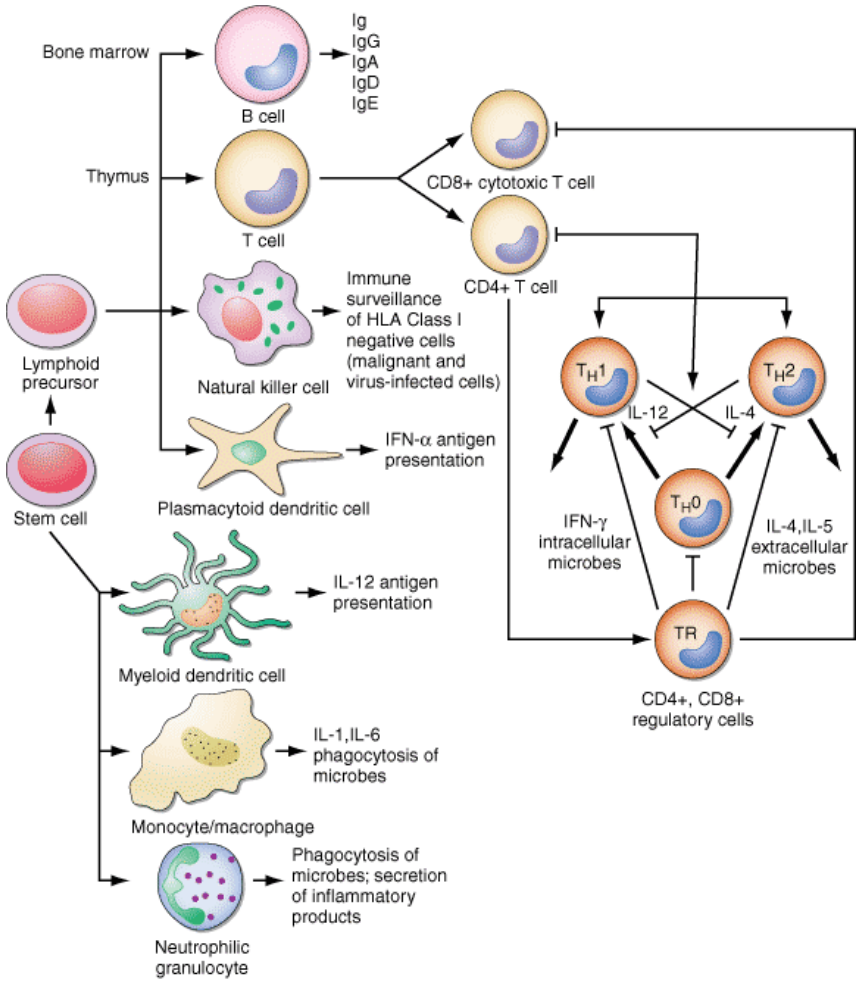

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Fig. 308-2
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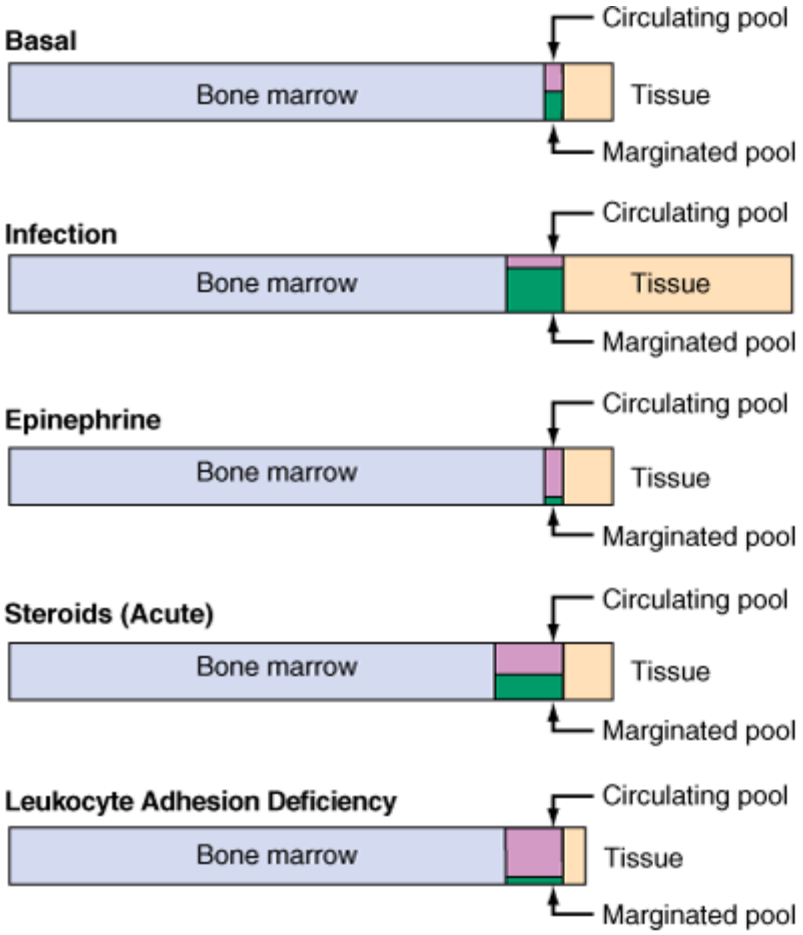
Neutrophil compartments



The freely flowing pool is about one-half the neutrophils in the basal state and is composed of those cells that are in the blood and not in contact with the endothelium.

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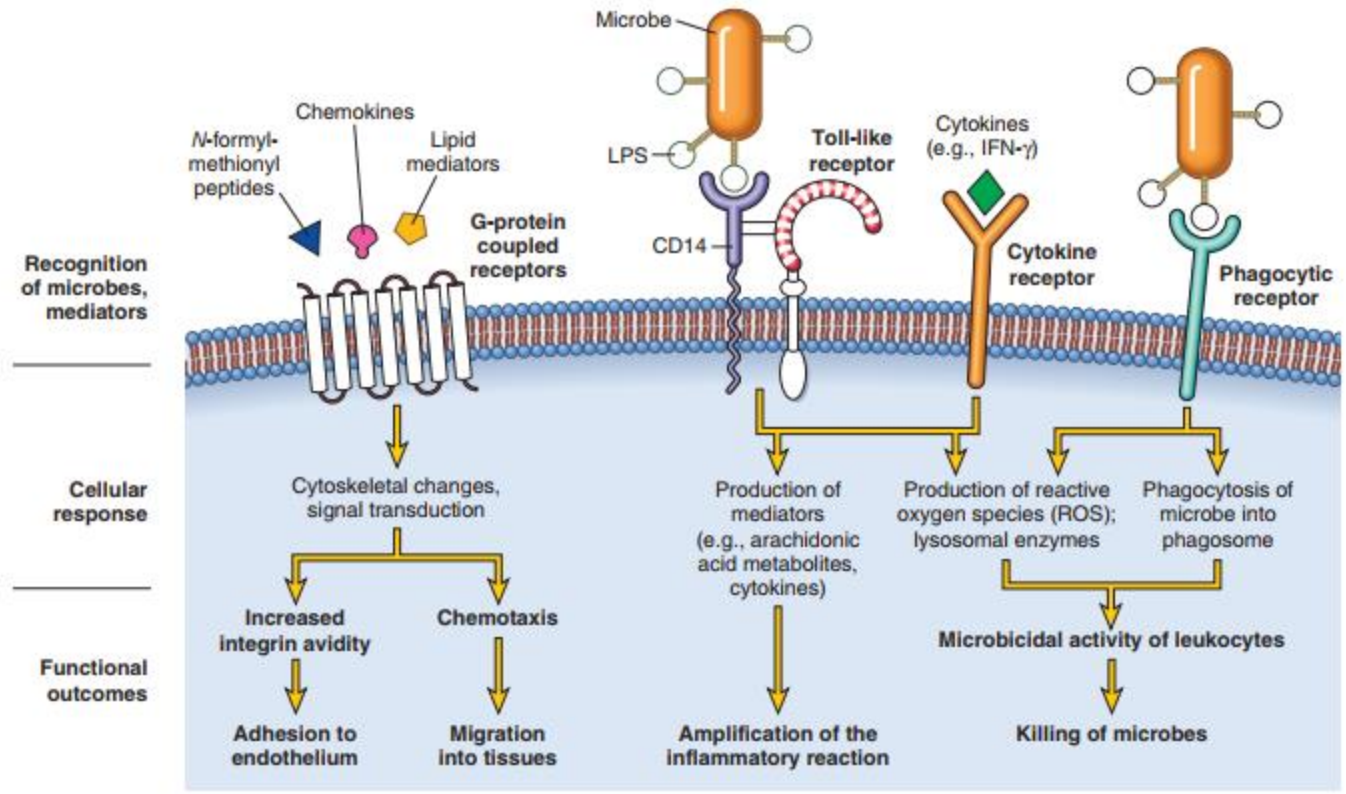

Figure 3.7 Leukocyte activation. Different classes of cell surface receptors of leukocytes recognize different stimuli. The receptors initiate responses that mediate the functions of the leukocytes. Only some receptors are depicted (see text for details). Lipopolysaccharide (*LPS*) first binds to a circulating LPS-binding protein (not shown). *IFN-* , Interferon-γ.

Leukocyte recruitment, adhesion and diapedesis

- First event is induction of adhesion molecules.
- Histamine, thrombin, and PAF stimulate redistribution of P-selectin from normal intracellular stores (Weible-Palade bodies of platelets) to cell surface .
- TNF and IL-1 cause stimulation of adhesion molecules of venular endothelial cells at site of injury.
- Second event is alteration of axial blood flow.
- Endothelial stimulation leads to Ca^{2+} influx and generation of nitric oxide species. Diminishes adhesion.

Leukocyte recruitment, adhesion and diapedesis

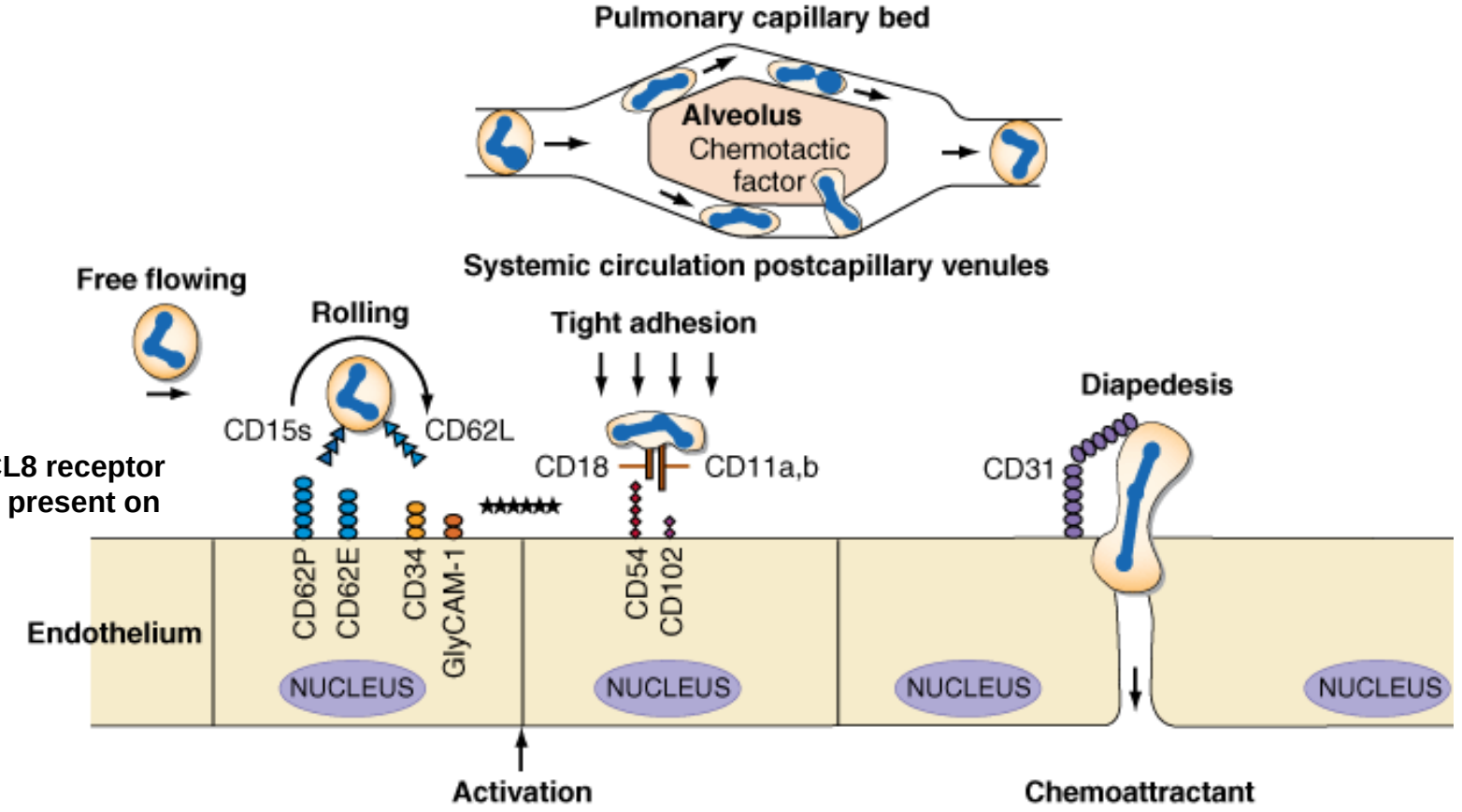

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Fig. 61-8 Accessed 07/01/2010

Table 3.4 Properties of Neutrophils and Macrophages

	Neutrophils	Macrophages
Origin	HSCs in bone marrow	HSCs in bone marrow (in inflammatory reactions) Many tissue-resident macrophages: stem cells in yolk sac or fetal liver (early in development)
Lifespan in tissues	Several days	Inflammatory macrophages: days or weeks Tissue-resident macrophages: years
Responses to activating stimuli	Rapid, short-lived, mostly degranulation and enzymatic activity	More prolonged, slower, often dependent on new gene transcription
Reactive oxygen species	Rapidly induced by assembly of phagocyte oxidase (respiratory burst)	Less prominent
Nitric oxide	Low levels or none	Induced following transcriptional activation of iNOS
Degranulation	Major response; induced by cytoskeletal rearrangement	Not prominent
Cytokine production	Low levels or none	Major functional activity; requires transcriptional activation of cytokine genes
NET formation	Rapidly induced, by extrusion of nuclear contents	No
Secretion of lysosomal enzymes	Prominent	Less

HSC, Hematopoietic stem cells; *iNOS*, inducible nitric oxide synthase; *NET*, neutrophil extracellular trap.

This table lists the major differences between neutrophils and macrophages. The reactions summarized above are described in the text. Note that the two cell types share many features such as phagocytosis, ability to migrate through blood vessels into tissues, and chemotaxis.

Key migration steps

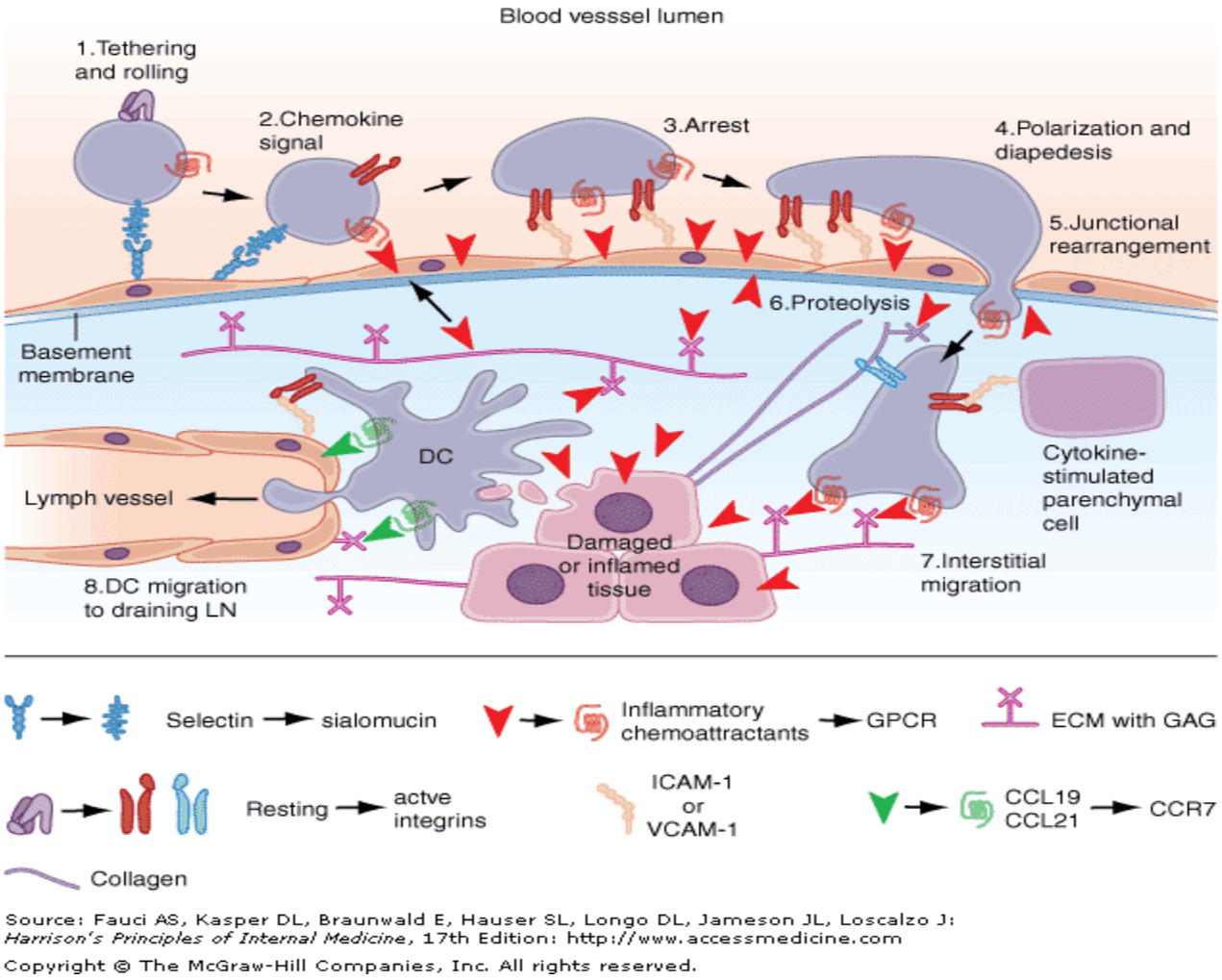

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Table 3.3 Endothelial and Leukocyte Adhesion Molecules

Family	Molecule	Distribution	Ligand
Selectin	L-selectin (CD62L)	Neutrophils, monocytes T cells (naïve and central memory) B cells (naïve)	Sialyl-Lewis X/PNAd on GlyCAM-1, CD34, MAdCAM-1, others; expressed on endothelium (HEV)
	E-selectin (CD62E)	Endothelium activated by cytokines (TNF, IL-1)	Sialyl-Lewis X (e.g., CLA) on glycoproteins; expressed on neutrophils, monocytes, T cells (effector, memory)
	P-selectin (CD62P)	Endothelium activated by cytokines (TNF, IL-1), histamine, or thrombin	Sialyl-Lewis X on PSGL-1 and other glycoproteins; expressed on neutrophils, monocytes, T cells (effector, memory)
Integrin	LFA-1 (CD11aCD18)	Neutrophils, monocytes, T cells (naïve, effector, memory)	ICAM-1 (CD54), ICAM-2 (CD102); expressed on endothelium (upregulated on activated endothelium)
	MAC-1 (CD11bCD18)	Monocytes, DCs	ICAM-1 (CD54), ICAM-2 (CD102); expressed on endothelium (upregulated on activated endothelium)
	VLA-4 (CD49aCD29)	Monocytes T cells (naïve, effector, memory)	VCAM-1 (CD106); expressed on endothelium (upregulated on activated endothelium)
	$\alpha 4\beta 7$ (CD49dCD29)	Monocytes T cells (gut homing naïve effector, memory)	VCAM-1 (CD106), MAdCAM-1; expressed on endothelium in gut and gut-associated lymphoid tissues
Ig	CD31	Endothelial cells, leukocytes	CD31 (homotypic interaction)

CLA, Cutaneous lymphocyte antigen-1; *GlyCAM-1,* glycan-bearing cell adhesion molecule-1; *HEV,* high endothelial venule; *Ig,* immunoglobulin; *IL-1,* interleukin-1; *ICAM,* intercellular adhesion molecule; *MAdCAM-1,* mucosal adhesion cell adhesion molecule-1; *PSGL-1,* P-selectin glycoprotein ligand-1; *TNF,* tumor necrosis factor; *VCAM,* vascular cell adhesion molecule.

Inflammation

- Rolling interactions reflect low affinity binding.
- P-selectin reacts to a ligand that is a Sialyl-Lewis X modified glycoprotein receptor on neutrophils, monocytes, lymphocytes;
- E-selectin plays a major role in rolling and adhesion of neutrophils, monocytes, and T cells.
- L-selectin homes T cells to high endothelial venules; CD34 as endothelial molecule, weakly expressed in other leukocytes (HEV).

Inflammation

- Integrins are of the immunoglobulin family
- Firm adhesion reflects high affinity binding.
- Integrin ICAM-1, as ligand for LFA-1, a β_2-integrin. Facilitates adhesion arrest and transmigration.
- VICAM-1 is a ligand for VLA-4, a β_1-integrin.
- LPAM-1 bind eosinophils and mononuclear cells.
- IL-1, TNF stimulate integrin production.

Inflammation

- PECAM-1 (CD31) recognizes leukocyte receptor, removes aged cells. Degrades in post-capillary venules. Found at intracellular junctions, principal site of chemokine production, and localizes cells.
- Leukocytes secrete collagenases and pierce basement membrane. Adhere to extracellular matrix.
- CD44 is potent ligand to hyaluronic acid as well as E-lectin.
- Hydrogen peroxide generation leads to axon stimulation and restoration of neural fibers in wounds

Inflammation

- All bacterial proteins (and only those few mammalian proteins synthesized in mitochondria) are initiated by N-formylmethionine.
- All leukocytes contain G-protein coupled receptors that recognize N-formylmethionyl residues.
- IL8, C5a, LTB_4 are other chemoattractants.
- Bind to G-protein coupled receptors on leukocyte surface.
- Activate RAC/RHO/CDC42 family of GTPs, induce polymerization of actin at leading edge of the cell.

Inflammation

- Leukocyte moves by extending filopodia that pull the back of the cell in the direction of the extension.
- Chemotaxis.

Inflammatory cells

- Neutrophils formed in bone marrow, released into blood, migrate 7-10hrs, then home to the tissue where they have a short-lived lifespan.
- Possess mannose, glycan, scavenger, and N-formylmethyl receptors as well as lipopolysaccharide receptor (CD14) and Fc receptor.
- Basophils, as mast cells, are found surrounding blood vessels.
- High affinity IgE Fc receptor on cell surface; when cross-linked by antigen, degranulates cell.

Inflammatory cells

- Histamine, proteoglycans, leukotrienes, tryptase released from granules.
- Produces IL-4.
- Histamine binds to endothelial H_1 receptors on endothelial cells, leading to increase vascular permeability.

Table 3.5 Principal Mediators of Inflammation

Mediator	Source	Action
Histamine	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion and activation
Cytokines (TNF, IL-1, IL-6)	Macrophages, endothelial cells, mast cells	Local: endothelial activation (expression of adhesion molecules) Systemic: fever, metabolic abnormalities, hypotension (shock)
Chemokines	Leukocytes, activated macrophages	Chemotaxis, leukocyte activation
Platelet-activating factor	Leukocytes, mast cells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst
Complement	Plasma (produced in liver)	Leukocyte chemotaxis and activation, direct target killing (membrane attack complex), vasodilation (mast cell stimulation)
Kinins	Plasma (produced in liver)	Increased vascular permeability, smooth muscle contraction, vasodilation, pain

IL, Interleukin; *TNF*, tumor necrosis factor.

Inflammatory cells

- Eosinophil granules contain major basic protein (leads to mast cell degranulation), cationic protein (a ribonuclease that punches holes in cell membranes), elastase, peroxidase, platelet activating factor, leukotrienes.
- These leukocytes predominate up to 24 hours following injury.
- Myeloid derived suppressor cells increase in sepsis.
- There is a shift away from T_{H1} to T_{H2} characterized by production of IL-10, produced by T_{reg}, inhibiting macrophage function.

Inflammatory cells

- Monocytes develop in bone marrow. Released into blood and home to other tissues where they become macrophages.
- First to respond to invading element.
- 2 day life span in circulation
- M1 monocytes respond to toll like receptor ligands as well as IFN-γ.
- Produce IL-1.
- Recruit neutrophils.
- Become tissue macrophages.

Inflammatory cells

- M2 monocytes respond to IL-4 and IL-13 (products of the T_{H2} T-cell subset) and are involved in wound repair and fibrosis.
- Arginase, proline polyaminase, and TGF-β as major products.

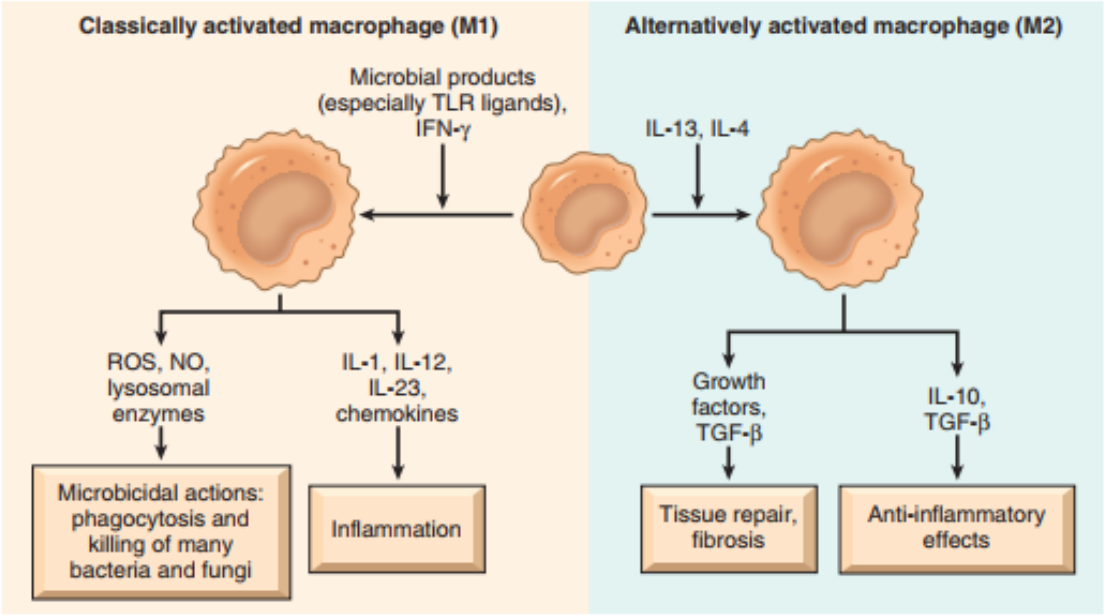


Figure 3.20 Classical and alternative macrophage activation. Different stimuli activate monocytes/macrophages to develop into functionally distinct populations. Classically activated macrophages are induced by microbial products and cytokines, particularly interferon-γ (IFN-γ). They phagocytose and destroy microbes and dead tissues and can potentiate inflammatory reactions. Alternatively activated macrophages are induced by other cytokines and are important in tissue repair and resolution of inflammation. *IL*, Interleukin; *NO*, nitric oxide; *ROS*, reactive oxygen species; *TGF-* , transforming growth factor-β; *TLR*, Toll-like receptor.

Inflammatory cells

- The high affinity phagocytic receptor FcγRI reacts to immunoglobulin (IgG) coating of particle.
- Other potent opsonins are C3 fragments; bind to type I complement receptor on phagocytes.
- Mannin binding lectin binds terminal mannose or fucose residues of glycoproteins or glycolipids as well as bacteria and delivers them to leukocyte for phagocytosis.

Cytokines secreted by macrophages

- TNF-α also produced by mast cells and T cells
- IL-1 also produced by endothelial cells. Production is controlled by a multi-protein cellular complex (inflammasome)
- Stimulate expression of endothelial adhesion molecules
- Stimulate synthesis of chemical mediators
- Stimulate production of enzymes associated with matrix remodeling

Cytokines secreted by macrophages

- Increase surface thrombogenicity of endothelium
- Increase serum amyloid A protein which acts as an opsonin (as does C-reactive protein) and also binds chromatin;
- Serum amyloid A protein also replaces α_2-lipoprotein A in HDL, altering targeting of HDL from hepatocytes to macrophages where it is used as an energy source
- PDGF, FGF, VEGF, TGF-β are products of activated macrophages.
- IL-4 maintains activation.

Cytokines secreted by macrophages

- IL-1β.
- Activates vascular endothelium.
- Activates lymphocytes.
- Local tissue destruction.
- Increases access of effector cells.
- Fever.

Cytokines secreted by macrophages

- TNF-α.
- Activates vascular endothelium and increases vascular permeability.
- Leads to increased entry of immunoglobulin, complement, and effector cells.
- Increases drainage to lymph nodes.
- Fever.

Cytokines secreted by macrophages

- IL-6.
- Lymphocyte activation.
- Increased antibody production.
- Induces hepatocyte production of acute phase proteins such as C-reactive protein and fibrinogen.
- Antiproteases α-1 antitrypsin and α-2 macroglobulin keep system in check.
- Fever.

Cytokines secreted by macrophages

- CXC are α-chemokines that have amino acid residue separating the first two of four conserved cysteine residues.
- Primarily affect neutrophils.
- IL-8 produced.
- Secreted by macrophages and endothelial cells.
- Activated T-cells homing is mediated by E or P selectins as well as integrins (LFA-1 oir VLA-4, ICAM-1 or VICAM-1).

Cytokines secreted by macrophages

- CXCL8.
- Chemotactic effector recruits T-cells, neutrophils, and basophils to site of inflammation.
- CXCL10.
- Soluble.
- Attaches to CXCR3, CXCR4.
- Facilitates cell attachment to endothelium.

Cytokines secreted by macrophages

- CC are β-chemokines whose first two conserved cysteine residues are adjacent.
- Primarily affect monocytes, eosinophils, basophils.
- Examples are MCP-1, MIP-1α, RANTES.
- Eotaxin only affects eosinophils.
- Naïve T-cells home to lymphocytes as a result fo L-selectin and integrin binding (LFA-1 or VLA-4, ICAM-1 or VICAM-1) to their ligands on endothelial surfaces.

Cytokines secreted by macrophages

- CCL19, CCL21 expressed in lymph nodes.
- Bind to CCR7 on naïve T-cells.
- Enhances integrin dependent adhesion and migration through high endothelial venules.

Cytokines secreted by macrophages

- CCR7, CXCR3, CXCR4 are implicated in metastasis.
- C are γ-chemokines that lack the first and third of the four conserved cysteines.
- Lymphotoxins.
- CX3C chemokines contain three amino acid residues separating the first two of four conserved cysteine residues.
- Fractalkine as example.
- Produced and secreted by endothelial cells.
- Adheres monocytes and T-cells.

Other cytokines

- E-adherin produced by T-cells as well as by macrophages.
- Also has soluble form.
- IL-12. Activates NK cells and induces the differentiation of CD4+ cells to T_{H1} cells.
- IL-17 is produced by T cells.
- Attracts leukocytes.

Table 3.7 Cytokines in Inflammation

Cytokine	Principal Sources	Principal Actions in Inflammation
In Acute Inflammation		
TNF	Macrophages, mast cells, T lymphocytes	Stimulates expression of endothelial adhesion molecules and secretion of other cytokines; systemic effects
IL-1	Macrophages, endothelial cells, some epithelial cells	Similar to TNF; greater role in fever
IL-6	Macrophages, other cells	Systemic effects (acute phase response)
Chemokines	Macrophages, endothelial cells, T lymphocytes, mast cells, other cell types	Recruitment of leukocytes to sites of inflammation; migration of cells in normal tissues
IL-17	T lymphocytes	Recruitment of neutrophils and monocytes
In Chronic Inflammation		
IL-12	Dendritic cells, macrophages	Increased production of IFN-γ
IFN-γ	T lymphocytes, NK cells	Activation of macrophages (increased ability to kill microbes and tumor cells)
IL-17	T lymphocytes	Recruitment of neutrophils and monocytes

IFN-γ, Interferon-γ; *IL*, interleukin; *NK*, natural killer; *TNF*, tumor necrosis factor.

The most important cytokines involved in inflammatory reactions are listed. Many other cytokines may play lesser roles in inflammation. There is also considerable overlap between the cytokines involved in acute and chronic inflammation. Specifically, all the cytokines listed under acute inflammation may also contribute to chronic inflammatory reactions.

Eicosanoids

- Produced in leukocytes, mast cells.
- Linoleic acid is he beginning of the arachinodate cascade.
- Phospholipase A_2 releases arachidonic acid from membrane-bound phospholipids.
- Cyclo-oxygenase converts arachidonic acid itno prostaglandin G_2 which then changes to PGH_2 and is converted to thromboxane A_2 (in platelets) , PGI_2, prostacyclin (in endothelial cells), and PGE_2.

Eicosanoids

- 5-Lipo-oxygenase converts arachidonic acid into 5-HPETE, the precursors of leukotrienes (LTA_4 is the parent molecule).
- Main path in neutrophils.
- Also produced by platelets, mast cells.

Eicosanoids

- Cyclo-oxygenase pathway leads to prostacyclin (PI_2) production.
- Cox-1 constitutively expressed in tissues.
- PGG_2 to $PGGH_2$ to PI_2.
- PGI synthase makes prostacyclins in vascular smooth muscle.
- PGI_2 inhibits platelet aggregation, leukocyte aggregation, T-cell proliferation.
- Potent vasodilator.

Eicosanoids

- PGD and PGE synthase are found in lymphocytes.
- PGD_2 produced by mast cells
- Chemoattractant for neutrophils
- PGD_2, PGE_2 stimulate vasodilation, increase cAMP levels.

Eicosanoids

- PGE_2 is hyperalgesic.
- Resets temperature set point at a higher level in hypothalamus.
- $PGF_{2\alpha}$ stimulates vasoconstriction, bronchoconstriction, and smooth muscle contraction of uterus
- Peroxidase requires reduced glutathione to generate active 15-OH metabolite.

Eicosanoids

- TXA synthase is found in platelets.
- Thromboxane, TXA_2, has a 6-member ring that contains an Oxygen atom.
- Thromboxanes increase vasoconstriction, platelet aggregation and bronchoconstriction.
- 5-HPETE to 5-HETE, promotes chemotaxis.
- 5-HPETE to LTA_4 to LTB_4
- LTB_4 increases neutrophil chemotaxis and adhesion.
- Increase IL-1, IL-2, IFN-γ.

Eicosanoids

- LTA_4 in platelets leads to production of LTC_4 (LTC_4 synthase) as well as lipoxin (12-lipoxygenase).
- LTC_4, LTD_4, LTE_4 increase venular vascular permeability, arteriolar vasoconstriction, bronchoconstriction.
- Lipoxins stimulate the production of superoxide ion, necessary for respiratory burst.
- Inhibit leukocyte recruitment and adhesion although are chemotactic.

Eicosanoids

- Lipoxygenase isoforms are issue specific:
- 5-lipoxygenase noted in neutrophils
- 12-lipoxygenase, platelets
- 15-lipoxygenase, eosinophils.
- Platelet activating factor (phospholipid) a potent vasodilator.

Table 3.6 Principal Actions of Arachidonic Acid Metabolites in Inflammation

Action	Eicosanoid
Vasodilation	Prostaglandins PGI_2 (prostacyclin), PGE_1, PGE_2, PGD_2
Vasoconstriction	Thromboxane A_2, leukotrienes C_4, D_4, E_4
Increased vascular permeability	Leukotrienes C_4, D_4, E_4
Chemotaxis, leukocyte adhesion	Leukotrienes B_4, HETE

HETE, Hydroxyeicosatetraenoic acid.

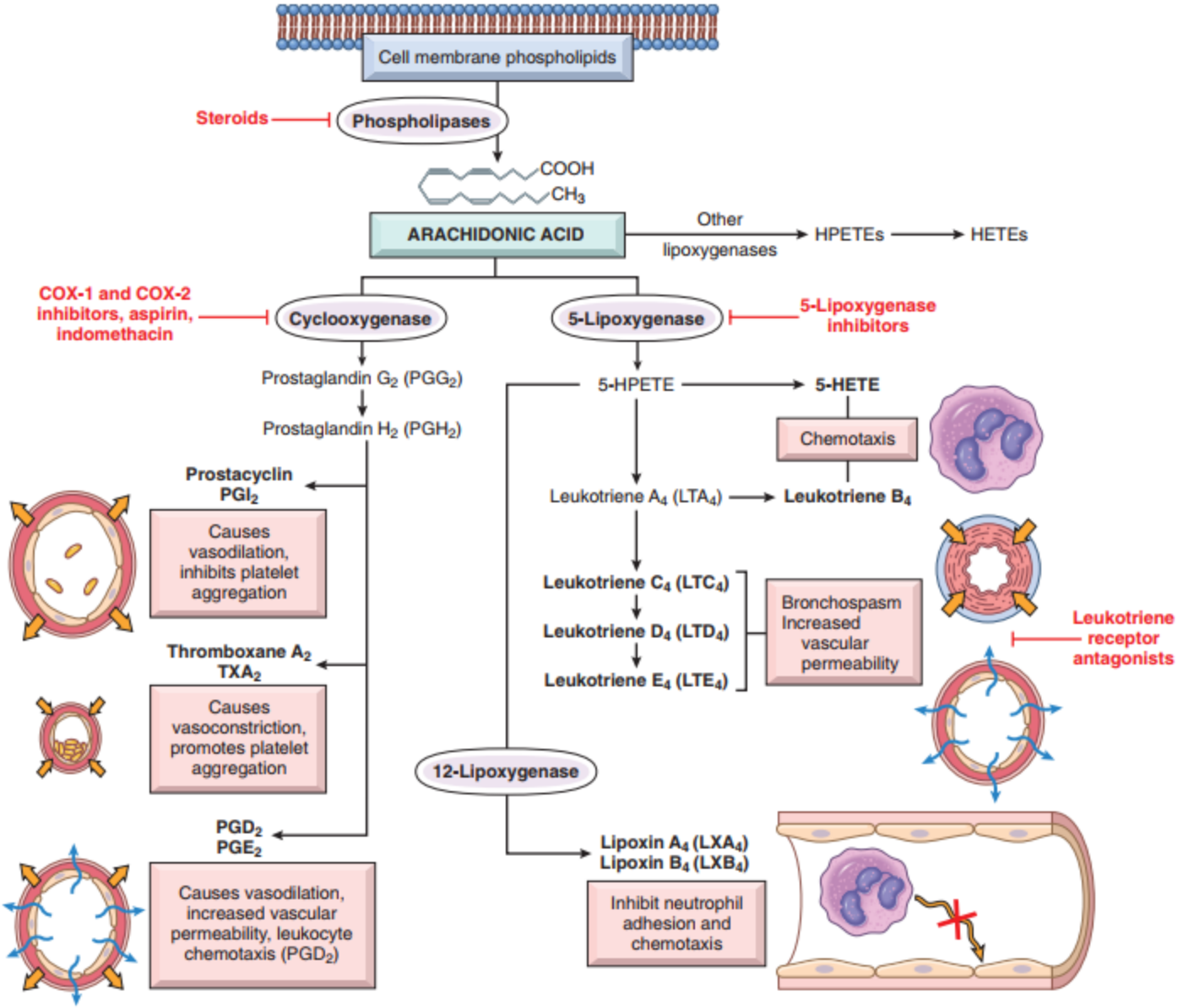


Figure 3.10 Production of arachidonic acid metabolites and their roles in inflammation. Note the enzymatic activities whose inhibition through pharmacologic intervention blocks major pathways (denoted with a red *X*). *COX-1*, *COX-2*, Cyclooxygenase 1 and 2; *HETE*, hydroxyeicosatetraenoic acid; *HPETE*, hydroperoxyeicosatetraenoic acid.

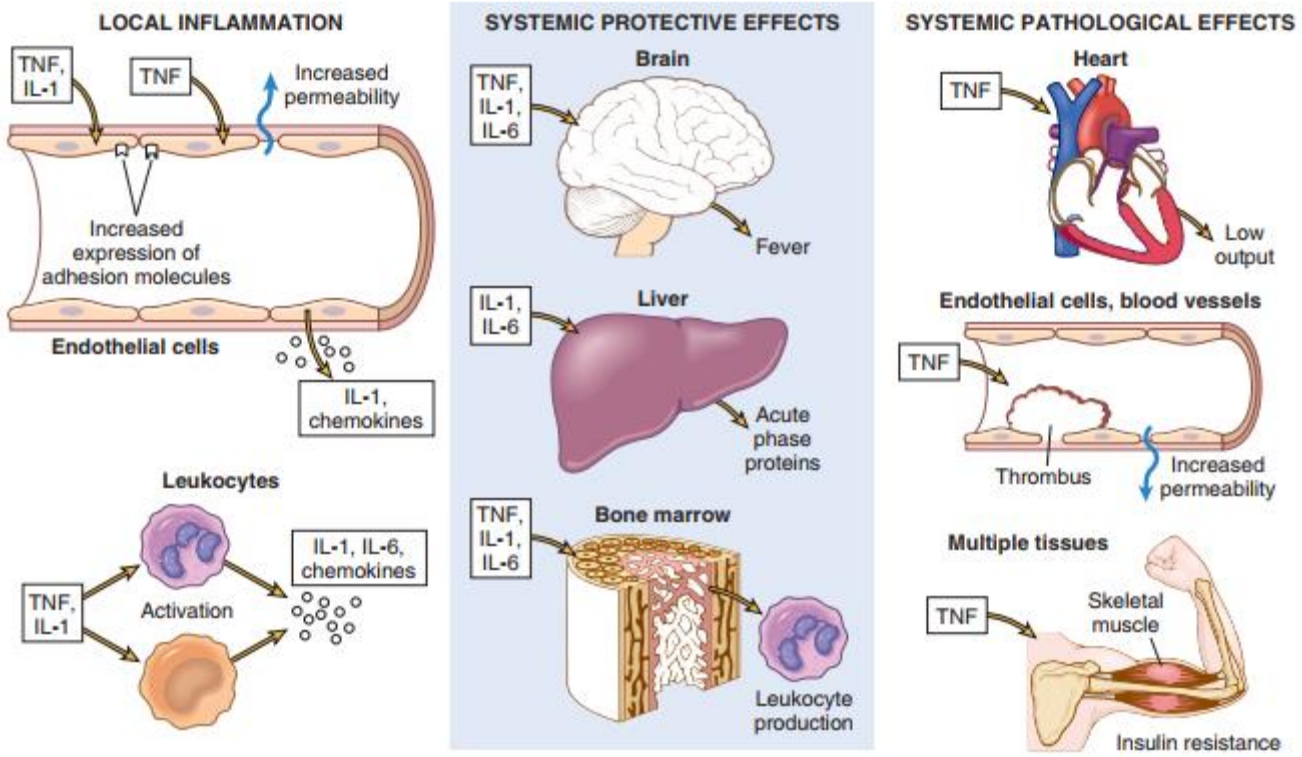


Figure 3.11 Major roles of cytokines in acute inflammation. *IL*, Interleukin; *TNF*, tumor necrosis factor.

PHAGOCYTOSIS
ROLE OF COMPLEMENT

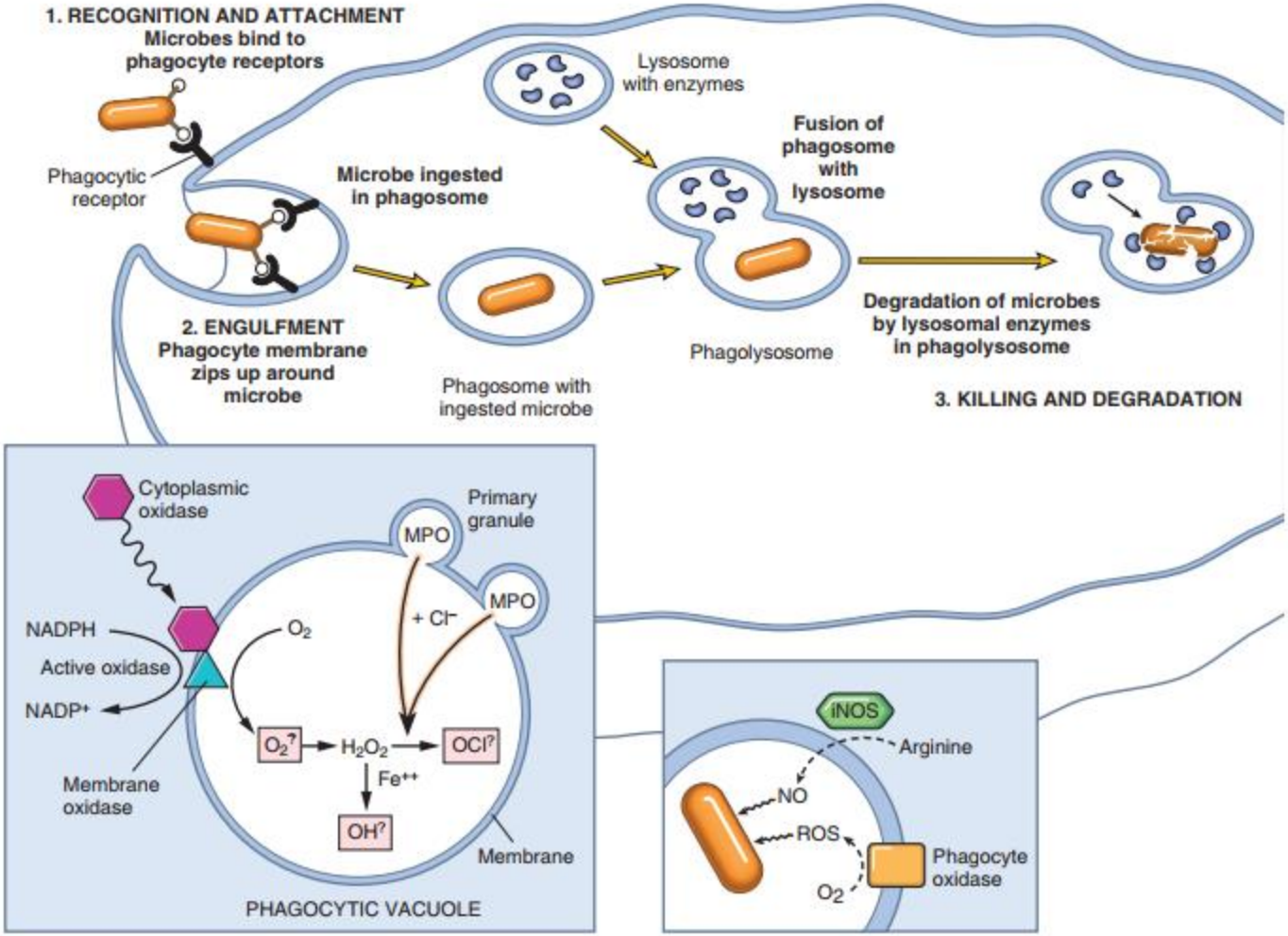


Figure 3.8 Phagocytosis and intracellular destruction of microbes. Phagocytosis of a particle (e.g., a bacterium) involves binding to receptors on the leukocyte membrane, engulfment, and fusion of the phagocytic vacuoles with lysosomes. This is followed by destruction of ingested particles within the phagolysosomes by lysosomal enzymes and by reactive oxygen and nitrogen species. Hypochlorite ($HOCl^-$) and hydroxyl radical ($^{\cdot}OH$) are microbicidal products generated from superoxide ($O_2^{\cdot-}$), and peroxynitrite ($OONO^-$) is generated from nitric oxide *(NO)*. During phagocytosis, granule contents may be released into extracellular tissues (not shown). *iNOS*, Inducible nitric oxide synthase; *MPO*, myeloperoxidase; *ROS*, reactive oxygen species.

Phagocytosis

- Neutrophil extracellular traps (NETs) are extracellular fibrillar networks that concentrate antimicrobial substances at the sites of infection, trapping microbes and preventing their spread.
- NETs are viscous meshworks of nuclear proteins that bind and concentrate granule proteins.
- ROS activation of conversion of arginine to citrulline leads to chromatin condensation.
- Other neutrophil enzymes enter the nucleus and lead to rupture of the nuclear envelope and cell death.

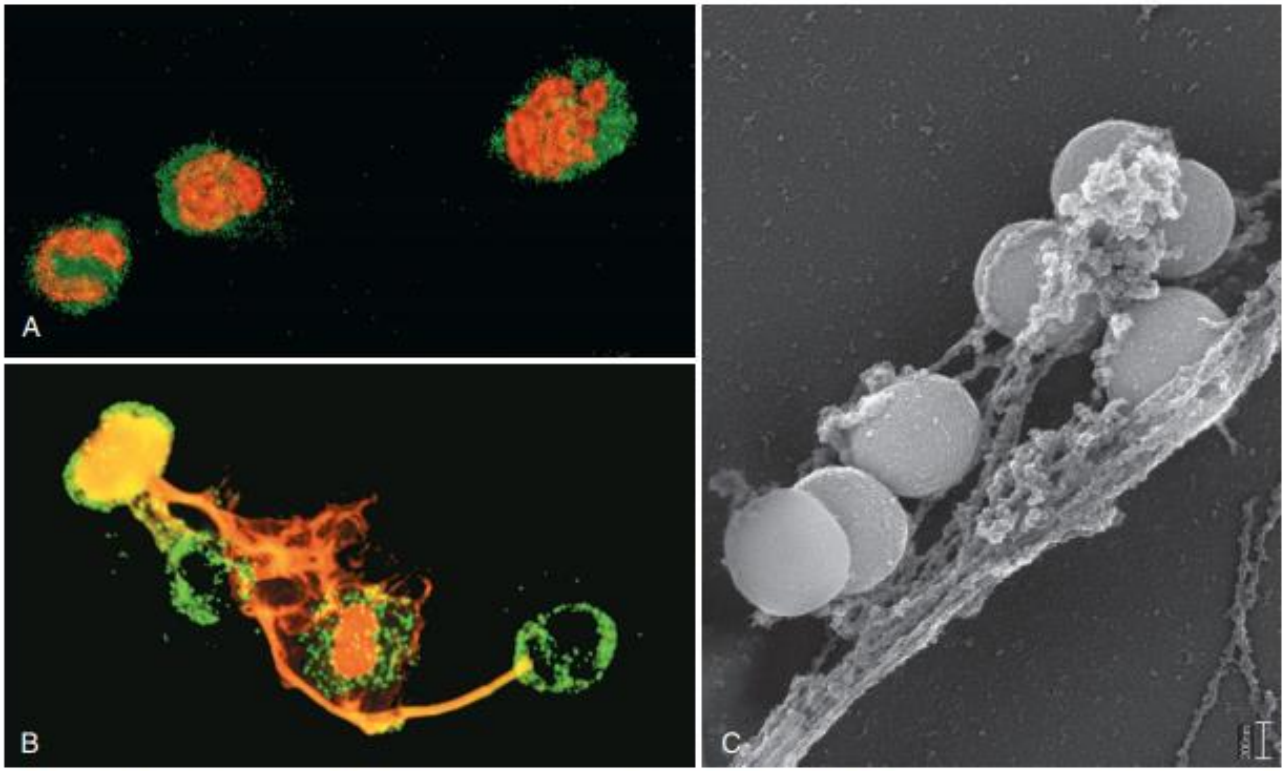


Figure 3.9 Neutrophil extracellular traps (NETs). (A) Healthy neutrophils with nuclei stained red and cytoplasm stained green. (B) Release of nuclear material from neutrophils (note that two have lost their nuclei), forming extracellular traps. (C) Electron micrograph of bacteria (staphylococci) trapped in NETs. (From Brinkmann V, Zychlinsky A: Beneficial suicide: why neutrophils die to make NETs, *Nat Rev Microbiol* 5:577, 2007, with permission.)

Phagocytosis

- Bacterial carbohydrate receptors on macrophages attach microbe.
- Mannose receptors are lectins that bind terminal mannose and fucose residues of glycolipids and glycoproteins.
- These residues are generally found on microbial cell walls.
- Sialic acid or N-acetylgalactosamine are the common terminations on mammalian cells.

Phagocytosis

- Scavenger receptors that bind and mediate oxidized or acetylated LDL particles also attach to microbes.
- Integrins such as MAC-1 (CD11b/CD18) also attach to microbes as well.
- IgG antibodies, the C3b breakdown product of complement, and mannose-binding lectin opsonize the microbe.
- Phagocytosis is enhanced by opsonization as phagocytes contain high affinity receptors for those products.

Phagocytosis

- The phagocytized microbe is engulfed.
- The vacuole formed fuses with the primary lysosomes of the macrophage (or granules of the neutrophil) to permit inactivation and digestion of contents.
- Lactoferrin and vitamin B_{12} binding protein competitively inhibit microbial proliferation.

Phagocytosis

- Oxygen dependent killing (burst) results from the interaction of NADPH oxidase with cytochrome b, reducing Oxygen to superperoxide anion O_2^+ which (in the presence of the catalyst, superoxide dismutase) is converted to hydrogen peroxide (H_2O_2).
- This respiratory burst occurs in the lysosome and phagolysosome.
- In the neutrophil, hydrogen peroxide with myeloperoxidase transform halides into hypohalides such as $HOCl_2$ that kill microbes.

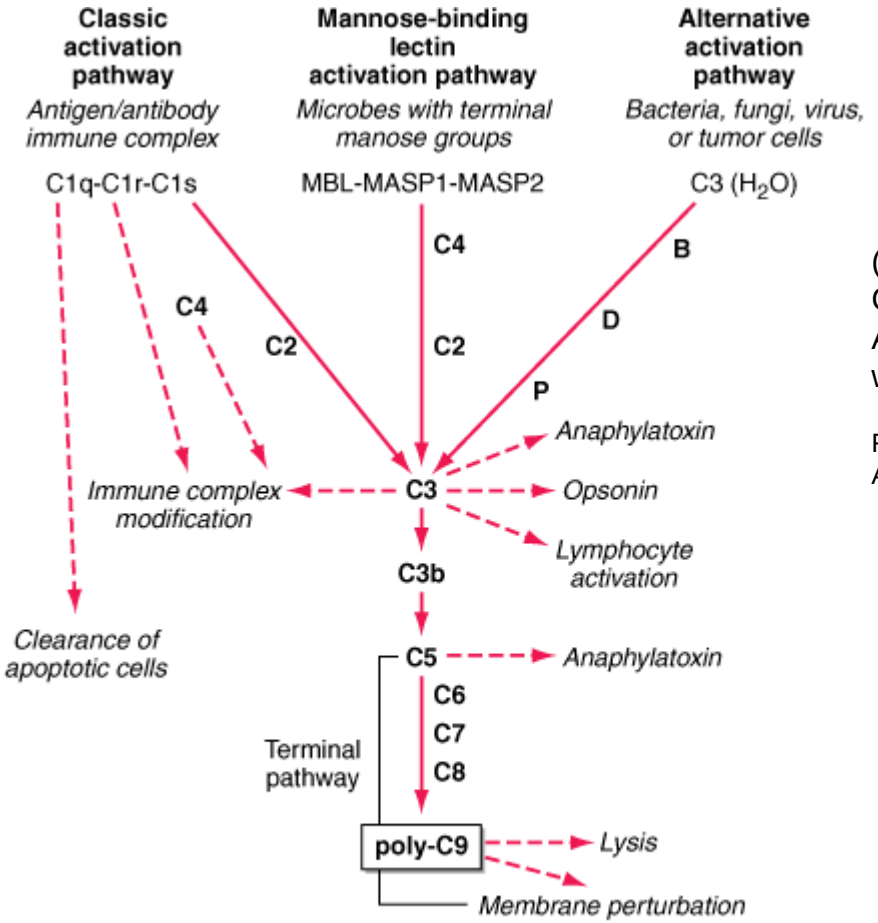
Phagocytosis

- NO produced during this response is also antimicrobial.
- Hydrogen peroxide may also be converted to hydroxyl ion (OH^-).
- Ferrous iron (Fe^{2+}) and Copper may catalyze these reactions.
- These free radicals may be release extracellularly and lead to tissue damage.

Phagocytosis

- Other microbicidial proteins include:
- Proteases
- Defensins (cationic arginine-rich peptides)
- Lactoferrin
- Cathelicidins
- Lysozyme (hydrolyzes the muramic acid– N-acetylglucosamine bond found in the peptide coat of all bacteria)
- Major basic protein (cationic protein found in eosinophils that is cytotoxic to many parasites).

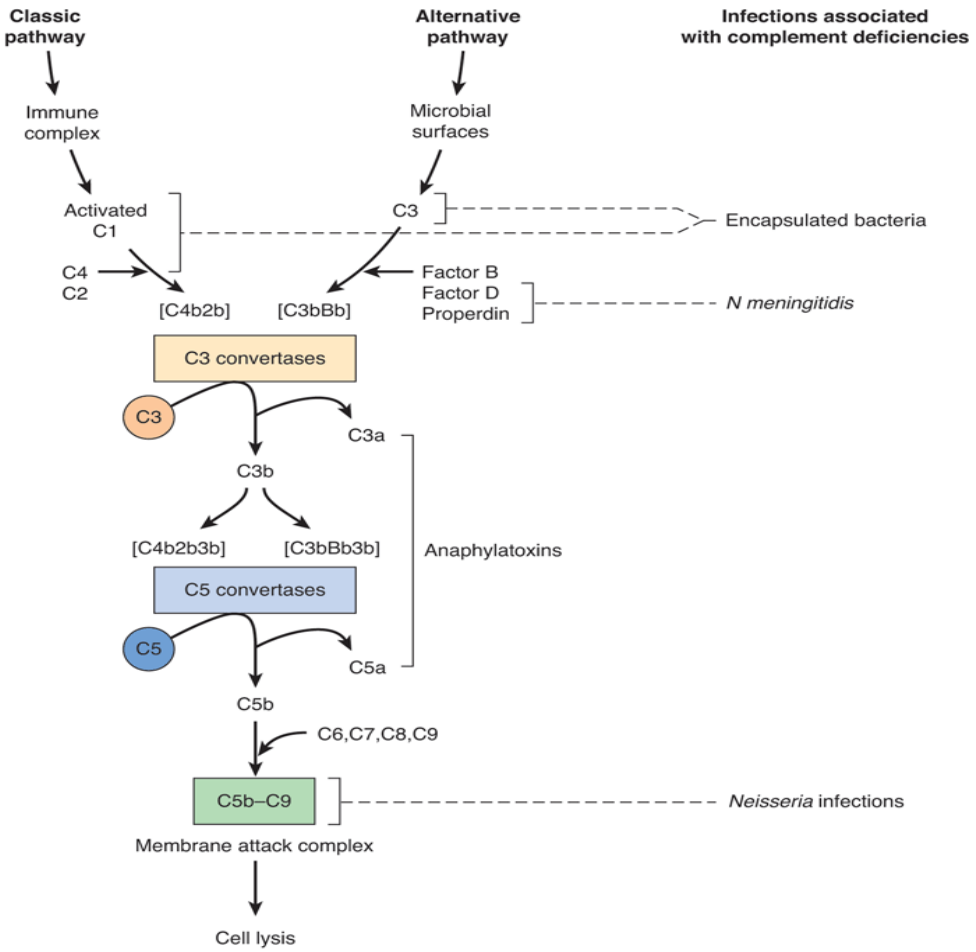
Complement pathways



(After BJ Morley, MJ Walport: The Complement Facts Books. London, Academic Press, Chap 2, 2000; with permission.)

Fig. 308-5
Accessed 07/01/2010

Complement pathways



(Redrawn, with permission, from Nairn R. Immunology. In: *Jawetz*, *Melnick*, *and Adelberg's Medical Microbiology,* 23rd ed. Brooks GF, Butel JS, Morse SA [editors]. McGraw-Hill, 2004.)

Fig. 4-4 Accessed 07/01/2010

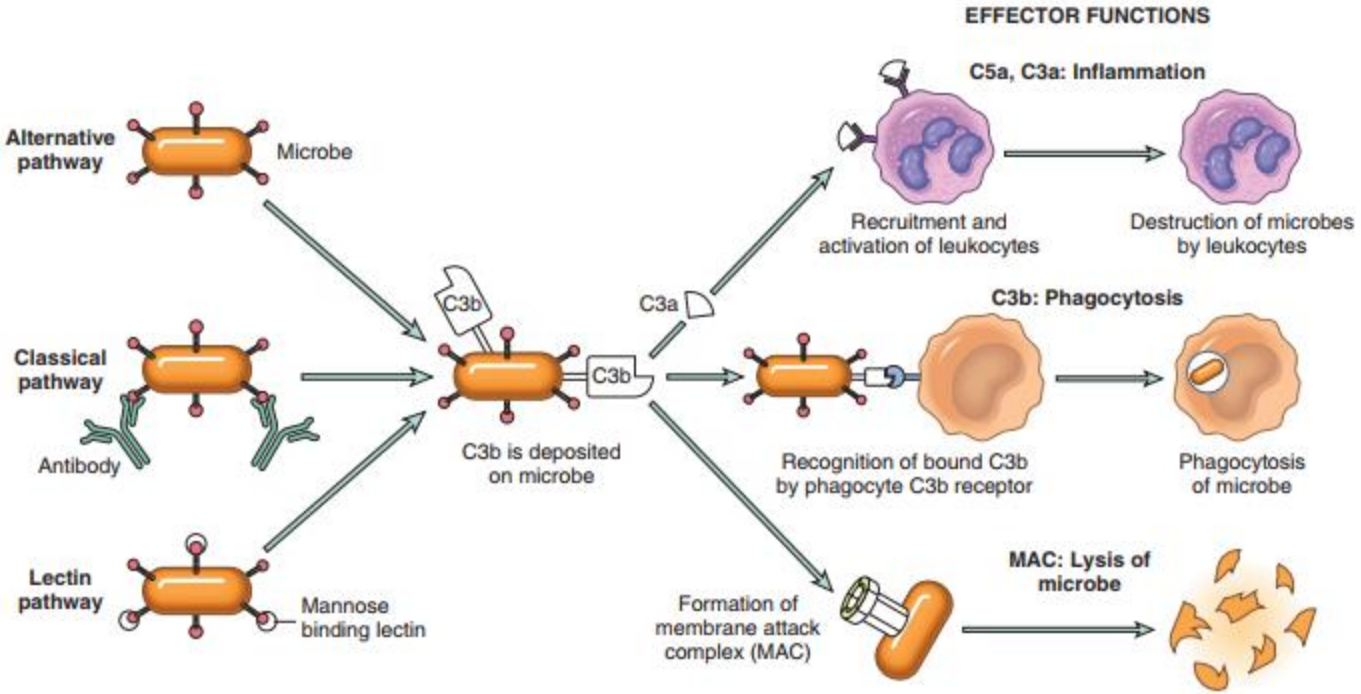


Figure 3.12 Activation and functions of the complement system. Activation of complement by different pathways leads to cleavage of C3. The functions of the complement system are mediated by breakdown products of C3 and other complement proteins and by the membrane attack complex *(MAC)*.

Complement

- Lectin pathway bypasses antibody requiring step. Mannose receptors necessary for phagocytosis. Effective early in infection.
- Classic pathway activated later by antibody-antigen complexes. Complement binding site on the heavy chain of IgG and IgM is unavailable to the C1 component of complement if antigen is not bound to these antibodies.
- Proteolysis of C3 to C3a and C3b by C3 convertase is critical step.

Complement

- Alternative pathway. Initiated by C3b. Factor B, Factor D, and Properdin are other initiators.
- Properdin is the only positive regulator in the complement system.
- Permits amplification of loop and leads to deposition of C3b onto the surface of activating cells, protein complexes, or particles in the immediate vicinity of the activation site. Properdin also delays decay of C3b not bound to surface.
- Time limitation (decay of C3b) another control mechanism in the pathway.

Complement

- C3b favors opsonization of gram positive bacteria. C3b recognized by macrophage receptor.
- Covalently attached to cell or molecule where complement activated.
- C3b binds to previously generated fragments to form C5 convertase to cleave C5 to release C5a and leave C5b attached to cell surface.
- C3b stimulates antibody production by B-cells.

Complement

- C5a attracts neutrophils and enhance adhesiveness of neutrophils to endothelium.
- C5,6,7 attract neutrophils.
- C3a, C4a, C5a cause degranulation of mast cells. May lead to anaphylaxis.
- C5b,6,7,8,9 complex inserts into cell wall, disrupting membrane. Lyse gram negative bacteria. (Membrane attack complex).

Complement

- C1 inhibitor inactivates C1 (serine protease).
- Decay accelerating factor (DAF), a glycoprotein on the surface of human cells, binds C3b and C4b, limiting the formation of C3 convertase.
- Factor H binds to C3b and the complex is cleaved to form Factor I (serine protease).
- Reduces amount of C5 convertase available.
- CD59 inhibits C5 convertase, preventing the formation of the membrane attack complex.
- DAF and CD59 are both linked to plasma membranes by a glycophosphatidyl anchor.

IMMUNITY

Lymphocyte traffic

- Lymphocytes (B or T cells) must be able to continuously circulate through the secondary lymphoid tissues in order to increase the chances that they will come in contact with antigen
- Once a lymphocyte recognizes antigen, within 24 hours of antigen localizing in the lymph node, antigen-specific lymphocytes are depleted from circulation, localize in the lymph node or spleen and become activated
- The flow of B and T cells through the secondary lymphoid tissues is directed by cell adhesion molecules (CAM's) and specialized endothelial cells in the high endothelial venules (HEV).

Transmigration

- Rolling
- Selectins on the lymphocyte bind CD34 on the vascular endothelium.
- Attachment
- Endothelial binding activates β_2 integrins.
- Arrest and Adhesion
- β_2 integrins change conformation and bind very strongly to intercellular adhesion molecules on the surface of the vascular endothelium.
- Transendothelial migration

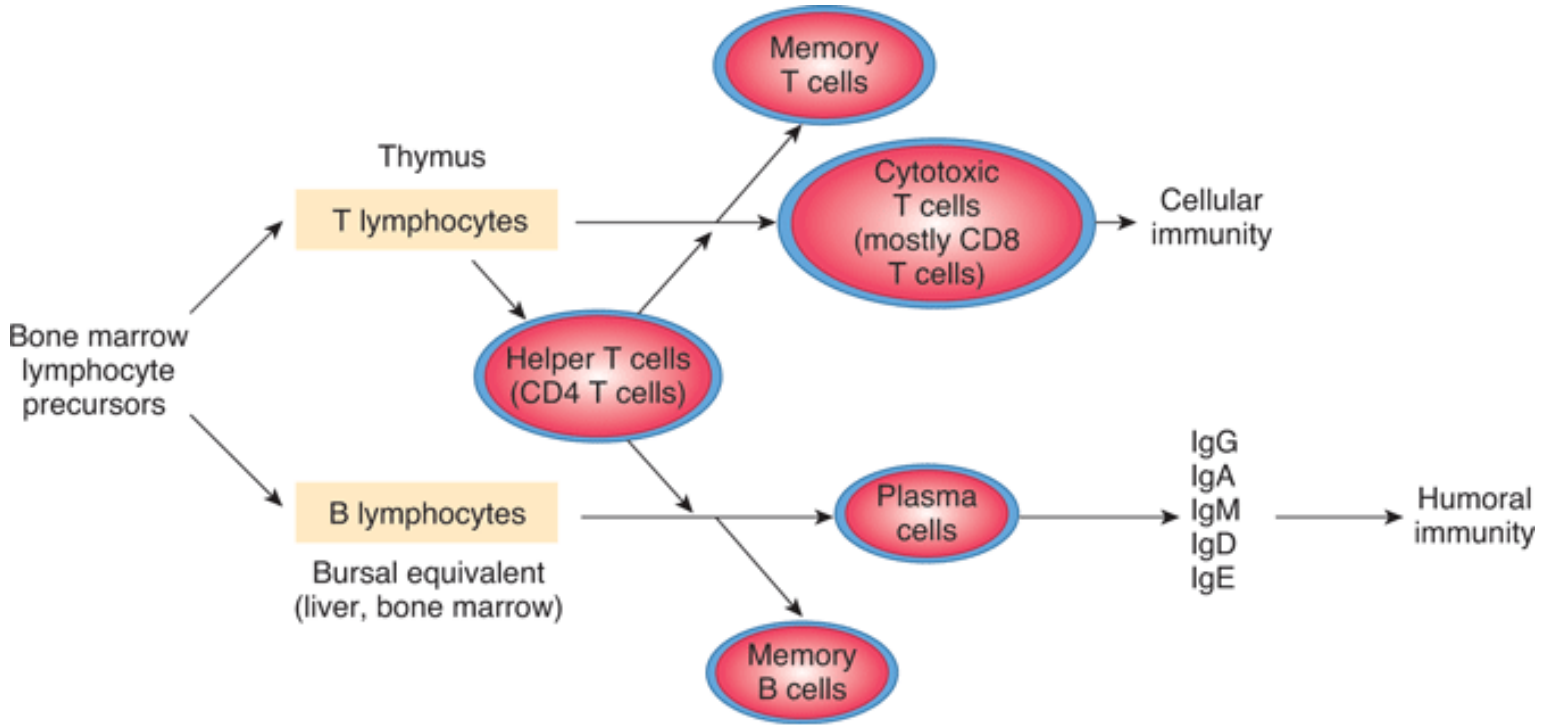
Lymphocyte differentiation

- Antigen enters the Peyers patches across specialized phagocytic cells (M cells).
- After activation, lymphocytes enter lymph, go through lymph node and thoracic duct, then pass from the blood back into the lamina propria and become IgA secreting plasma cells
- A large concentration of activated T cells with a CD4:CD8 ratio of 7:3, B cell blasts and IgA+ plasma cells are found in the lamina propria.
- Intraepithelial lymphocytes are principally T cells, but 10-40% are TCR γδ cells.

Antigen

- An antigen is any macromolecule (protein or polysaccharide) that can combine with a T-cell receptor.
- Each receptor is unique.
- If the antigen is too small to generate an immune response by itself, but must be linked to a macromolecule to stimulate a response, it is called a hapten.
- If the antigen is large enough to generate a response, it is called an immunogen.
- Epitopes are the areas on the immunogen where the antigens reside. They are haptens attached to a macromolecule.

Mediation of immunity



Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Fig. 3-5 Accessed 07/01/2010

Acquired immune response

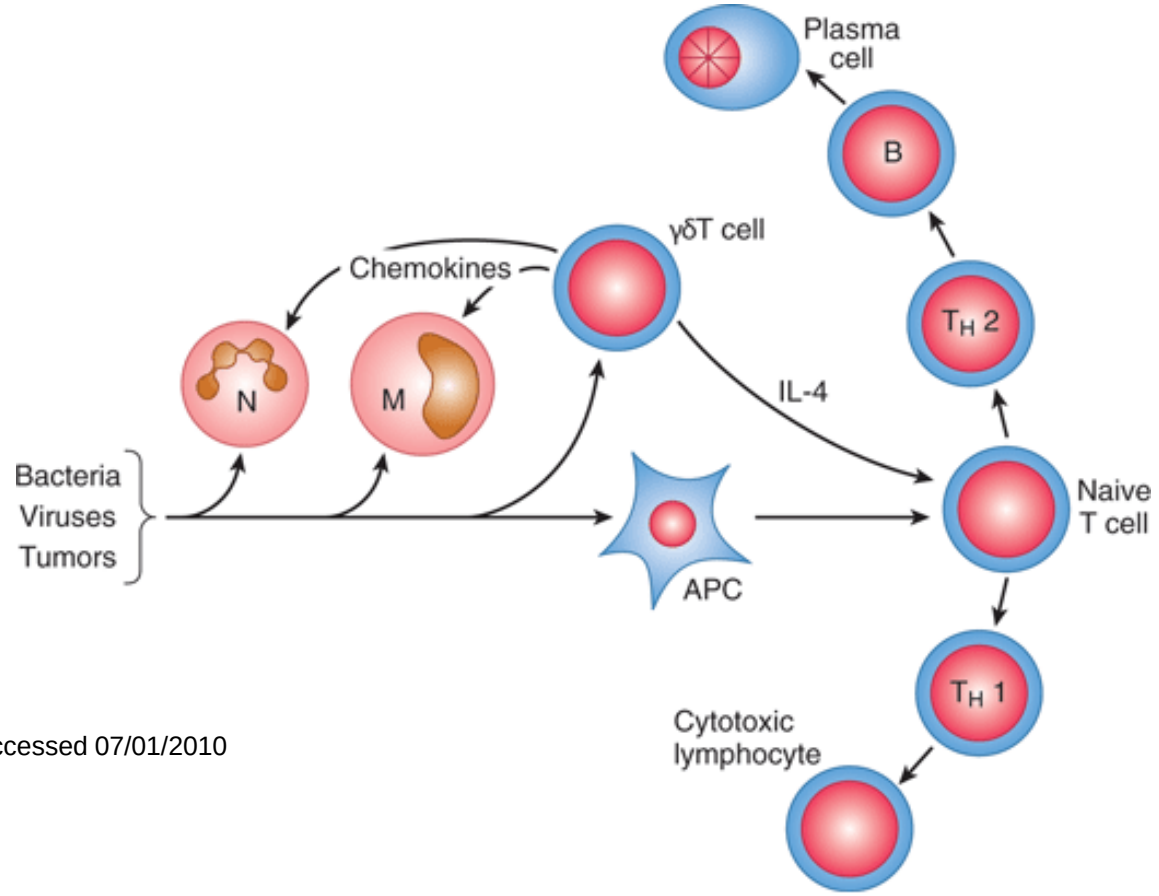


Fig. 3-3 Accessed 07/01/2010

Antigen presentation (old model)

- According to the traditional model, T_H cells and T_C cells recognize antigen on the same antigen processing cell (APC).
- The APC-activated T_H cell produces interleukin-2 (IL-2), which contributes to the activation of T_C cells while in simultaneous interaction with the same APC.
- If the APC recognizes CD47 on the cell membrane in conjunction with SIRPa, the entering agent is not identified as foreign and no reaction ensues.

T-cell antigen recognition

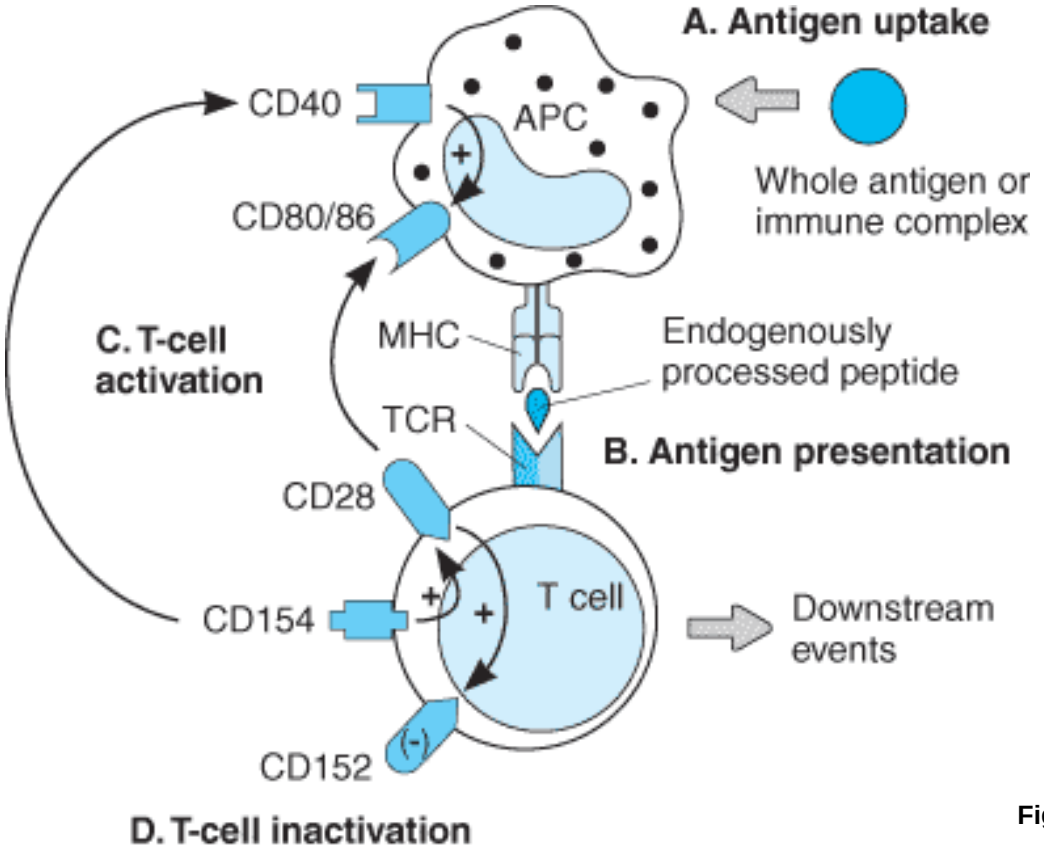


Fig. 3-3 Accessed 07/01/2010

Antigen presentation (new model)

- According to the proposed new model**,** APCs are allowed to activate T_C cells by T_H or other stimuli (lipopolysaccharides, IFN-, viruses).
- APCs first interact with T_H cells.
- The association of CD154 (CD40 L) on the T_H cell and CD40 on the APC allows the latter to activate T_C cells directly.
- Once so prepared, APCs are capable of activating cytotoxic T cells without the need of simultaneous interaction with T_H cells.

Antigen presentation (new model)

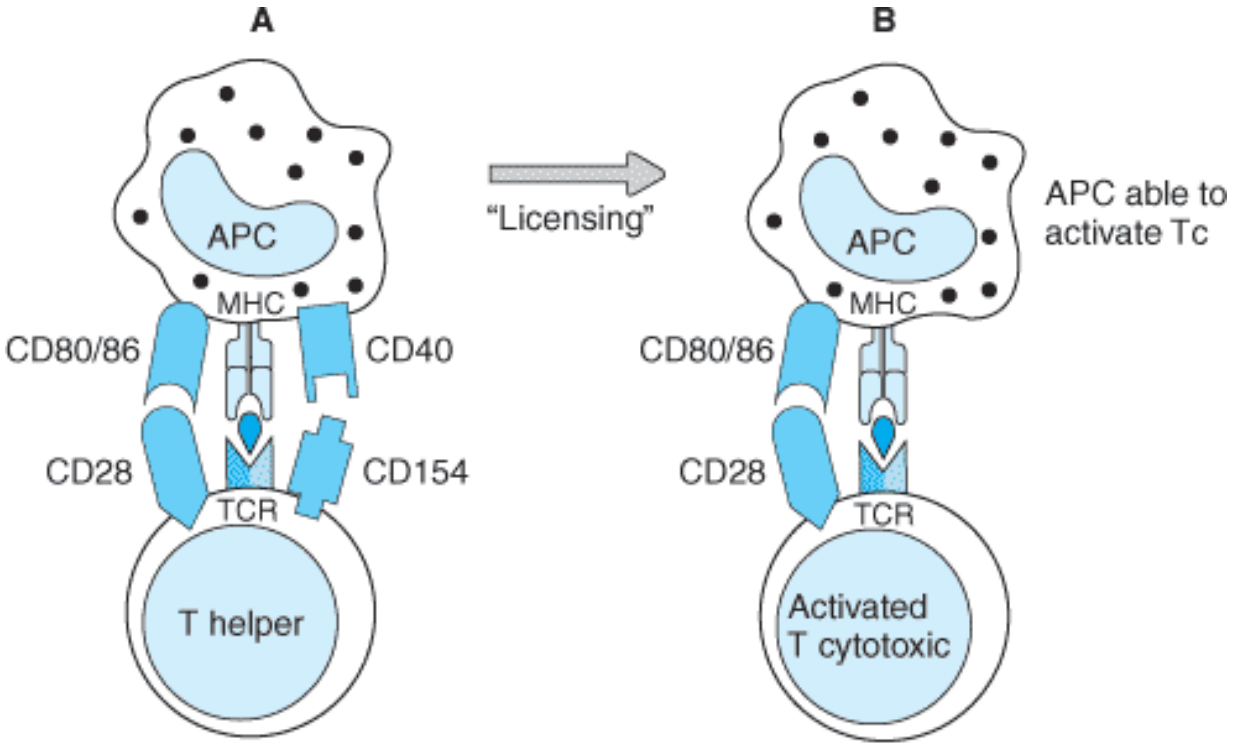


Fig. 3-4 Accessed 07/01/2010

Macrophage activation

- Classic activation (M1 macrophage).
- Induced by endotoxin (engage toll-like receptors) or IFN-γ, crystals or particulate matter.
- Produce NO and other reactive oxygen species as well as upregulate lysosomal enzymes.
- Produce IL-1, IL-12, IL-23 and other inflammatory chemokines.

Macrophage activation

- Alternative activation (M2 macrophage).
- Induced by IL-4 and IL-13.
- Not actively microbicidal.
- Produce TGF-β and other growth factors damping inflammation and promoting fibrosis.
- Produce IL-10 and other anti-inflammatory cytokines.

T-cell receptor

- The T-cell receptor (TCR) is never secreted. It must recognize antigen coupled with MHC presentation.
- Receptor is a heterodimer composed of either αβ or γδ chains.
- Resembles an Fab fragment (arm of an antibody molecule).
- Each TCR non-covalently lined to δε polypeptide where they form the CD3 complex as well as the ζ-chain dimer
- When recognized, activates NF-κB

T-cell receptor

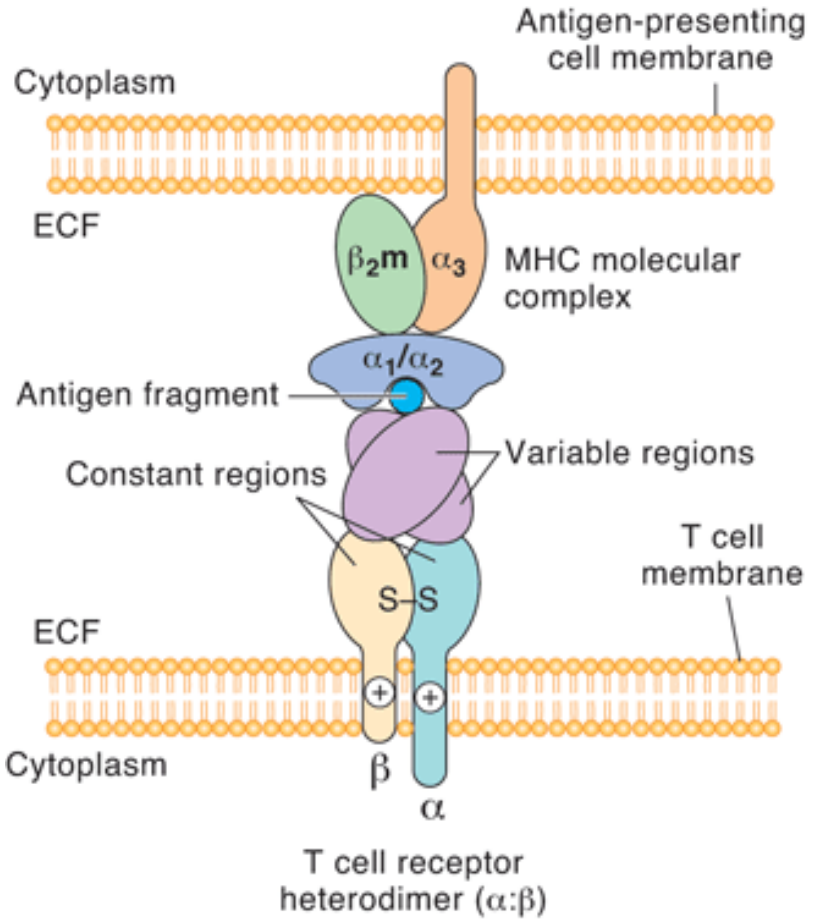
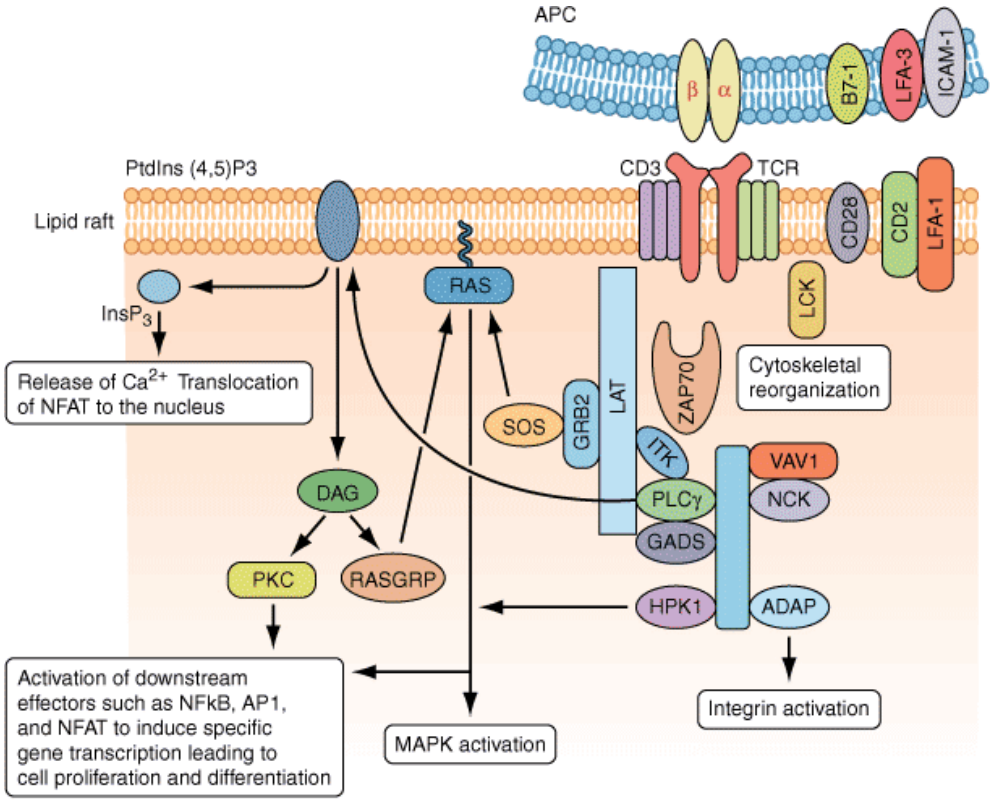


Fig. 3-7 Accessed 07/01/2010

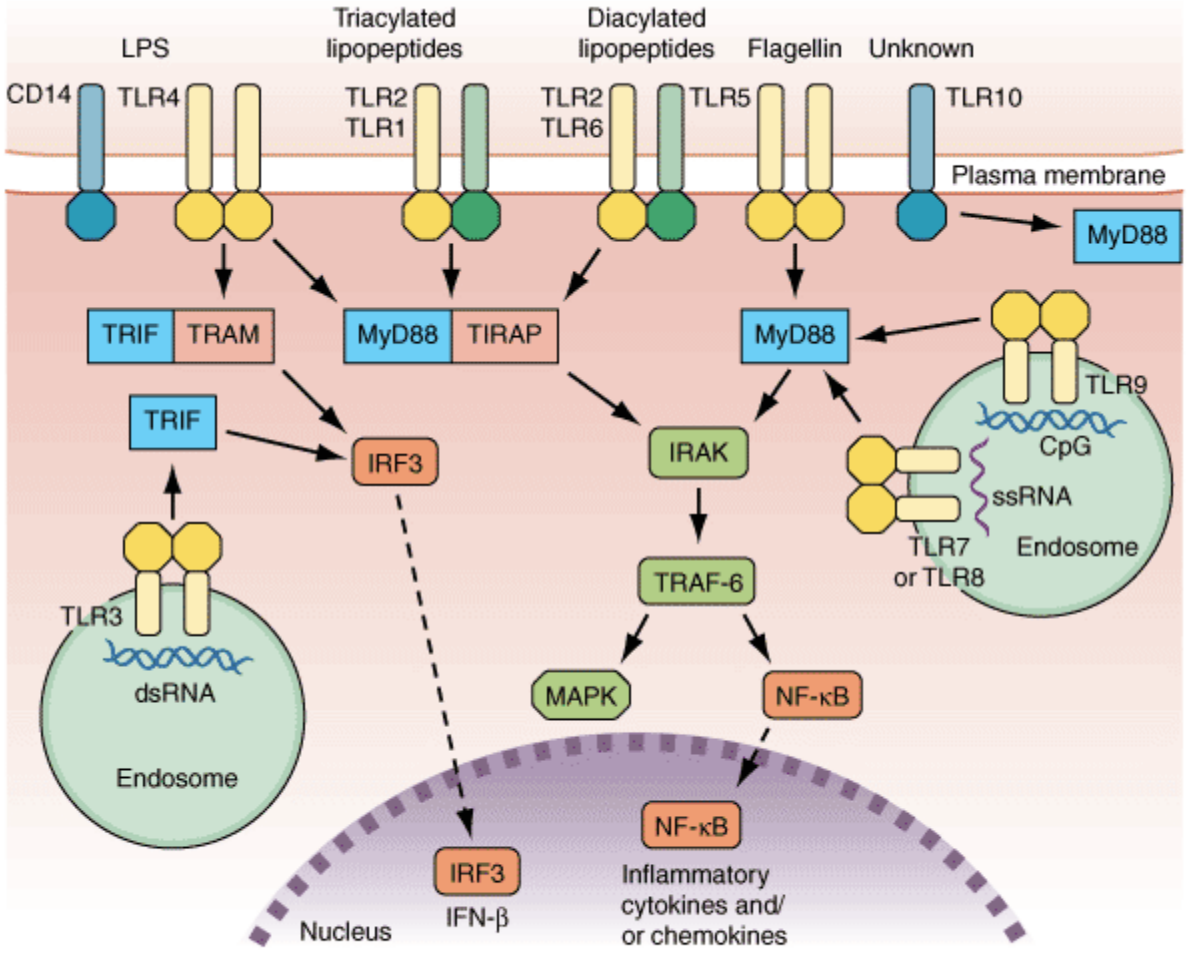
Signaling through the T cell receptor



[Adapted from GA Koretzky, F Abtahaian, MA Silverman, SLP76 and SLP65: Complex regulation of signalling in lymphocytes and beyond. Nature 6(1):67–78, 2006; with permission.]

Fig. 308-7 Accessed 07/01/2010

Pathogen associated molecular patterns



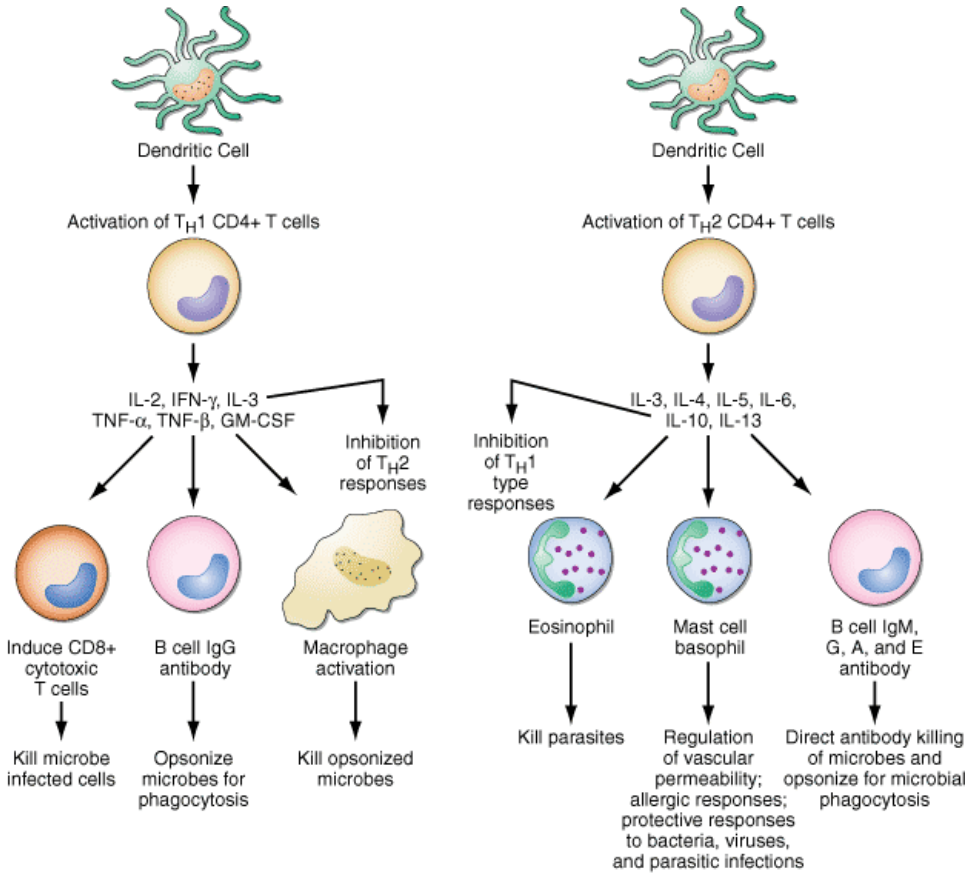
(Adapted from D van Duin, R Medzhitov, AC Shaw, Triggering TLR signaling in vaccination. Trends Immunol 27:49, 2006, with permission.)

Fig. 308-1
Accessed 07/01/2010

T cell

- 95% possess TCR αβ.
- CD4+ recognize MHC class II molecules. (CD4)
- T_{H1}, T_{H2} cytokine patterns.
- CD8+ recognized MHC class I molecules (cell processed peptides).
- IL2R-α chain, IL-10, TGF-β characterize suppressor cells. (CD25)
- 5% possess TCR γδ. (C23)
- Recognize low molecular weight polysaccharides from bacterial biosynthesis pathways.
- CD2 is the earliest pan-T-cell marker.

CD4+ cell classes



Adapted from S Romagnani: CD4 effector cells, in J Gallin, R Snyderman (eds): Inflammation: Basic Principles and Clinical Correlates, 3d ed. Philadelphia, Lippincott Williams & Wilkins, 1999; with permission.)

Fig. 308-2
Accessed 07/01/2010

T_H cell subsets

- T_{H0}
- Secrete IL-3, GM-CSF, IFN-γ, IL-2, IL-4, and IL-5.
- May differentiate into T_{H1} or T_{H2}.
- T_{H1} (Cell Mediated Immunity)
- Delayed type hypersensitivity; development of $CD8^+$ T_C cells.
- Produces IFN-γ, IL-2, TNF-β, IL-3 and GM-CSF.
- Down regulates T_{H2} response via IFN-γ.
- IFN-γ, IL-2 induce

T_H cell subsets

- T_{H2} (Humoral Immunity)
- Help produce antibody. Class switching.
- Promote eosinophil and mast cell production.
- Produce IL-4, IL-5, IL-10, and IL-13.
- Downregulate T_{H1} responses via IL-10.
- The three subsets are differentiated based on cytokine production as well as cell function.
- IL-4 needed for class switching
- IL-5 induces eosinophil production
- IL-13 induces IgE production

T_H cell subsets

- T_{H17} recruits neutrophils
- Macrophages produce IL-17, IL-22, chemokines
- Production induced by TGF-β, IL-1, IL-6, IL-23

T_H cell activation

- T_H cell and antigen presenting cell exchange mutual activation signals:
- IL-4, IFN-γ from the T_H cell activate B cells and macrophages.
- Macrophages secrete IL-1, IL-6 and TNF-α.
- T_H cell express IL-2R and secrete IL-2.
- Co-stimulatory molecules CD28 (T_H) and B7 (B) as well as adhesion molecules are expressed.
- DNA binding proteins produced regulate the cell cycle.

T_H cell activation

- CD3 is the signaling molecule for T cells (analagous to Igα and Igβ on B cells).
- Composed of 5 proteins lodged in the membrane: γε, δε, and either ζζ homodimer or ζη heterodimer.
- Non-covalently attached to T cell receptor.
- Once the T-cell receptor interacts with MHC ligand, phosphorylation of ITAM on CD3 by receptor associated kinases occurs.

T_H cell activation

- When the co-receptor binds to the MHC ligand, Zap-70 binds to phosporylated ITAM and is phosphorylated and activated
- Increase in intracellular Ca^{2+}.
- T cell proliferates, produces IL-2.
- When no further activation is needed, the APC presents a CTLA-4 protein that interacts more strongly with the CD28 protein, stopping the synthesis of interleukin 2.

T_C CELL

- CD8+.
- MHC I restricted.
- Clonal expansion and full effector function requires IL2 (from an activated T_H1 cell).
- CD80/CD86 co-stimulatory molecules from B-cell. Interact with CD28 and CD 152 (CTLA-4) on T-cell.
- Produces perforins and granzymes that damage target cell.
- Secrete IFN-γ and TGF-β. Trigger metabolic pathway that promotes apoptosis.
- Express FAS ligand and target cells with FAS ligand receptors (promote apoptosis).

Natural killer cells

- Large granular lymphocyte.
- CD16 (Fc receptor fo IgG confers ability to lyse IgG coated cells) and CD56 receptors on surface.
- No immunologic memory
- Lack T-cell receptors
- Lack surface IgM and IgD.
- NKG2D receptors activate.

Natural killer cells

- Two classes of inhibitory receptors:
- Express C-type lectin (CD94)
- Immunoglobulin like, recognize self-class MHC I. Are not specific.
- Thymus not required for development.

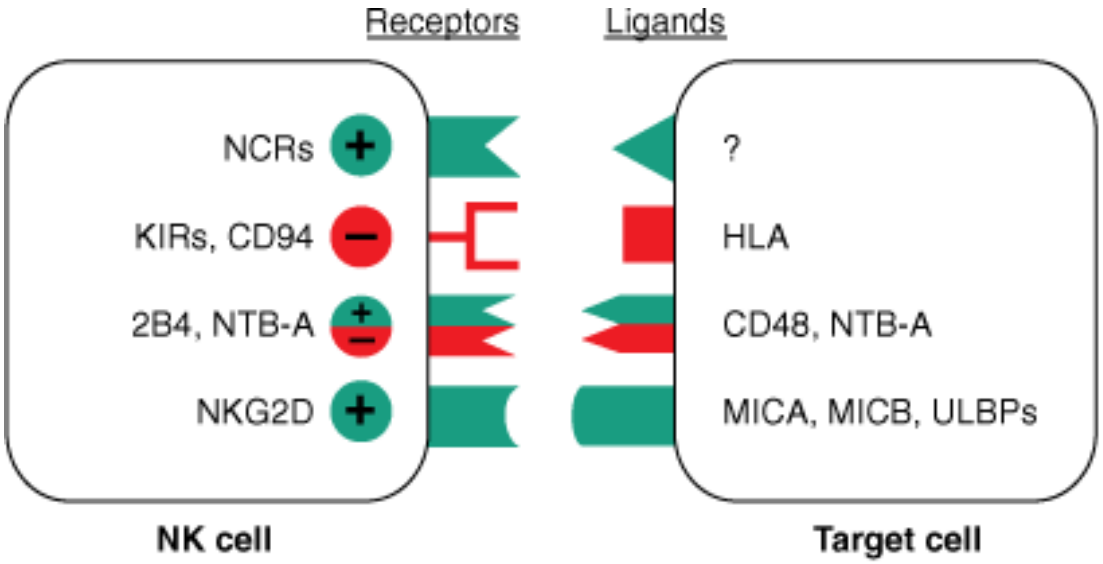
Natural killer cells

- Recognize MHC-α chains (Class I)
- Damage cells that have little MHC I expression.
- Not enhanced by exposure.
- Express FAS ligand and target cells with FAS ligand receptors (promote apoptosis).
- IL12 activates, leads to secretion of IFN-γ.
- IL-2 and IL-15 stimulate proliferation of NK cells.
- IgG antibody enhances cell killing.

NK cell killing

- NK cells kill cells that have little MHC class I expression.
- Produces perforins and granzymes (lead to apoptosis).
- FAS ligand on NK cell interacts with FAS on target cell
- A Ca^{2+} dependent endonuclease is activated, splitting cellular DNA, inducing apoptosis.
- NK cells have receptors that recognize MHC-α chains and inhibit cell function
- NK cells have receptors that recognize lectins, stimulating cell function.

NK cell receptor activation



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Fig. 308-4 Accessed 07/01/2010

NK cells

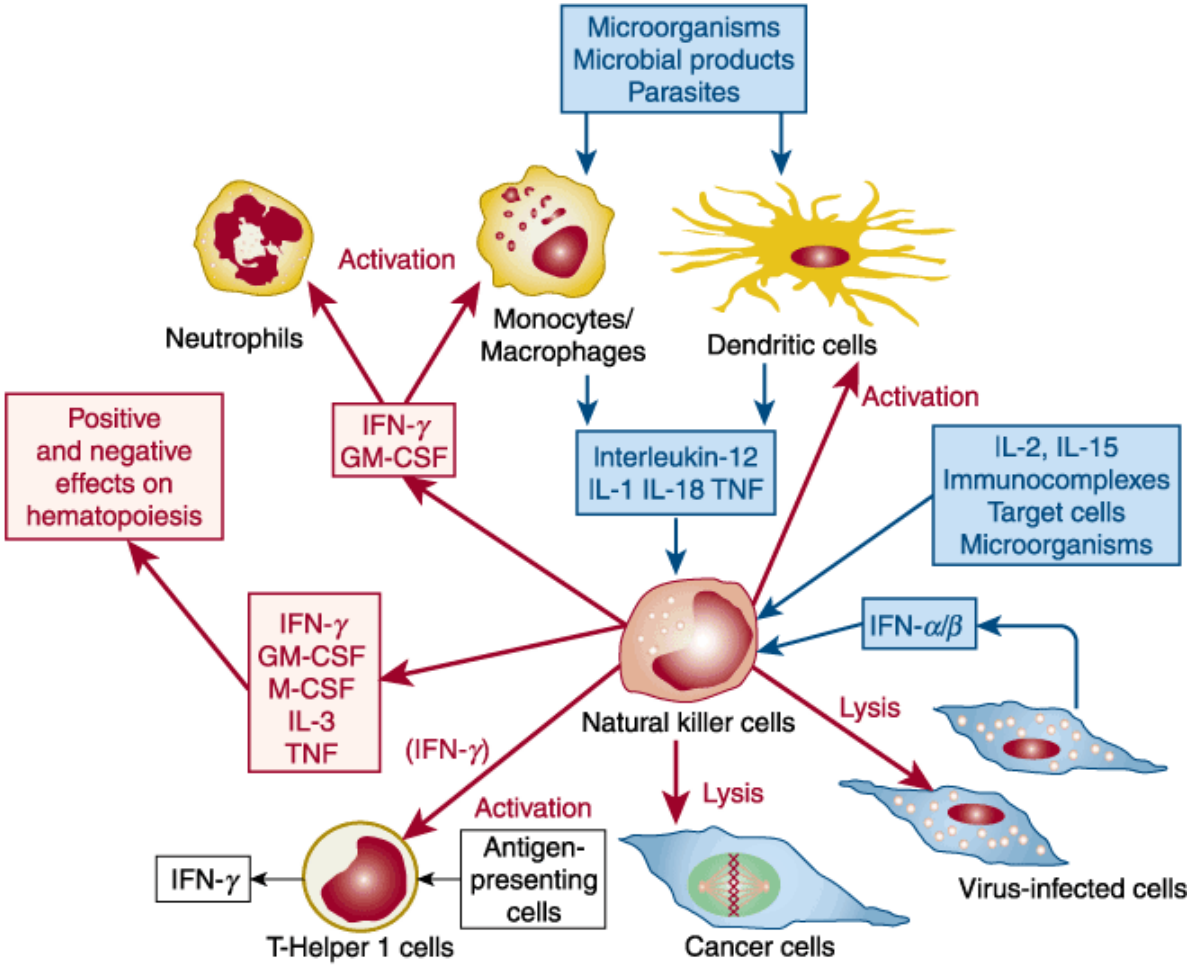


Fig.79-2 Accessed 07/01/2010

T-cell kinetics

- T cells turnover every 19 hours.
- Though recruited by IL2, the lag time to division remains unchanged at 2 hours.
- Recirculation constant between tissues and home sites.
- Unprimed naïve cells: 25CD8+ to 1 CD4+.
- In the spleen, the ratio is 1:1
- In the periphery, 1:2.

T-cell kinetics

- Naïve CD4+ cells have a 118 day half-life in the periphery.
- If in continuous antigen contact in the periphery, the half-life is 7.5 weeks.
- Naïve CD8+ cells have a 154 day half-life in the periphery.
- If in continuous antigen contact, in the periphery, the half-life is 41 days.
- Total mature T cells: 3^{11}.

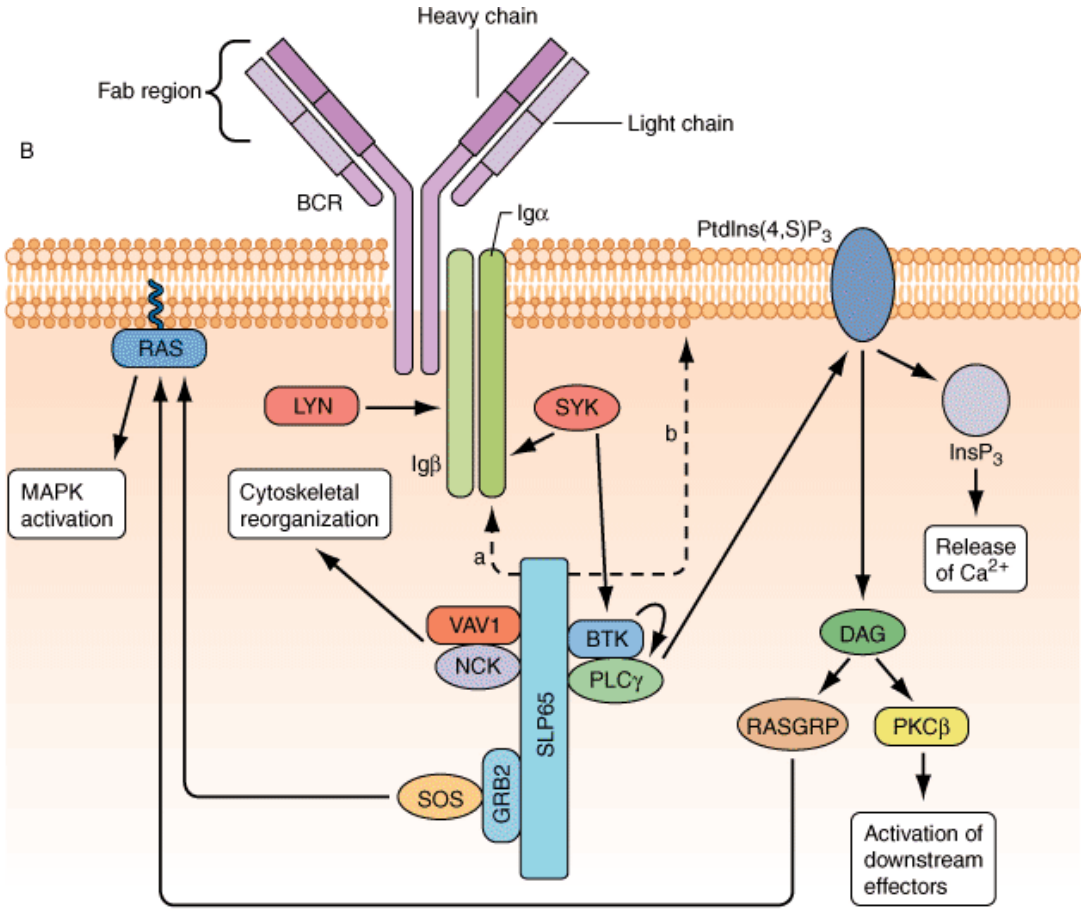
B-cell

- The B-cell receptor for antigen are the heavy and light chain surface immunoglobulins.
- Signal transduction molecules associated with the Ig H chain are Igα (CD79a) and Igβ(CD79b).
- CD21 is the B-cell co-receptor; accepts complement component. CD19, and CD81 are additional transduction molecules.

B-cell

- CD40 interacts with CD154 (CD40 L) on T-cell to induce antibody class switch.
- B7 (CD80 and CD86) are co-stimulatory molecules that interact with CD28 and CD152 (CTLA4) on T-cells.
- CD32 is a low affinity Fc receptor for IgG (Fcγ/RIIb).

B cell receptor activation



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

[Adapted from GA Koretzky, F Abtahian, MA Silverman, SLP76 and SLP65: Complex regulation of signalling in lymphocytes and beyond. Nat Rev Immunol 6(1):67–78, 2006; with permission.]

Fig. 308-8 Accessed 07/01/2010

Plasma cell and memory cell formation

- Mature B-cell in lymph node contacts antigen presented by T-cell and is activated.
- Some B-cells establish a germinal center in a lymphoid follicle , “fine tune” the B-cell receptor, and turn into memory cells.
- They may migrate to the periphery, turn into plasma cells, or remain at site of exposure to await arrival of the same pathogen.

B –cell kinetics

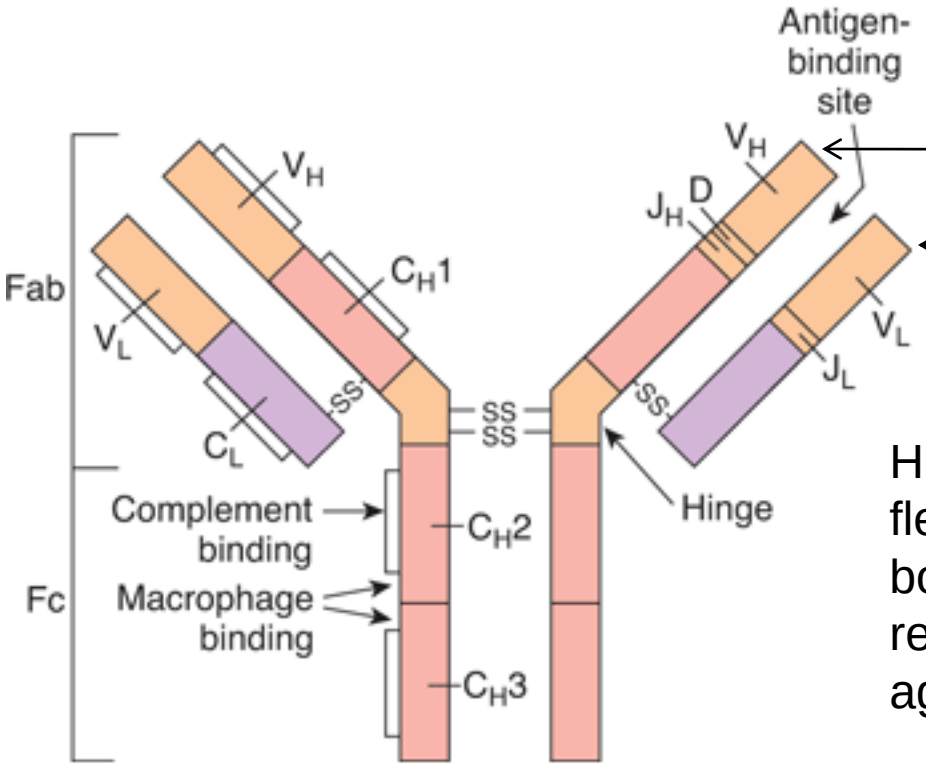
- Naïve cells as well as memory cells have a 5-6 week half-life if not in plasma.
- An unprimed B cell divides monthly; a primed B cell divides every 6 hours.
- A naïve B cell lives 3.5 years.
- A plasma cell lives >1 year even if B-memory cells are present.
- Total mature B cells: $1.3e^{11}$.

Diversity

- Immunoglobulin genes are found in B cells.
- 5 heavy chain classes (μ,δ,γ,α, and ε).
- 2 light chain classes (κ and λ).
- The heavy and light chains are encoded by multiple gene segments.
- Variable (V), Diversity (D) and joining (J) segments compose the variable region of the heavy chain, while only V and J segments are used to generate the light chains.

Immunoglobulin molecule

Fab region binds antibody; Fc region binds complement



Hinge provides flexibility. Carbohydrate bound to complement regions protects against degradation.

Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Fig. 3-10 Accessed 07/01/2010

Allelic exclusion

- B cells are diploid.
- A given B cell, however, will only express the rearranged H chain genes from only 1 chromosome and the L chain from only 1 chromosome.
- This ensures antigenic specificity.

Class switching

- This is the hallmark of humoral immunity.
- Occurs after a B cell has come in contact with antigen.
- Requires T cell help (CD40-CD40L).
- After communication with the T_H cell, the B cell rearranges the constant region.
- Irreversible.
- Does not effect variable domain (same specificity maintained).

Affinity maturation

- In the course of a humoral response, the average affinity of the antibodies produced increases by 100-10,000 fold as a result of point mutations, deletions, or insertions into the V,D, or J region of rearranged immunoglobulin genes, and the selection of high affinity clones.
- Higher affinity antibodies are positively selected; lower affinity antibodies are signaled to die by apoptosis.

Response

- T_{H1} response is driven primarily by IFN-γ and leads to macrophage activation.
- T_{H2} response is driven primarily by IL-4 and IL-5, and leads to the production of IgE and IgG_4 and to the activation of mast cells and eosinophils.
- IFN-γ, IL-4 and TGF-β in conjunction with IL-5 determine which different classes of immunoglobulins are made.

Affinity maturation

- IgM response to invasive bacteria (primary response) occurs after first exposure, requires 5-10 days to generate.
- With second exposure, IgG produced in quantity; requires 1-3 days as B-memory cells primed during first response.
- The IgG response is much more specific (affinity maturation).

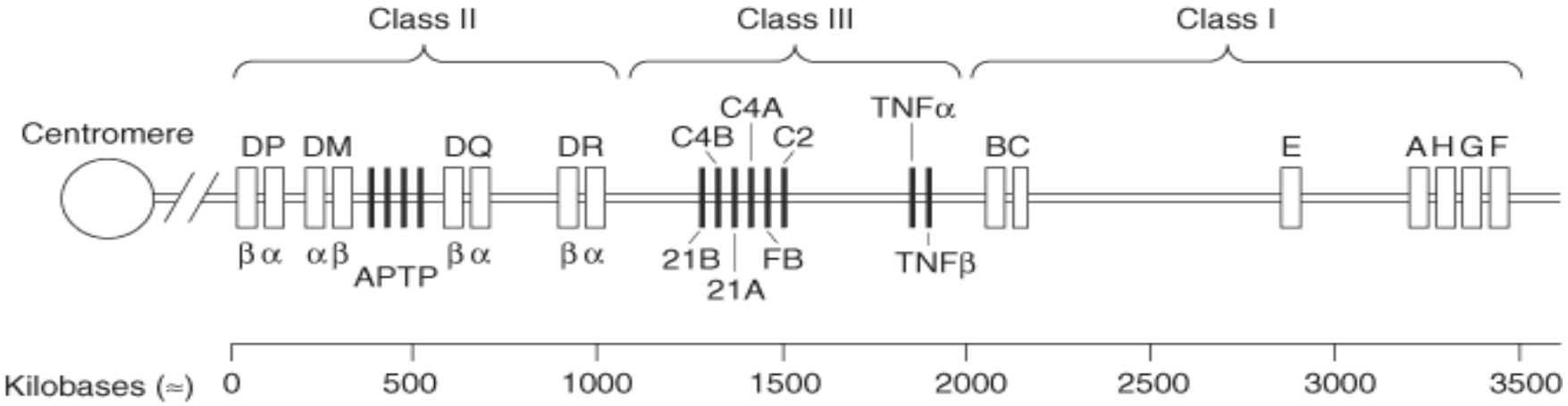
Isotype	Serum Form	Subclasses	Functions
IgG	monomer	IgG1 IgG2,3,4	Produced in secondary response (high affinity); activate classic complement pathway; crosses placenta via Fcγ receptors; opsonizes antigen for uptake by Fcγ receptors; on memory B cell as antigen receptor
IgA	monomer	IgA1 IgA2 is dimer with J chain in secretions	Primary antibody in mucosal secretions; J chain prevents degradation of dimer on mucosal surface; on memory B cell as antigen receptor
IgM	pentamer		Monomer on memory B cell as antigen receptor; secreted as pentamer during primary response (high avidity); activates classic complement pathway; first antibody produced in neonates; on naïve B cell as antigen receptor
IgD	monomer		Membrane bound on mature B cells; on naïve B cell as antigen receptor; never secreted
IgE	monomer		Bound to surface of mast cells and basophils by Fcε receptors (triggers degranulation); on memory B cell as antigen receptor

HLA function

- Bind antigenic peptides and present them to T cells.
- HLA class I (A,B,C)
- Presents antigens to T_H cells.
- Responsible for presentation of exogenous antigens (extracellular bacteria).
- HLA class II (D)
- Present antigens to T_C cells.
- Responsible for presentation of endogenous antigens (viral).

HLA complex

- Regions encoding the three classes of MHC proteins on top. APTP denotes a cluster of genes within the class II region encoding genes related to antigen processing, transport, and presentation. Class III region encodes genes unrelated to class I or class II not involved in antigen presentation (TNF-α and -β, complement factors C2, C4, B, and 21-hydroxylase and others).



Source: Gardner DG, Shoback D: *Greenspan's Basic and Clinical Endocrinology*, 8th Edition: http://www.accessmedicine.com

Fig. 3-2 Accessed 07/01/2010

MHC I

- MHC I are found on all nucleated cells apart from sperm, neurons.
- Each allele of the MHC complex encodes for a single α-chain.
- The α-chain confers antigen specificity.
- There is a hydrophobic trans-membrane domain.
- The antigen binding cleft is formed by the first two α-regions of the three domain α-chain.
- The α-chain is non-covalently bound to β2-microglobulin (coded outside the major histocompatibility complex).

MHC I

- β-2 microglobulin is required for proper folding of the molecule.
- Protein degradation of the antigen intracellularly generates a peptide of 8-10 amino acid length that is bound in the cleft for presentation to CD8 cells

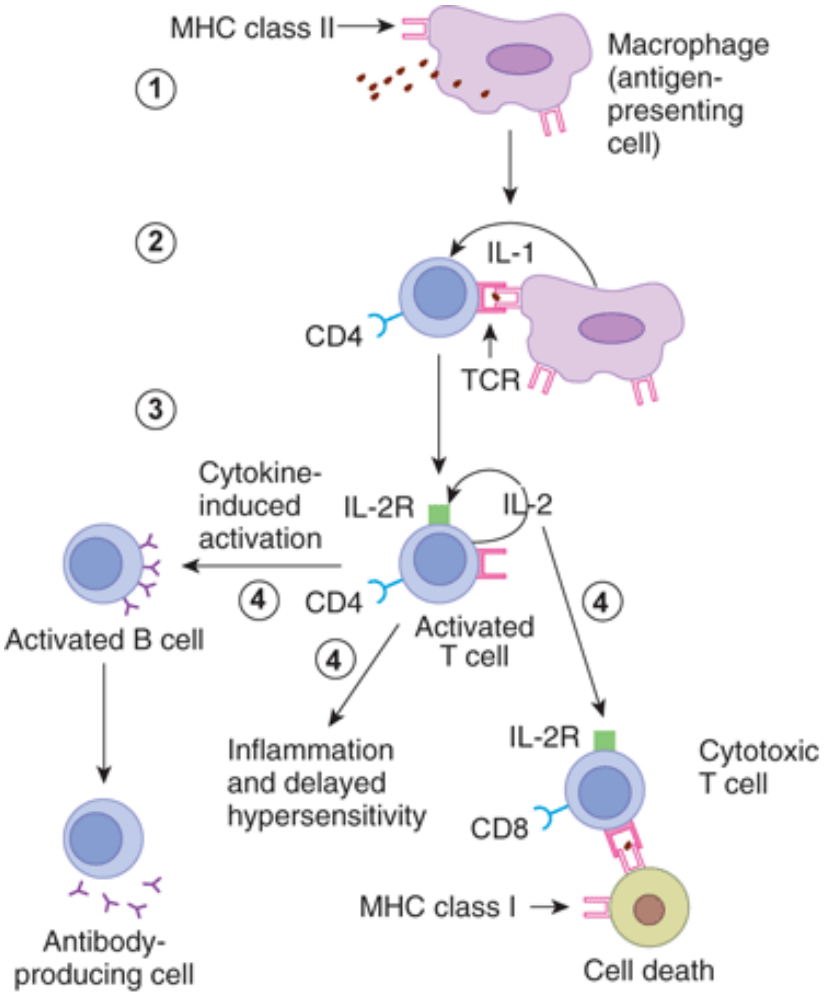
MHC II

- Expressed on antigen presenting cells.
- Hydrophobic transmembrane region.
- Present exogenous antigens to T_H cells.
- Highly polymorphic glycoproteins.
- Composed of α and β chains each of two domains. (both encoded in the major histocompatibility complex).
- Juxtaposition of the α1 and β1 domains not covalently bound at the cell surface form a a cleft that binds peptides of 12-15 amino acids in length.
- Invariant chain prevents endogenous peptides from binding in the peptide binding groove.

Cytokine and viral regulation of MHC expression

- Interferon-γ and tumor necrosis factor can increase the expression of class II MHC by inducing the formation of specific transcription factors that bind to promoters of MHC genes.
- MHC expression is also increased by some viruses (Cytomegalovirus, Hepatitis B virus, and Adenovirus).
- More commonly MHC expression is decreased.
- CMV proteins can bind to β2 microglobulin preventing the proper assembly of class I MHC molecules.

Acquired immunity



Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

(Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF (editors): *Pathophysiology of Disease,* 4th ed. McGraw-Hill, 2003.)

Fig. 3-9 Accessed 07/01/2010

Antigen presenting cells

- Macrophages, B cells, Langerhans, and interdigitating dendritic cells are the only cells that can present antigens to T_H cells.
- Antigen presenting cells express a molecule (B7) that is only constitutively expressed on interdigitating dendritic cells.
- Interdigitating dendritic cells are the only cells that can activate naïve T cells.
- Macrophages can only activate naïve T cells when activated by IFN-γ.
- B cells can activate naïve T cells after contact with antigen.

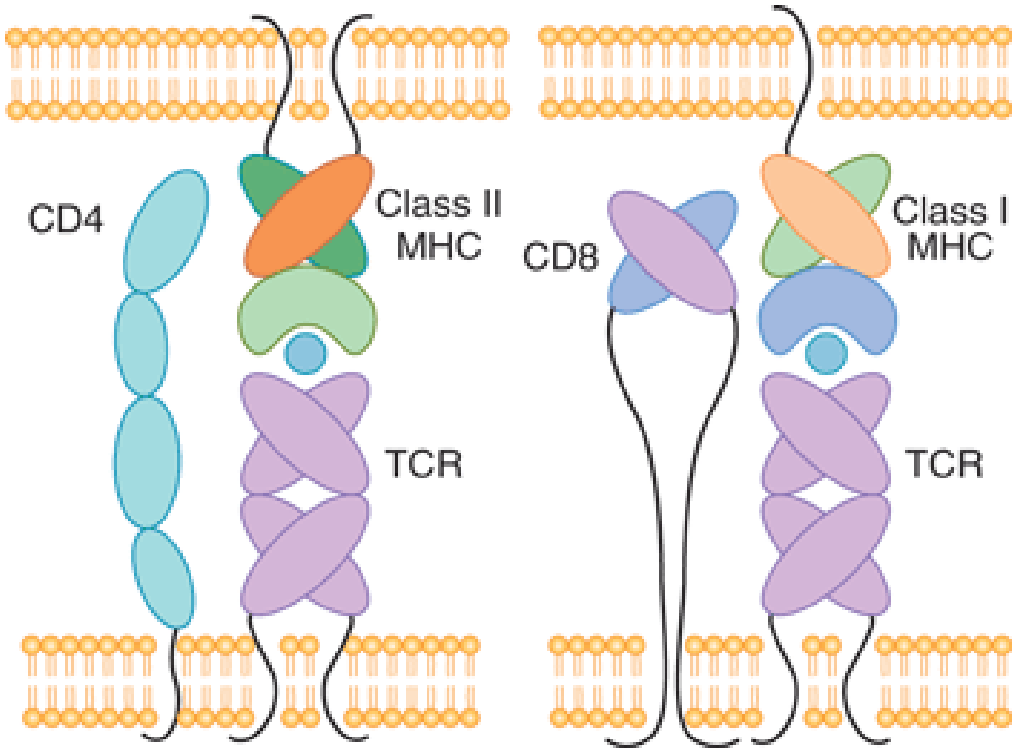
Antigen processing

- Macrophage engulfs antigen (endocytosis).
- In lysozome.
- The release of cytokines IFN-γ, from T_H1 (T_C cells) activates macrophages.
- Antigen cleaved to peptides.
- MHC class II molecule combines with peptide and migrates to cell surface.
- Helper T cell (CD4) T cell receptor recognizes macrophage with antigen because of presence of MHC II molecule with antigen on cell surface.
- Helper T cell secretes IL-2 which recruits B cells.
- Plasma cells secrete IgG, opsonize bacteria.

Antigen processing

- Helper T cells also secrete TNF and IFN to activate cytotoxic T cells (CD8).
- Cytotoxic T cells attack intracellular antigen when MHC type I molecule presents antigen on cell surface.
- Cytotoxic T cell does not recognize intracellular antigen without MHC I molecule.
- Cytotoxic T cell recognizes antigen attached to MHC I inside cell because of presence of MHC I and antigen on cell surface.

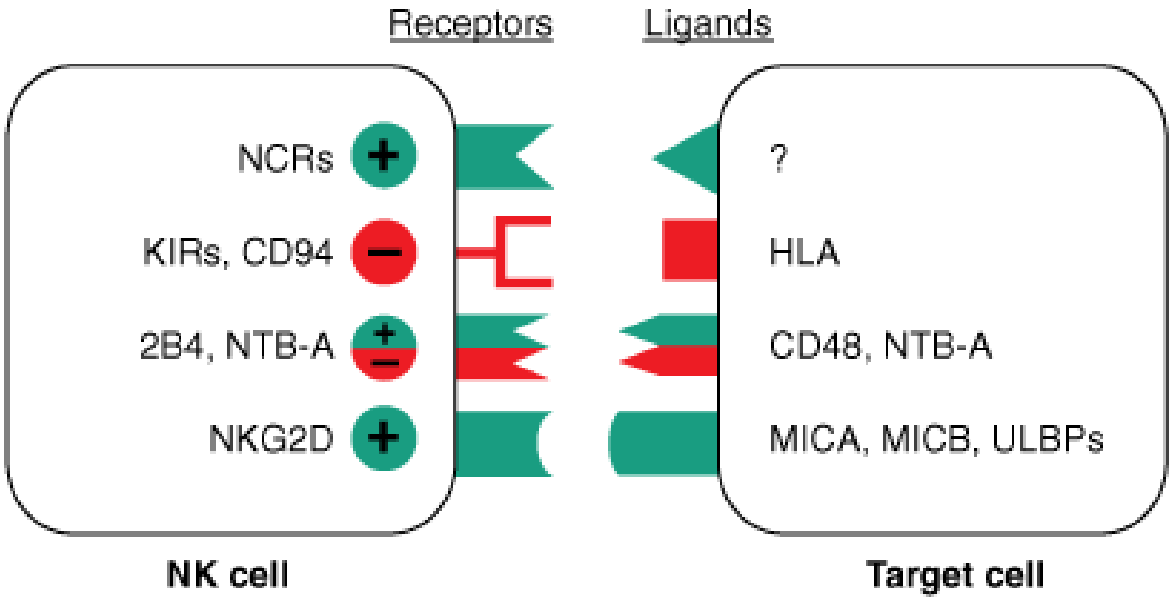
T-cells and MHC receptors



Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

Fig. 3-8 Accessed 07/01/2010

NK cell mediated cytotoxicity



(From Moretta A. et al. Nature Immunol., vol. 3, 2002; with permission.)

Fig. 308-4 Accessed 07/01/2010

Acquired immunity

- An antigen-presenting cell ingests and partially digests an antigen, then presents part of the antigen along with MHC II peptides on the cell surface.
- An "immune synapse" forms with a naive CD4 T cell, which is activated to produce IL-2.
- IL-2 acts in an autocrine fashion to cause the cell to multiply, forming a clone.
- The activated CD4 cell may promote B cell activation and production of plasma cells or it may activate a cytotoxic CD8 cell.
- The CD8 cell can also be activated by forming a synapse with an MCH I antigen-presenting cell.

Passive immunity

- Response to antigen recognition.
- Toll-like receptors are germline encoded and permit brisk, rapid response to foreign antigen.
- No memory, however.
- Antigen-specific receptors undergo recombination during lymphocyte differentiation and maturation in thymus.
- Slow response to foreign antigen.
- However, memory response is rapid and memory remains.

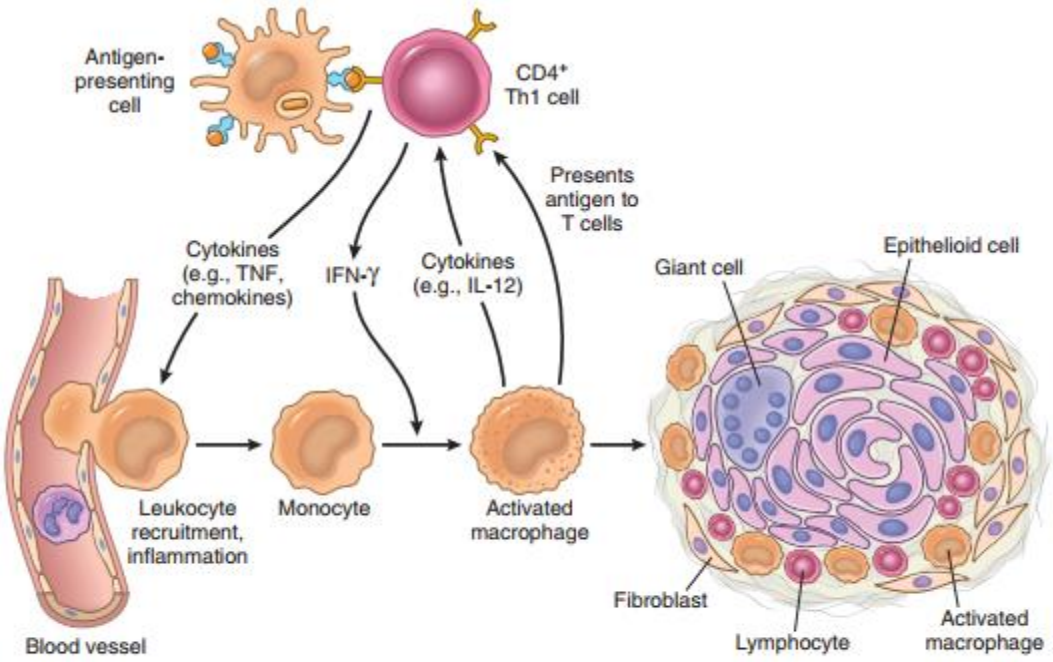


Figure 3.21 Macrophage–lymphocyte interactions in chronic inflammation. Activated T cells produce cytokines that recruit macrophages (tumor necrosis factor *[TNF]*, interleukin-17 *[IL-17]*, chemokines) and others that activate macrophages (interferon-γ *[IFN-]*). Activated macrophages in turn stimulate T cells by presenting antigens and via cytokines such as IL-12. Prolonged reactions involving T cells and macrophages may result in granuloma formation.

CELL DEATH

Indicators of cell damage

- Without neutralization of free radicals during the inflammatory process, mitochondrial dysfunction leads to increased intracellular Ca^{2+} and to activation of ATPases, proteases, phospholipases, and endonucleases.
- Cellular swelling, disaggregated ribosomes, vesicular endoplasmic reticulum, mitochondrial swelling, and cytoskeleton aggregation are noted in damaged cells.

Apoptosis

- Plasma membrane of dead cell is intact.
- Cleared before cytoplasmic components leak out.
- Not inflammatory.
- Normal path in embryogenesis as well when cells have outlived usefulness (senescence).
- Triggered by p53
- Also by increased expression of p16INK4a, a cell cycle inhibitor.

Apoptosis

- Cells have limited ability to replicate
- Regulated by length of the telomere.
- Telomeres are TTAGGG repeats at the linear ends of chromosomes and are not replicated.
- Repeats serve to protect termini from fusion and degradation.

Apoptosis

- Telomerase, an RNA enzyme complex, maintains telomere length by base addition.
- When ends are viewed as broken DNA, are removed.
- Senescence results.

Apoptosis

- Internal pathway:
- BCL family of proteins reside in cytoplasm and in mitochondrial membranes.
- Endoplasmic reticulum stress sensors (BIM, BIN, BAD) respond to protein misfolding.
- Are BH3 only proteins as they possess only a single BCL-2 homology domain instead of the usual four.
- BH3 activation leads to activation of BAX-BAK and blocking of BCL-2 and BCL-X, leading to channel creation at BCL-2 in the mitochondrial membrane, with resultant leakage of cytochrome-c.

Apoptosis

- Death domain receptor independent.
- Mitochondria leaks BCL-2 and BCL-X anti-apoptotoic molecules .
- Activates apoptosis activating factor-1 which leads to production of pro-apoptotic moleules (BAK, BAX, BIM) and increased mitochondrial membrane permeability.

Apoptosis

- External pathway:
- May also be triggered by T-cells as organism defense as FAS ligand (FASL) present on T-cells that recognize self-antigen.
- When FASL binds to FAS, three FAS molecules line up to form FAS associated death domain (FADD), binding inactive caspases (cysteine proteases that cleave aspartic acid residues) 8 and 10, cleaving and initiating nuclear DNA degradation.

Apoptosis

- Tumor necrosis factor receptor 1 (death domain) and associated FAS protein (CD95) trigger apoptosis.
- TNFR1 associated with TRADD, activates transcription factor (NF-κβ) by degrading inhibitor

Apoptosis

- Cytochrome c binds to apoptosis activating factor-1 and forms the apoptosome complex (hexamer).
- Complex activates capsase-9 (with capsase-8, initiates process).
- FLIP blocks pro-capsase-8 as it lacks protease domain, cannot cleave capsase, and, thus, blocks apoptosis.
- Capsases 3, 6 cleave an inhibitor of cytoplasmic DNAase leading to DNA degradation and cytoskeleton breakdown.
- SMAC/DIABLO are other mitochondrial proteins that leak out and bind to and block inhibitors of apoptosis.

Table 3.2 Features of Acute and Chronic Inflammation

Feature	Acute	Chronic
Onset	Fast: minutes or hours	Slow: days
Cellular infiltrate	Mainly neutrophils	Monocytes/macrophages and lymphocytes
Tissue injury, fibrosis	Usually mild and self-limited	Often severe and progressive
Local and systemic signs	Prominent	Less

Morphology of acute inflammation

- Dilatation of small blood vessels
- Accumulation of fluids and leukocytes in the extravascular tissue.
- Serous inflammation is marked by the exudation of leukocyte poor fluid into spaces created by inflammation or into body cavities.
- Fibrinous inflammation is noted where vascular leaks are large and proteins such as fibrinogen pass out of the blood or there has been local procoagulant stimulus

Morphology of acute inflammation

- Purulent inflammation is marked by tissue necrosis and an intense neutrophilic inflammatory infiltrate. Microbes are often present.
- If the collection is in a confined space, it is an abscess.
- An ulcer is a local defect or excavation of the surface of an organ or tissue that is caused by the loss of tissue due to inflammation or necrosis.

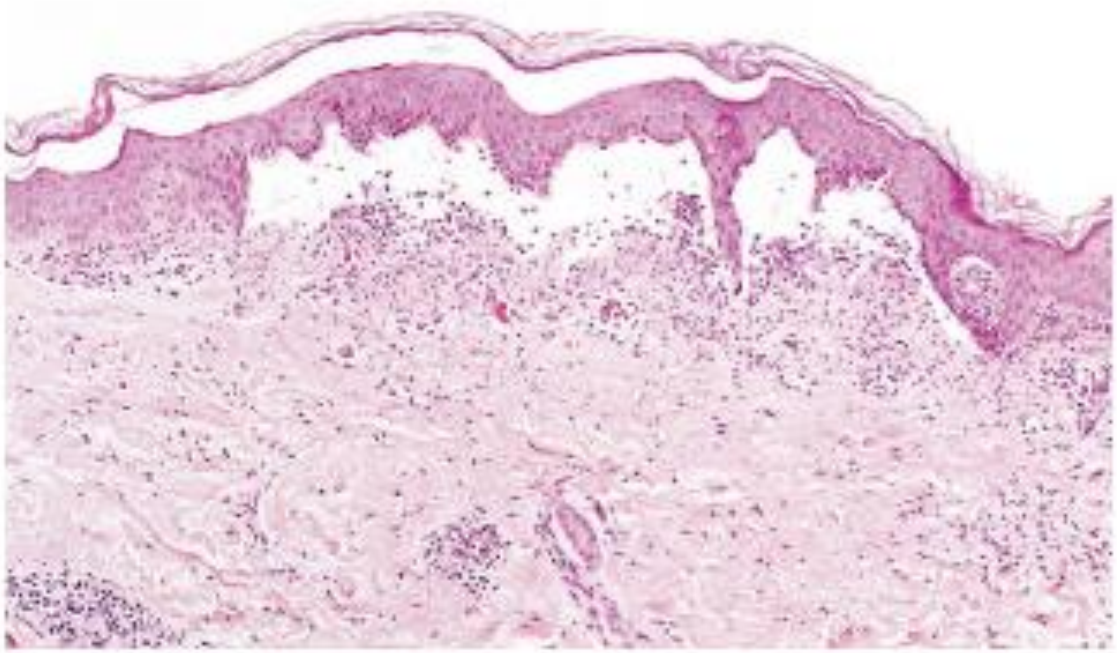


Figure 3.13 Serous inflammation. Low-power view of a cross section of a skin blister showing the epidermis separated from the dermis by a focal collection of serous effusion.

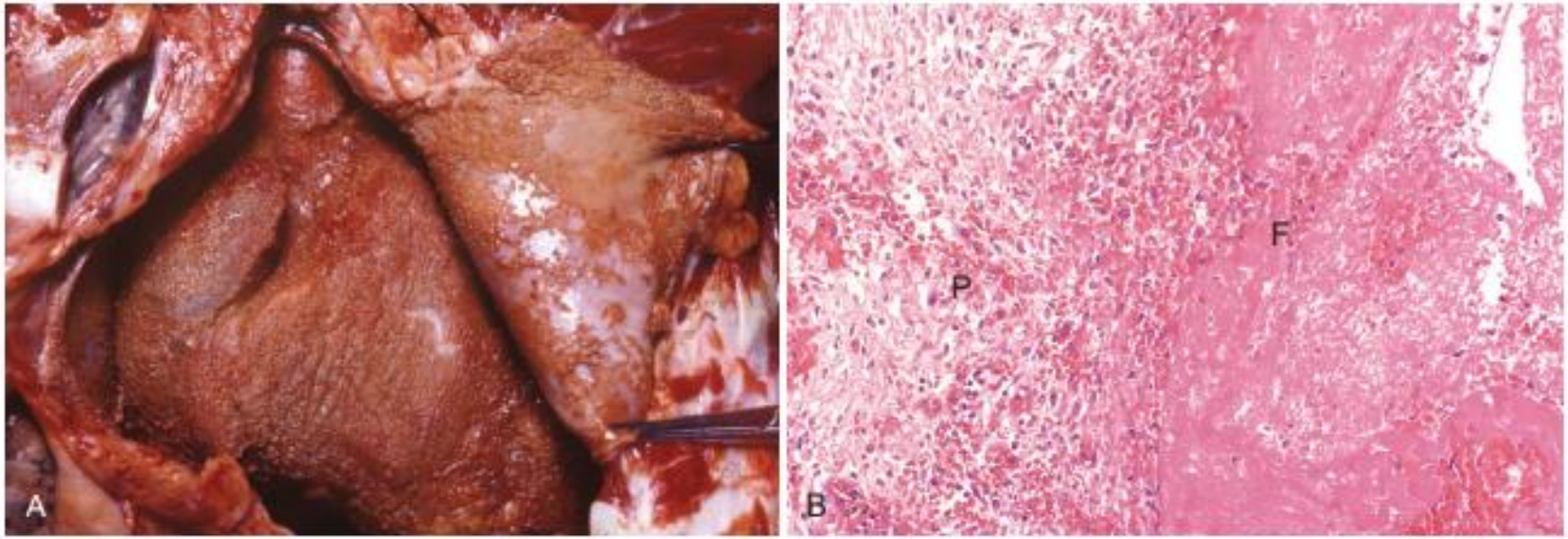


Figure 3.14 Fibrinous pericarditis. (A) Deposits of fibrin on the pericardium. (B) A pink meshwork of fibrin exudate *(F)* overlies the pericardial surface *(P)*.

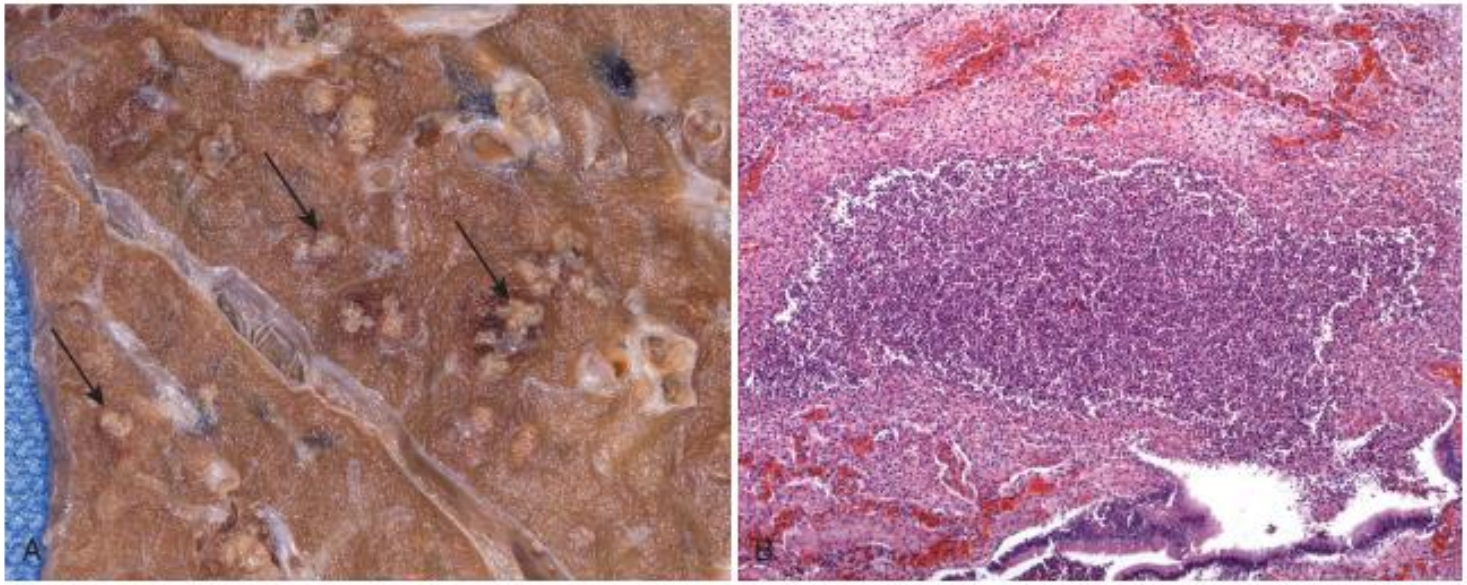


Figure 3.15 Purulent inflammation. (A) Multiple bacterial abscesses *(arrows)* in the lung in a case of bronchopneumonia. (B) The abscess contains neutrophils and cellular debris and is surrounded by congested blood vessels.

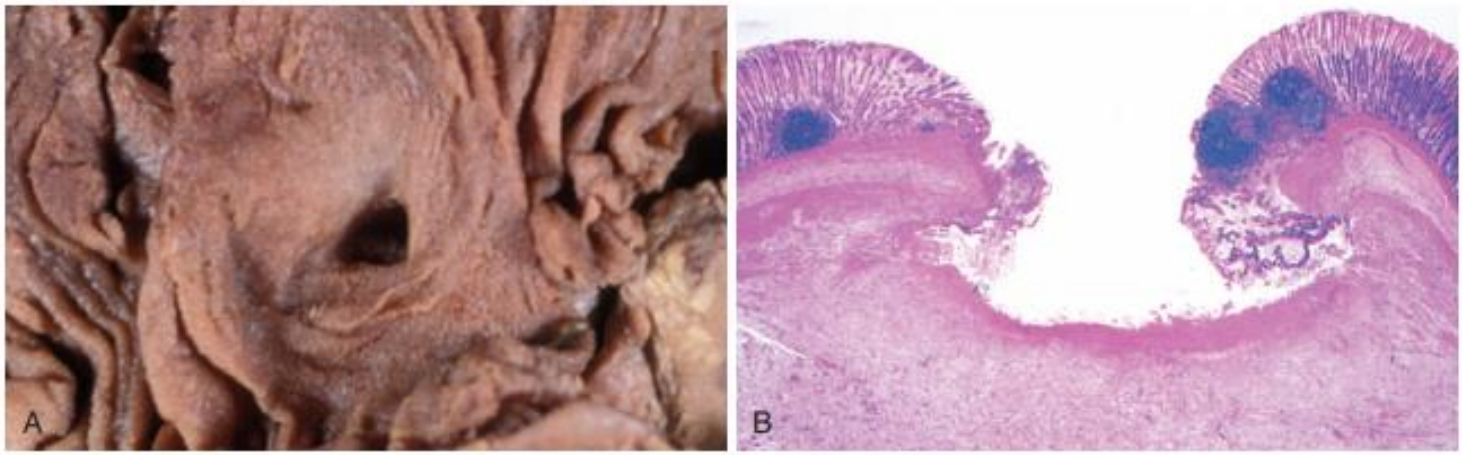


Figure 3.16 The morphology of an ulcer. (A) A chronic duodenal ulcer. (B) Low-power cross-section view of a duodenal ulcer crater with an acute inflammatory exudate in the base.

Morphology of chronic inflammation

- Characterized by:
- Mononuclear cell inflammation
- Tissue destruction
- Attempts at healing with evidence of angiogenesis and fibrosis
- Granulomatous inflammation is characterized by collections of activated macrophages, T cells, and may be associated with necrosis.
- May be initiated by foreign body
- May result from microbe persistence

Chronic inflammation

- If the source of antigen is not eradicated, inflammation persists.
- Activation of macrophages continues.
- Granuloma forms.
- Granulomas wall off infectious material from the body.
- May also be precipitated by the presence of indigestible antigen-antibody complexes, as well as persistent reaction to antigen.
- Alternative macrophage activation induced by IL-4 and IL-13, may lead to fibrosis.
- Insulin growth factor-1 is required for muscle repair.

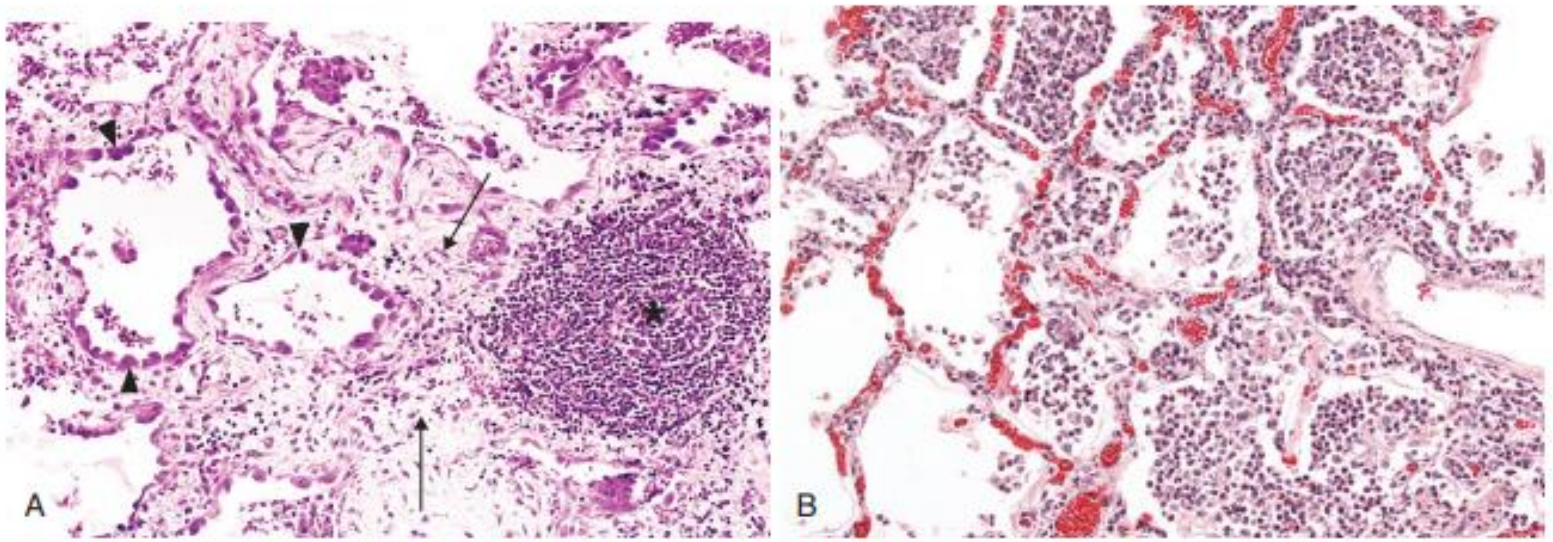


Figure 3.18 (A) Chronic inflammation in the lung, showing all three characteristic histologic features: (1) collection of chronic inflammatory cells *(asterisk)*, (2) destruction of parenchyma (normal alveoli are replaced by spaces lined by cuboidal epithelium) *(arrowheads)*, and (3) replacement by connective tissue (fibrosis) *(arrows)*. (B) In contrast, in acute inflammation of the lung (acute bronchopneumonia), neutrophils fill the alveolar spaces, and blood vessels are congested.

Table 3.9 Examples of Diseases With Granulomatous Inflammation

Disease	Cause	Tissue Reaction
Tuberculosis	*Mycobacterium tuberculosis*	Caseating granuloma (tubercle): focus of activated macrophages (epithelioid cells), rimmed by fibroblasts, lymphocytes, histiocytes, occasional Langhans giant cells; central necrosis with amorphous granular debris; acid-fast bacilli
Leprosy	*Mycobacterium leprae*	Acid-fast bacilli in macrophages; noncaseating granulomas
Syphilis	*Treponema pallidum*	Gumma: microscopic to grossly visible lesion, enclosing wall of histiocytes; plasma cell infiltrate; central cells are necrotic without loss of cellular outline
Cat-scratch disease	Gram-negative bacillus	Rounded or stellate granuloma containing central granular debris and recognizable neutrophils; giant cells uncommon
Sarcoidosis	Unknown etiology	Noncaseating granulomas with abundant activated macrophages
Crohn disease (inflammatory bowel disease)	Immune reaction against intestinal bacteria, possibly self antigens	Occasional noncaseating granulomas in the wall of the intestine, with dense chronic inflammatory infiltrate

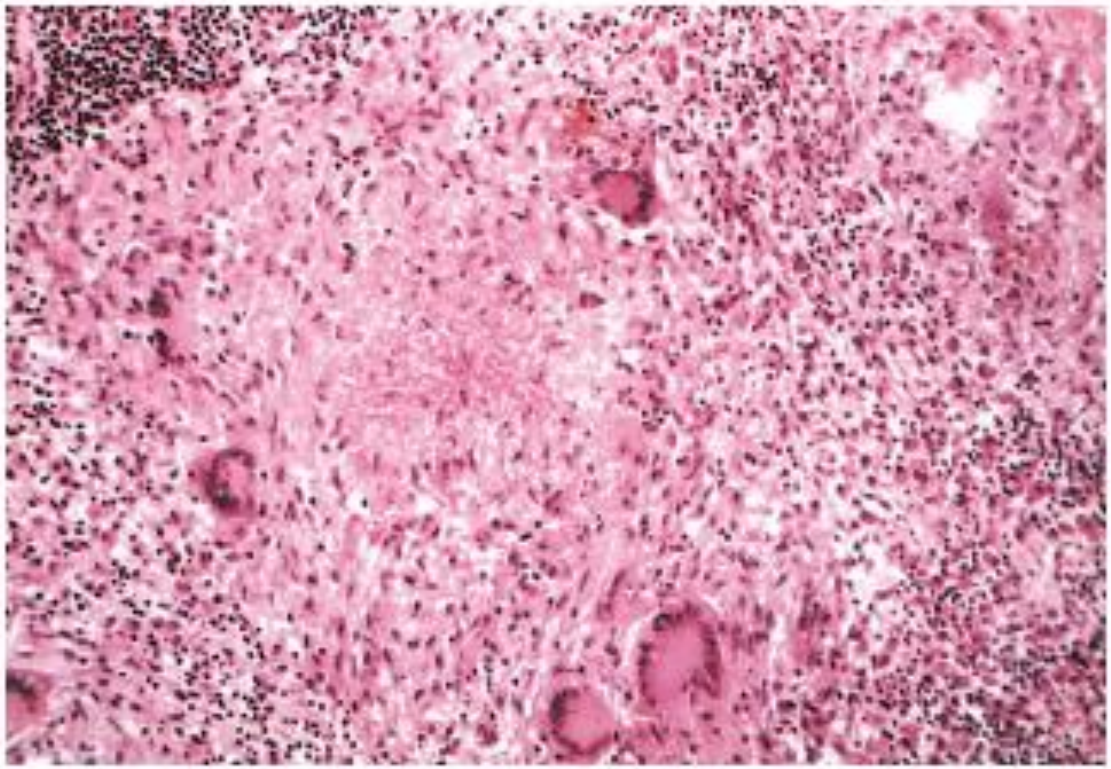


Figure 3.23 Typical tuberculous granuloma showing an area of central necrosis surrounded by multiple Langhans-type giant cells, epithelioid cells, and lymphocytes.

Table 3.1 Diseases Caused by Inflammatory Reactions

Disorders	Cells and Molecules Involved in Injury
Acute	
Acute respiratory distress syndrome	Neutrophils
Asthma	Eosinophils; IgE antibodies
Glomerulonephritis	Antibodies and complement; neutrophils, monocytes
Septic shock	Cytokines
Chronic	
Arthritis	Lymphocytes, macrophages; antibodies?
Asthma	Eosinophils; IgE antibodies
Atherosclerosis	Macrophages; lymphocytes
Pulmonary fibrosis	Macrophages; fibroblasts

IgE, Immunoglobulin E.

REGENERATION AND REPAIR

Regeneration and repair

- Intact extracellular matrix essential for regeneration. Else, healing is by fibrosis.
- Most mature tissues are composed of some combination of stem cells, dividing cells, and terminally differentiated cells, and quiescent cells.
- Stem cells are required for regeneration.
- Alteration in size as a result of functional need (hypertrophy) is response when there are few stem cells to proliferate (hyperplasia).
- Uncommitted (embryonic) stem cells are CD451+.

Regeneration and repair

- Derived from adult stem cells and continuously replaced are:
- Surface epithelium
- Lining mucosa of excretory ducts
- The columnar mucosa of the gastrointestinal tract and the uterus
- Transitional epithelium of the urinary tract
- Hematopoietic tissues

Regeneration and repair

- Generally quiescent are:
- Parenchymal cells of the liver, kidneys, and pancreas
- Fibroblasts
- Smooth muscle cells
- Vascular endothelial cells
- Cardiac muscle does not regenerate
- Lymphocytes

Regeneration and repair

- Adult stem cells are found in niches composed of mesenchymal, endothelial, and other cell types related to niche site.
- Adult stem cells are able to differentiate into diverse lineages.
- Mesenchymal stem cells are found about pericytes.
- In the liver, stem cells are found in the canals of Herring.

Regeneration and repair

- In the skin, stem cells are found at the bulge area of the hair follicle, in sebaceous glands, and scattered individually in the epidermis (interfollicular cells).
- The epidermis turns over every 4 weeks.
- The small intestine villus contains cells from multiple crypts (monoclonal structures derived from a single stem cell).
- Every 3-5 days the crypts regenerate.
- WNT stimulates; BMP inhibits proliferation.

Regeneration and repair

- Stem cells are also present at the junction of the cornea and the conjuctiva.
- Adult stem cells generate transit amplifying cells (lose self-perpetuative capacity) and give rise to progenitor cells.
- Neurogenesis is limited (subventricular zone and dentate gyrus of the hippocampus)
- Skeletal muscle regenerates from satellite cell differentiation (satellite cells are attached to endomysial sheath).
- Activated by Notch signalling and upregulation of δ-like ligands (Dll).

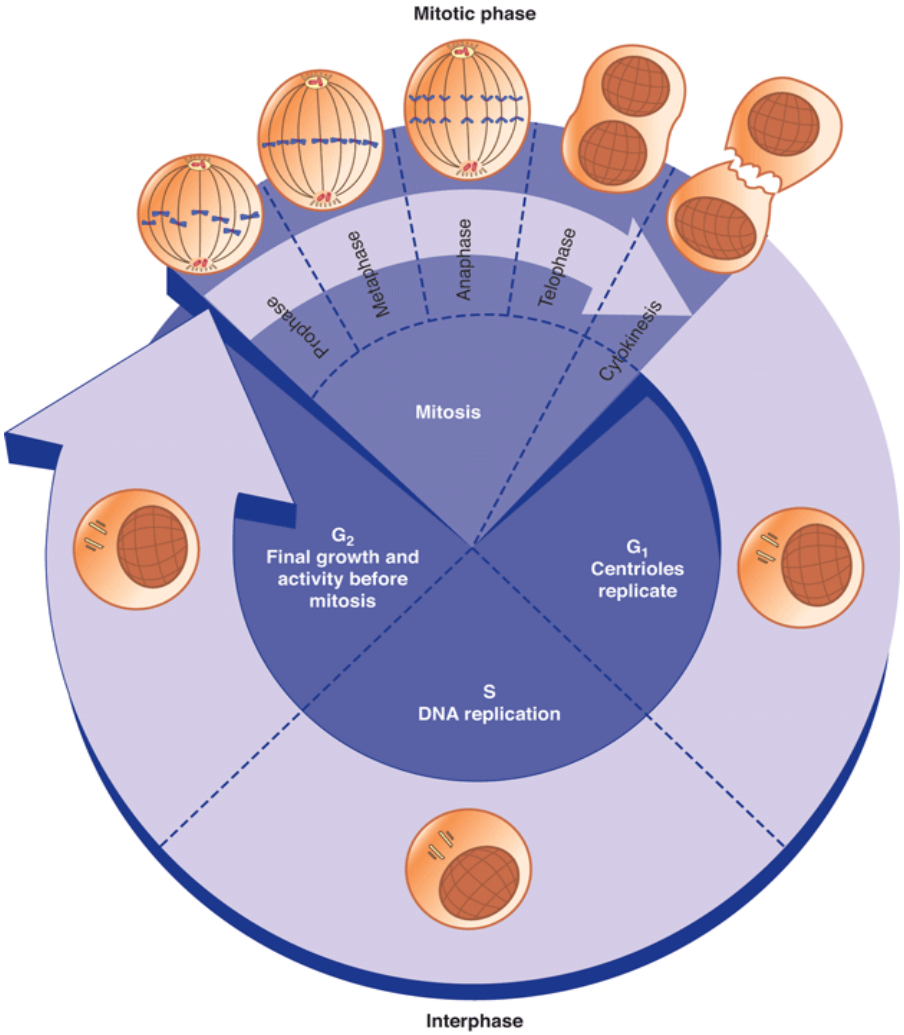
Pluripotent stem cells

- Embryonic stem cells comprise blastocyst. Are pluripotent.
- OCT 3/4, SOX2, NANOG, and LIN28 genes must be activated in adult cells to induce pluripotency.
- NANOG is a Homeobox protein that prevents differentiation.
- Stem cell division gives rise to one daughter retaining a self-renewing capacity and a second daughter that enters the differentiation pathway (obligatory asymmetric reproduction).
- Stem cell population is maintained by balance between renewal and differentiation (Stochastic differentiation).

Cell cycle

CDK1 kinase produced constitutively. Cyclin E produced in G1 phase; cyclin A, S phase. Cyclin B synthesized at G_2. When posphorylated, controls G_2/M transition.

CDK inhibitors include p16, p18, p19, p21, p27, p57.



Source: Barrett KE, Barman SM, Boitano S, Brooks H: *Ganong's Review of Medical Physiology, 23rd Edition*: http://www.accessmedicine.com

TNF-α and IL-6 are involved in G_0/G_1 transition.

G_1/S and G_2/M are critical steps where accuracy of synthesized DNA is determined.

Fig. 1-13 Accessed 07/01/2010

Growth factors

- Epithelial growth factor (EGF) and transforming growth factor (TGF-α) are principal stimulants to epithelial cell proliferation.
- Share the same receptor that also binds to heparin binding epithelial growth factor and to amphiregulin.
- EGF is mitogenic for keratinocytes and fibroblasts.
- EGF derived from platelets and from macrophages.

Growth factors

- TGF- α is found in body secretions
- Produced by keratinocytes, macrophages, T-cells.
- Binds to EGF receptor, triggering tyrosine kinase signaling.
- Stimulates replication of hepatocytes, most epithelial cells.
- Heparin binding epithelial growth factor stimulates keratinocytes.
- It is derived from macrophages and mesenchymal cells.

Growth factors

- Hepatocyte growth factor (fibroblast scatter factor) is mitogenic for hepatocytes, epithelial cells, endothelial cells.
- Derived from mesenchymal cells.
- Serine protease needed to convert to active form.
- C-MET is the hepatocyte growth factor receptor.
- Vascular endothelia growth factor (VEGF), platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) are needed to promote angiogenesis and repair.

Growth factors

- VEGF (isoforms A-D as well as placental growth factor) promotes angiogenesis.
- Signals through three tyrosine kinase receptors (VEGFR 1-3).
- VEGF-C and VEGF-D bind to VEGFR-3 and act on lymphatic endothelial cells.
- VEGF transcription regulated by hypoxia induced factor.

Growth factors

- PDGF produced by activated macrophages, endothelial cells, and smooth muscle cells.
- Stored in platelet granules and released upon platelet activation.
- PDGF (AA, AB, BB, CC, DD isoforms).
- CC, DD isoforms require proteolytic cleavage.
- Bind to PDGFR α and β ligands.
- B and C isoforms involved in hepatocyte regeneration and wound contraction.

Growth factors

- PDGF stimulates production of matrix metalloproteinases, fibronectin, and hyaluronate; activates and is mitogenic for smooth muscle cells.
- Chemotactic for neutrophils, macrophages, fibroblasts, and smooth muscle cells.

Growth factors

- FGFs are either acidic (FGF-1, for example) or basic (FGF-2, for example).
- Signals through four tyrosine kinase receptors (FGF 1-4)
- FGF-1 binds to all receptors.
- FGF-2 and FGF-7 (keratinocyte growth factor) contribute to re-epithelialization of skin wounds.
- FGF-2 induces new vessel formation.
- Also involved in keratinocyte proliferation.
- Released FGFs associate with heparan sulfate in the extracellular matrix.

Growth factors

- TGF-β (three isoforms) is part of the superfamily that includes BMP (bone morphogenetic protein) and avidin.
- TGF-β1 is a homodimeric protein produced in platelets, endothelial cells, macrophages, lymphocytes.
- Precursor protein proteolytically cleaved to active form.
- Binds to types I and II cell receptor with serine/threonine kinase activity and triggers phosphorylation of SMAD (1, 2, 3, 5, 8).
- These form heterodimers with SMAD 4 and enter nucleus to regulate cell transcription.

Growth factors

- TGF-β inhibits epithelial cell proliferation by increasing the expression of CIP/KIP and INK4/ARF families of cell cycle inhibitors.
- Inhibit leukocyte proliferation as well.
- Stimulates fibroblast chemotaxis.
- Production of fibronectin, collagen, and proteoglycans stimulated.
- Decreases matrix proteases and increases protease inhibitor activities.
- Also enhances development of T_{H17} cells; stimulates production of IgA in gut mucosa.
- TNF and IL-6 are involved in the initiation of liver regeneration.

Growth factors

- Also enhances development of T_{H17} cells
- Stimulates production of IgA in gut mucosa.
- TNF and IL-6 are involved in the initiation of liver regeneration.

TGF-β1 subfamily

- GDF11 (also known as BMP11) reverses age-related dysfunction in skeletal (and cardiac) muscle as well as vascular and neurogenic function in the brain.
- Target are cells with stem cell activity
- Gene is at 12q32
- HDAC3 promotes
- PSKS5 activity required for maturation
- Involved in anterior/posterior axial skeleton development
- GDF8 inhibits muscle growth
- GDF15 is better prognostic factor than is CA19-9 in pancreatic cancer

Extracellular matrix

- Collagen forms extracellular framework.
- Requires, Vitamin C.
- Polypeptide characterized by repeating Gly-X-Y structure where X,Y cannot be cysteine or tryptophan.
- Contains four hydroxyproline and hydroxylysine.
- Prolyl residues to hydroxyproline in the Y position stabilize triple helix. Cross-linking provides strength.
- Types I, II, III, V, and IX are fibrillar collagens and have >1000 uninterrupted residues.
- Found in extracellular fibrillar structures.

Extracellular matrix

- Type I collagens found in hard and soft tissues
- Type II, cartilage, vitreous, and intervertebral disk
- Type III, hollow organs, soft tissues
- Type V, soft tissues, blood vessels
- Type IX, cartilage, vitreous.
- Type IV collagens have interrupted triple helical domains and form sheets rather than fibrils.
- With laminin, form basement membranes.

Extracellular matrix

- Type VII is also interrupted and forms anchoring fibrils between epithelial and mesenchymal structures such as epidermis and dermis.
- Type VI is found in myofibrils of muscles.
- Type XI collagen is found in cartilage, intervertebral disks
- Type XVII, transmembrane collagen in epidermal cells (bulla)
- Types XV, XVIII form endostatin and are found in endothelial cells.

Extracellular matrix

- Elastin is major core molecule in those tissues requiring elasticity.
- Elastin core surrounded by fibrillin (glycoprotein) scaffolding.
- Associates with itself or with other components of the extracellular matrix.
- Serves as scaffolding.
- Influences the effects of TGF-β.

Extracellular matrix

- Cell adhesion molecules classified into four main families:
- Immunoglobulin family
- Cadherins
- Integrins
- Selectins.
- Function as trans-membrane receptors and are sometimes stored in the cytoplasm.
- Homotypic or heterotypic (same or different cell type) binding.

Extracellular matrix

- Fibronectin.
- Two glycoprotein chains held together by disulfide bonds.
- Adhesion molecule that is cell surface receptor.
- Plasma form binds to fibrin.
- Cell form forms fibrillar aggregates.

Extracellular matrix

- Laminin is a glycoprotein that binds matrix and cell surface receptor.
- Mediates cell attachment to connective tissue.
- Polymers of laminin and collagen Type IV form tightly bound networks (found in basement membranes).
- Cadherin and integrin link cell surface with cytoskeleton.
- Ca^{2+} dependent adherence protein (cadherin) participate in homotypic binding.
- Connect plasma membranes of adjacent cells (zonula adherens, adjacent cells).

Extracellular matrix

- Linkage of cadherins with cytoskeleton through β-catenin
- Links cadherin with α-catenin which then connects with actin, completing cytoskelton connection.
- Free β-catenin acts independently of cadherin in WNT signaling pathway.
- Integrins provide mechanism for transmission of mechanical force as well as activate intracellular signal transduction pathways.

Extracellular matrix

- Ligand binding to integrins cause clustering of the receptors in the cell membrane with formation of focal adhesion complexes.
- Talin, vinculin, pasillin co-locate with integrins at these complexes.
- Activate MAPK, PKC, PI3K pathways and cross-talk with growth factor pathways.

Extracellular matrix

- Other adhesion molecules include:
- SPARC (osteonectin), an angiogenesis inhibitor
- Thrombospondins, angiogenesis inhibitors
- Osteopontin, regulates calcification and mediates leukocyte migration
- Temascin, multimeric proteins involved in morphogenesis and cell adhesion.

Extracellular matrix

- Glycosaminoglycans are long repeating disaccharide polymers.
- Linked to a core protein as are produced in Golgi and endoplasmic reticulum
- Heparin sulfate, chondroiton/dermatan sulfate, keratin sulfate, and hyaluron as distinct families.
- Hyaluronan is produced at plasma membrane.
- Hyaluron binds water; permits resistance to compression
- Low molecular weight hyaluronates produced through hyaluronidases (from endothelial cells).
- These bind to CD44 receptor on leukocytes, recruit same (inflammatory response)

HEALING

Healing and repair

- Refers to the restoration of tissue architecture and function after an injury.
- Repair is a term used for parenchymal and connective tissues
- Healing is a term used for surface epithelia
- Regeneration by proliferation of uninjured cells AND maturation of tissue stem cells
- Driven by growth factors
- Dependent upon integrity of extracellular matrix
- Deposition of connective tissue to form a scar
- If it occurs in a space occupied by an inflammatory exudate, it is referred to as "organization."

Healing

- The ability of tissues to repair themselves is determined in part by their intrinsic proliferative capacity.
- Continuously dividing (labile) tissues
- Proliferation of mature cells and maturation of stem cells
- Hematopoietic cells in the marrow
- Surface epithelium (usually squamous)
- Cuboidal epithelium of ducts draining exocrine organs
- Columnar epithelium
- Uterus and fallopian tubes
- Transitional epithelium (urinary tract)

Healing

- Stable tissues
- G_0 (resting phase)
- Divide in response to injury or diminished cell mass
- Parenchyma of solid tissues
- Endothelium
- Smooth muscle
- Fibroblasts
- Limited capacity to regenerate (liver as an exception)

Healing

- Permanent tissues
- Terminally differentiated
- Majority of neurons
- Limited replication in some areas of adult brain
- Majority of cardiac muscle cells
- Normal stem cell replacement is 1%/year
- Skeletal muscle
- Satellite cells attached to endomysial sheath provide limited regenerative capacity

Role of stem cells

- Tissue stem cells are multipotent or lineage committed (not pluripotent or embryonal)
- OCT 3/4, SOX2, NANOG, and LIN28 genes must be activated in adult cells to induce pluripotency
- Examples of niches where found:
- Subventricular zone and dentate gyrus of brain (neural)
- Bulge region of hair follicle
- Limbus of cornea
- Crypt cells of the small intestine
- Canals of Herring in the liver
- Marrow and fat (mesenchymal)

Role of stem cells

- Characterized by self-renewal
- One daughter cell enters differentiation pathway; the second remains undifferentiated and maintains stem cell pool (Asymmetric division; stochastic differentiation)
- WNT pathway stimulates; respond to tension
- BMP pathway inhibits (GDF11)
- Activation is growth factor driven
- Growth factors activate signaling pathways

Role of stem cells

- WNT and YAP/TAZ have also been linked to the pathophysiology of fibrosis.
- Tissue mechanics have been shown to orchestrate WNT-dependent human embryonic stem cell differentiation.

Growth factors

- Methods of delivery
- Endocrine
- Factors secreted into the blood and conveyed to a distant site
- Insulin-like factors I and II are examples of this.
- Paracrine
- The secretory product of one cell acts directly on another.
- Cell to cell distances are therefore important for paracrine factors to act efficiently.
- PDGF, TGF-α, and TGF-β

Growth factors

- Autocrine
- Perform self-regulatory functions.
- TGF-β is an example of an autocrine factor whose secretory cell is both the source and target of its activity.

Growth factor	Source	Wound healing related functions
PDGF	Platelets, macrophages, endothelial cells, injured cells	Chemotaxis, fibroblast proliferation, collagenase production
TGF-β	Macrophages, platelets, neutrophils, lymphocytes, fibroblasts, epithelial and endothelial cells, injured cells	Fibroblast proliferation, chemotaxis, collagen metabolism
EGF	Plasma, platelets, macrophages, epithelial cells	Epithelial cell proliferation, granulation tissue formation
TGF-α	Activated macrophages, platelets, epithelial cells, injured cells	Epithelial cell proliferation, granulation tissue formation
KGF	Fibroblasts	Epithelial cell proliferation
IL-1	Macrophages	Fibroblast proliferation
FGF	Pituitary, macrophages, fibroblasts, endothelial cells	Fibroblast proliferation, matrix deposition, wound contraction angiogenesis
TNF-α	Macrophages, T lymphocytes	Fibroblast proliferation
IGF-1	Plasma, liver, fibroblasts	Synthesis of sulfated proteoglycans and collagen, fibroblast proliferation
IFNs	Lymphocytes, fibroblasts	Inhibition of fibroblast proliferation and collagen synthesis

doi: 10.4103/0975-7406.100294

Growth factors

- EGF and TGF-α affect mesenchymal and epithelial cells
- Derived from transmembrane proteins and act through a paracrine mechanism on the EGF receptor.
- Wound macrophages contain significant amounts of TGF-α that add to the significance of this cell in the initial tissue response to injury.
- The main effect of TGF-α and EGF appears to be on granulation tissue development, with epidermal re-growth and modulation of angiogenesis being unique features of TGF-α activity

Growth factors

- PDGF is stored in the α granules of platelets and released after activation of the platelets at sites of tissue injury.
- Macrophages, endothelial cells, vascular smooth muscle cells, and fibroblasts also express PDGF
- Chemotactic for fibroblasts and monocytes as well as mitogenic for fibroblasts and vascular smooth muscle cells.
- The production of PDGF at wound sites is not constant, and increases in concentration have been correlated with augmented connective tissue formation.

Growth factors

- PDGF acts through paracrine and autocrine mechanisms that enable it to function not only as a stimulator of cellular activity but also in a homeostatic feedback fashion.
- Stimulate tyrosine kinase, and increase transcription of C-FOS and C-MYC
- Its effects on angiogenesis are indirect

Growth factors

- TGF-β
- Identified in a wide variety of cells including platelets, macrophages, bone cells, monocytes, lymphocytes, and platelets.
- Has both mitogenic effects as well as regulatory functions over matrix production.
- Chemotactic for fibroblasts and monocytes and is capable of stimulating or inhibiting fibroblasts.
- Stimulate angiogenesis through the induction of IL-1 and TNF-α production in macrophages

Growth factors

- Basic fibroblast growth factors (bFGFs)
- High affinity for heparin.
- Release of occurs due to the action of the enzyme heparinase found in platelets
- Found in many different tissues including endothelial cells, macrophages, and fibroblasts
- Chemotactic toward endothelial cells and leukocytes as well as mitogenic for endothelial cells.
- Initiates release of basement membrane degrading enzymes that liberate endothelial cells before new vessel formation.

Growth factors

- Derived from M1 monocytes and macrophages:
- Epidermal growth factor (EGF)
- Mitogenic for keratinocytes and fibroblasts
- Also produced by platelets
- Transforming growth factor (TGF-α)
- Stimulates cell proliferation (especially, hepatocytes)
- Also produced by keratinocytes and T cells.

Growth factors

- Derived from M1 monocytes and macrophages:
- Fibroblast growth factors (FGF)
- FGF1 (acidic) and FGF2 (basic)
- Chemotactic and mitogenic for fibroblasts
- Stimulates angiogenesis (FGF2)
- Stimulates extracellular matrix (ECM) protein synthesis
- Produced also by endothelial cells and mast cells

Growth factors

- Derived from M2 monocytes:
- Transforming growth factor (TGF-β)
- Chemotactic for fibroblasts
- Stimulates ECM protein synthesis
- Suppresses acute inflammation
- Inhibits epithelial cell proliferation
- Increase expression of INK4/ARF
- Increase expression of $p21^{cip1}$ and $p27^{kip1}$
- Also produced by keratinocytes

Growth factors

- Produced from mesenchymal cells:
- Vascular endothelial growth factor (VEGF)
- Isoforms A-D involved in angiogenesis
- Simulates proliferation of endothelial cells
- Increases vascular permeability
- Produced from fibroblasts:
- Keratinocyte growth factor (KGF or FGF7)
- Stimulate keratinocyte migration
- Stimulate keratinocyte proliferation
- Stimulate keratinocyte differentiation

Growth factors

- Derived from fibroblasts and stromal cells in the liver (mesenchymal origin)
- Hepatocyte growth factor (HGF, scattering factor)
- Enhances proliferation of hepatocytes (and other epithelial cells)
- Enhances proliferation of endothelial cells
- Increases cell motility

Growth factors

- Produced from Platelets:
- Platelet derived growth factor (PDGF)
- Chemotactic for:
- Neutrophils
- Macrophages
- Fibroblasts
- Smooth muscle cells
- Activates and stimulates proliferation of:
- Fibroblasts
- Endothelial cells
- Stimulates ECM protein synthesis

Growth factors

- PDGF also produced by:
- Macrophages
- Endothelial cells
- Smooth muscle cells
- Keratinocytes.
- Isoforms CC and DD require cleavage
- Isoforms BB and CC involved in hepatocyte regeneration and in wound contraction
- Stored in platelet granules

Cytokines

- IL-1 is chemotactic for neutrophils
- Produced by endothelial cells
- Stimulates metalloproteinases
- Stimulates production and release of acute phase reactants from liver
- The cytokine IL-4 sustains growth factor production

Cytokines

CYTOKINE	FUNCTION
IL-1	First cytokine to be released. Fever Neutrophil chemotaxis IgA secretion on mucosal surfaces
IL-2	Maintains T cell production and activity
IL-4	Shifts to Th2 cells Stimulate B cells Class switches to IgG and IgE Maintains growth factor production
IL-5	Class switch to IgA Eosinophil chemotaxis
IL-6	Stimulates production of acute phase reactants
IL-8	Neutrophil chemotaxis
IL-10	Inhibits Th1 cells
IL-12	Shifts to Th1 cells Activates NK cells
IFN-γ	Activates NK cells

WOUND REPAIR

Repair

- Tissue repair begins with 24 hours of injury.
- Fibroblast emigration as well as induction of fibroblast and endothelial cell proliferation.
- By 3-5 days of tissue repair a specialized type of tissue appears (granulation tissue) characterized by fibroblast proliferation and new, thin walled delicate capillaries.
- (Angiogenesis allows formation of capillary sprout, migration of endothelial cells followed by epithelial cells, with maturation of endothelial cells into pericytes or smooth muscle cells as capillaries are organized).

Repair

- Vasodilatation in response to nitrous oxide, VEGF production.
- Increased permeability results.
- Proteolytic degradation of the basement membrane by metalloproteinases follows and endothelial cell to cell contact is disrupted by plasminogen activator.
- Migration of endothelial cells occurs to the stimulus. Proliferate behind the leading front.
- Later, pericytes and vascular smooth muscle cells are recruited, and form the mature vessel.

Repair

- Endothelial precursor cells can be recruited from marrow.
- Express C-KIT ,VEGFR-2 (KDR), and vascular endothelial-cadherin.
- Increase in ischemic conditions.

Repair

- Notch signaling pathway promotes proper vessel branching and prevents excessive angiogenesis by decreasing responsiveness to VEGF.
- Notch consists of 5 ligands (jagged 1,2; DLL 1, 3, 4) and four transmembrane receptors (Notch 1-4) with EGF-like repeats on the extracellular surface.
- DLL-4 endothelial cell specific; only expressed in arteries and capillaries.

Repair

- VEGF induces Notch-4 in tip cell while Notch-1 and Notch-4 expressed in lagging stalk cells of new vessel (that maintain contact with the existing vessel).
- Interaction leads to extracellular proteolytic cleavage of the receptor by ADAM and intracellularly by γ-secretase, releasing the Notch intracellular domain that translocates to the nucleus and dampens responsiveness to VEGF.

Repair

- Angiopoietin-1 interacts with the endothelial cell receptor TIE2 to recruit pericytes and (with PGDF) vascular smooth muscle cells.
- This interaction mediates vessel maturation and maintains endothelial quiescence.
- Endothelial cells are more responsive to VEGF.
- TIE2 mutation leads to venous malformation

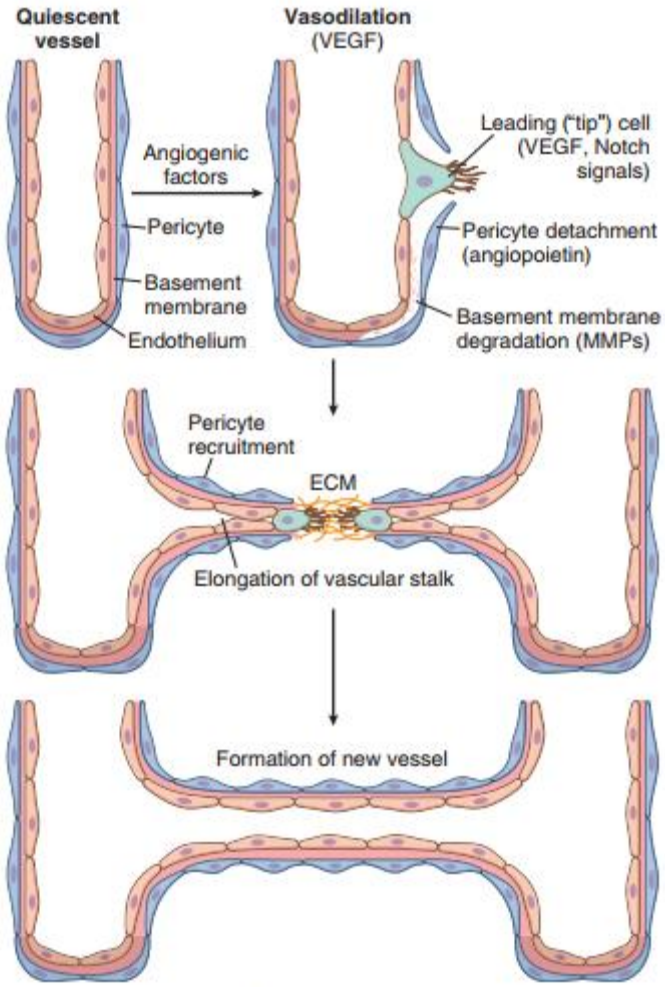


Figure 3.28 Angiogenesis. In tissue repair, angiogenesis occurs mainly by sprouting of new vessels. The steps in the process and the major signals involved are illustrated. The newly formed vessel joins up with other vessels (not shown) to form the new vascular bed. *ECM*, Extracellular matrix; *MMPs*, matrix metalloproteinases; *VEGF*, vascular endothelial growth factor.

Repair

- Wound healing involves formation of blood clot on surface.
- Fibrin, fibronectin serve as scaffolding for neutrophils (present in first 24 hours).
- Fibroblasts proliferate in first 24-72 hours.
- Granulation tissue fills wound area by 5-7 days.
- Neovascularization maximal.
- Macrophages replace neutrophils at 48-96 hours.

Repair

- Vertically oriented collagen fibers present at wound margin.
- Re-epithelialization from wound margin begins at 24-48 hours, depositing basement membrane components.
- Provisional matrix contains type III collagen, later replaced by type IV

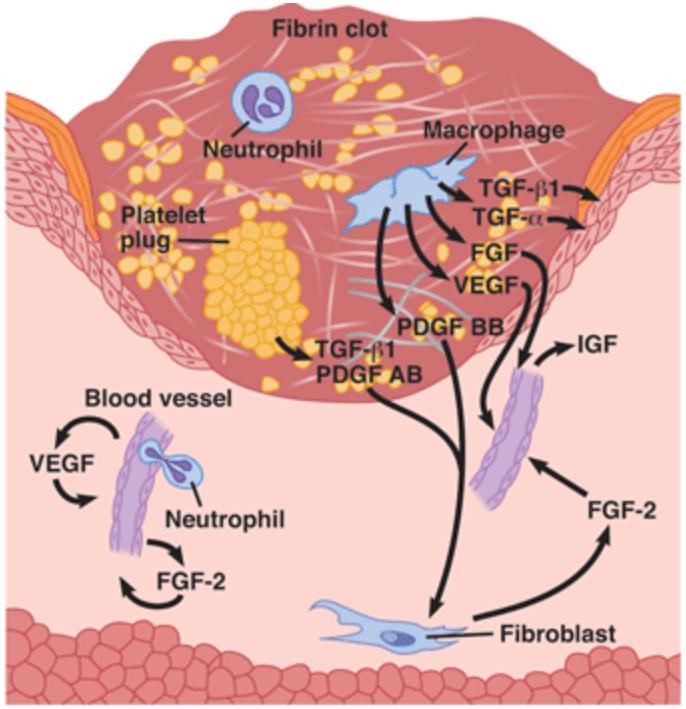
Repair

- At 7 days, tensile strength of fibrous tissue network is 10% of pre-injury state.
- At 3 weeks, reaches a maximum of 70-80%.
- Wound contraction results from myofibroblast network formed at edge of wound; express smooth muscle α-actin and vimentin.

Repair

- Remodeling to restore tissue function occurs with degradation of collagen and other extracellular matrix components by Zinc dependent metallo-proteinases (collagenases, gelatinases, stromelysins).
- ADAM-17 cleaves membrane bound TNF and TGF-α.
- Scar consists of fibroblasts and dense collagen.

Wound repair (3 days)



(Modified from Singer AJ, Clark RAF: Cutaneous wound healing. *N Engl J Med* 1999;341:738.)

Fig. 3-13 Accessed 07/01/2010

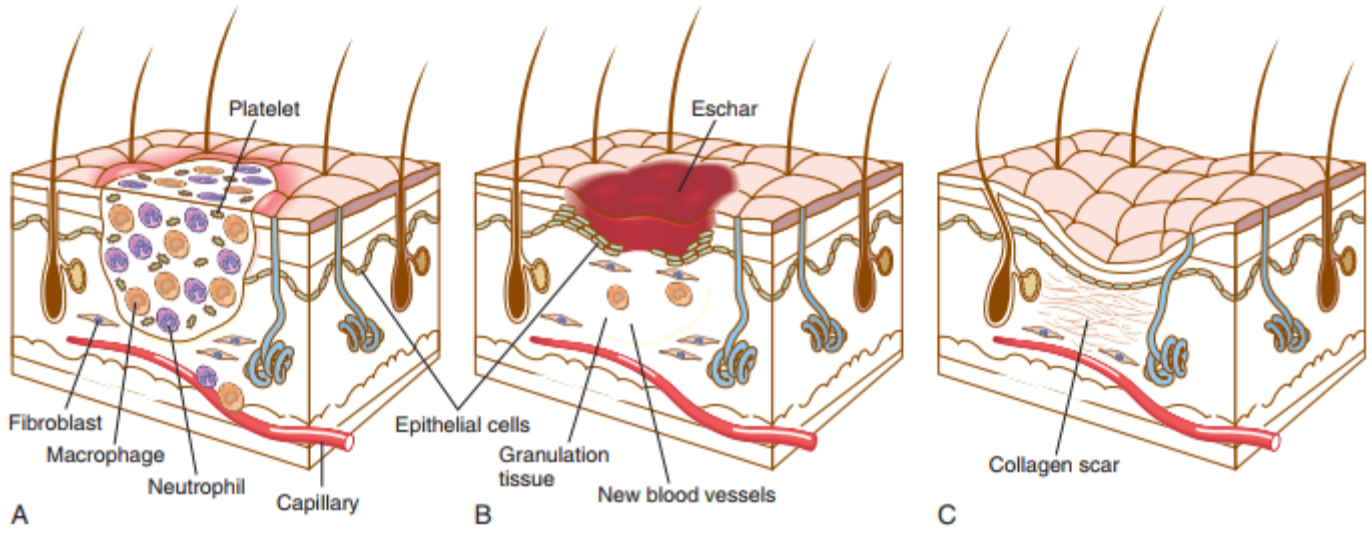


Figure 3.26 Steps in repair by scar formation: wound healing in the skin. (A) Inflammation. (B) Proliferation of epithelial cells; formation of granulation tissue by vessel growth and proliferating fibroblasts. (C) Remodeling to produce the fibrous scar.

Burns

- In the adult, the head and arms each constitute 9% of body surface area; the trunk, back, and legs each constitute 18% of body surface area; genitalia, 1%.
- In the child, the arms each constitute 9% of body surface area; the head, trunk, and back each constitute 18% of body surface area; each leg, 14%; genitalia, 1%.
- Inhalation injury is associated with facial burns. Laryngospasm may be the presenting problem or hoarseness, coughing, and wheezing may be present. May require airway support. The development of ARDS may follow.

Burn management

- Carboxyhemoglobin level may provide severity of injury.
- Provide supplemental Oxygen.
- Immunize all patients who have not had a tetanus booster in the past year.
- Hospitalize if second degree burns involve >20% of body surface area or third degree burns involve >10% of body surface area.
- Hospitalize if any burns involve >10% of body surface area in children or the elderly.
- Hospitalize if any burns involving the hands, face, feet, or perineum.
- Hospitalize if any burns involving inhalation injury.

Burn management

- Stop the burning process.
- Resuscitate using isotonic solutions without glucose. 4ml/kg per % body surface area burned is target amount with half infused in the first 8 hours.
- Colloid (albumin) may be given after 24 hours as capillary integrity returns to normal. 0.3ml/kg per % body surface area burned is target amount.
- Debride.
- Apply sulfamyon (painful, may cause acidosis) or silvadene (less penetrating but not painful) antibiotic cream.
- Skin graft.

Burn management

- Circumferential burn may require incision of burned area to prevent vascular compression.
- Myoglobinuria is a possibility.
- Will have positive urine dipstick test for blood.
- Send urine for definitive myoglobin determination if dipstick positive.
- Hydrate aggressively and institute mannitol diuresis to increase myoglobin excretion.
- Failure to do so may lead to renal failure.

Burn management

- Administer proton pump inhibitor to prevent Curling's ulcer (gastric or duodenum).
- Patients with electrical burns are hospitalized.
- There may be brainstem injury or cardiac disturbances as a result of injury.

Repair by connective tissue deposition

- If repair cannot be accomplished by regeneration alone, it occurs by replacement of injured cells with connective tissue.
- Patches rather than restores tissue
- Scar usually refers to wound healing
- Granulation tissue intermediate remodeling phase
- β-catenin upregulation facilitates epithelial-mesenchymal transformation

Steps in granulation tissue formation

- Angiogenesis
- VEGF driven
- Vasodilatation in response to NO
- Hypoxia inducible factor-1 (HIF-1) promotes new vessel formation, stimulates cell survival pathways, and enhances anerobic glycolysis
- Production stimulated by VEGF A
- VEGFR 2 critical receptor
- Increased vascular permeability associated with post-inflammation edema

Steps in granulation tissue formation

- Angiogenesis 1 and 2 growth factors lead to:
- Pericyte separation from vessel wall and
- Basement membrane break down, lead to sprout
- Endothelial cells migrate to sprout
- Remodel sprout into capillary tube

Steps in granulation tissue formation

- FGF2
- Stimulates endothelial cell proliferation
- Promotes fibroblast migration to site
- Promotes macrophage migration to site
- Activated macrophages clear dead tissue
- Produce growth factors and cytokines
- Stimulate fibrous tissue deposition
- PDGF recruits smooth muscle cells for vessels
- Notch signaling regulates branching and spacing of new vessels (through cross-talk with VEGF)

Steps in granulation tissue formation

- TGF-β stabilizes process
- Suppresses endothelial cell proliferation and migration
- Suppresses leukocyte migration
- Stimulates collagen and fibronectin synthesis
- Intact laminin necessary for repair
- With Type IV collagen, form basement membranes
- Enhances production of ECM proteins
- Integrins and scaffolding proteins
- Post-transcriptional activation of latent TGF-β regulates levels

Steps in granulation tissue formation

- Metalloproteinases degrade ECM to permit remodeling and extension of vascular tube
- Degrade Type III collagen for replacement by Type I
- Zinc dependent removal of Type III collagen
- Activity tightly controlled
- Produced as inactive precursors (zymogens)
- Activated by proteases at site (e.g., plasmin)
- Inhibited by specific tissue inhibitors (TIMPs)
- Produced by mesenchymal cells

Steps in granulation tissue formation

- Type III collagen is the initial collagen produced
- Tropocollagen is a triple helix of α-chains
- It is the structural unit of collagen
- Hydroxylation of proline and lysine requires ascorbic acid
- Hydroxyproline residues stabilize the triple helix
- Hydroxylysine residues oxidized to aldehyde and covalently cross-link the triple helix
- Copper is a co-factor with lysine oxidase
- Cross-linking increases tensile strength
- Type I collagen in skin, bone, tendons has greatest tensile strength

Steps in granulation tissue formation

- ADAMs (disintegrin and metalloproteinase)
- Plasma membrane anchored
- Cleave and release extracellular domains of cell-associated cytokines and growth factors

- Maturation and reorganization of connective tissue is end result of repair (scar)

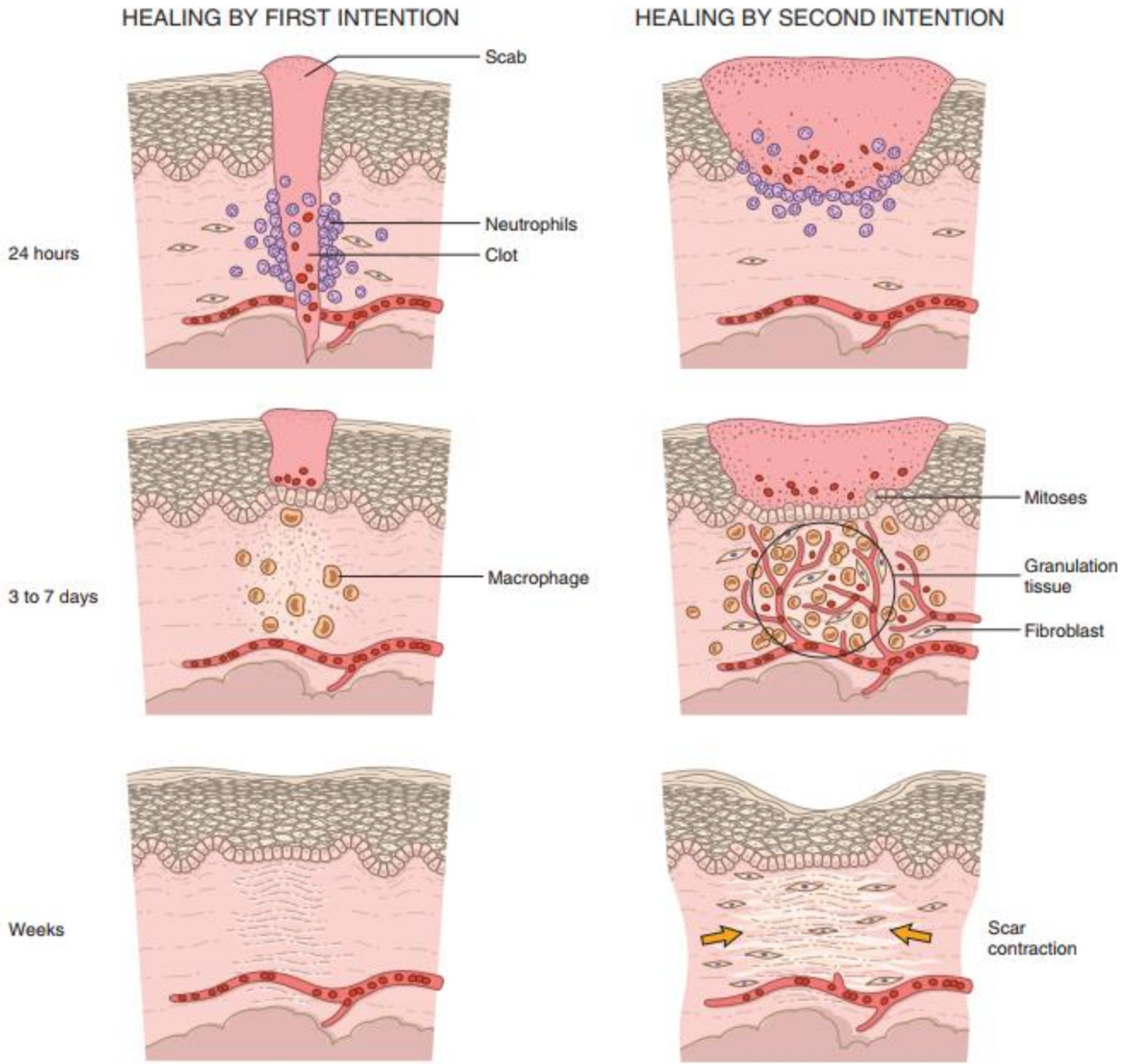


Figure 3.29 Steps in wound healing by first intention (left) and second intention (right). In the latter, note the large amount of granulation tissue and wound contraction.

Skin wound healing
Primary intention

- Injury only involves epithelial layers
- 24 hours clot
- 24-48 hours Neutrophils prominent
- 48 hours epithelial layers migrate from both edges and proliferate along the dermis
- Meet in midline beneath clot
- 48-72 hours Macrophages predominate
- Type III collagen evident at incision margins

Skin wound healing
Primary intention

- 120 hours Neovascularization
- Collagen bridges site
- Type I collagen
- Tensile strength 10% as compared to pre-injury
- With sutures in place, tensile strength is 70% that of pre-injury
- 30 days Scar comprises acellular tissue devoid of inflammation and adnexal cells
- Type III collagen
- Tensile strength 80% as compared to pre-injury

Skin wound healing
Secondary intention

- Gaping wound
- Loss of dermal tissues
- More intense inflammatory response and granulation tissue formation
- Fibroblast conversion to myofibroblasts at wound periphery lead to contraction of wound site
- 90% wound closure by 6 weeks largely as result of contraction

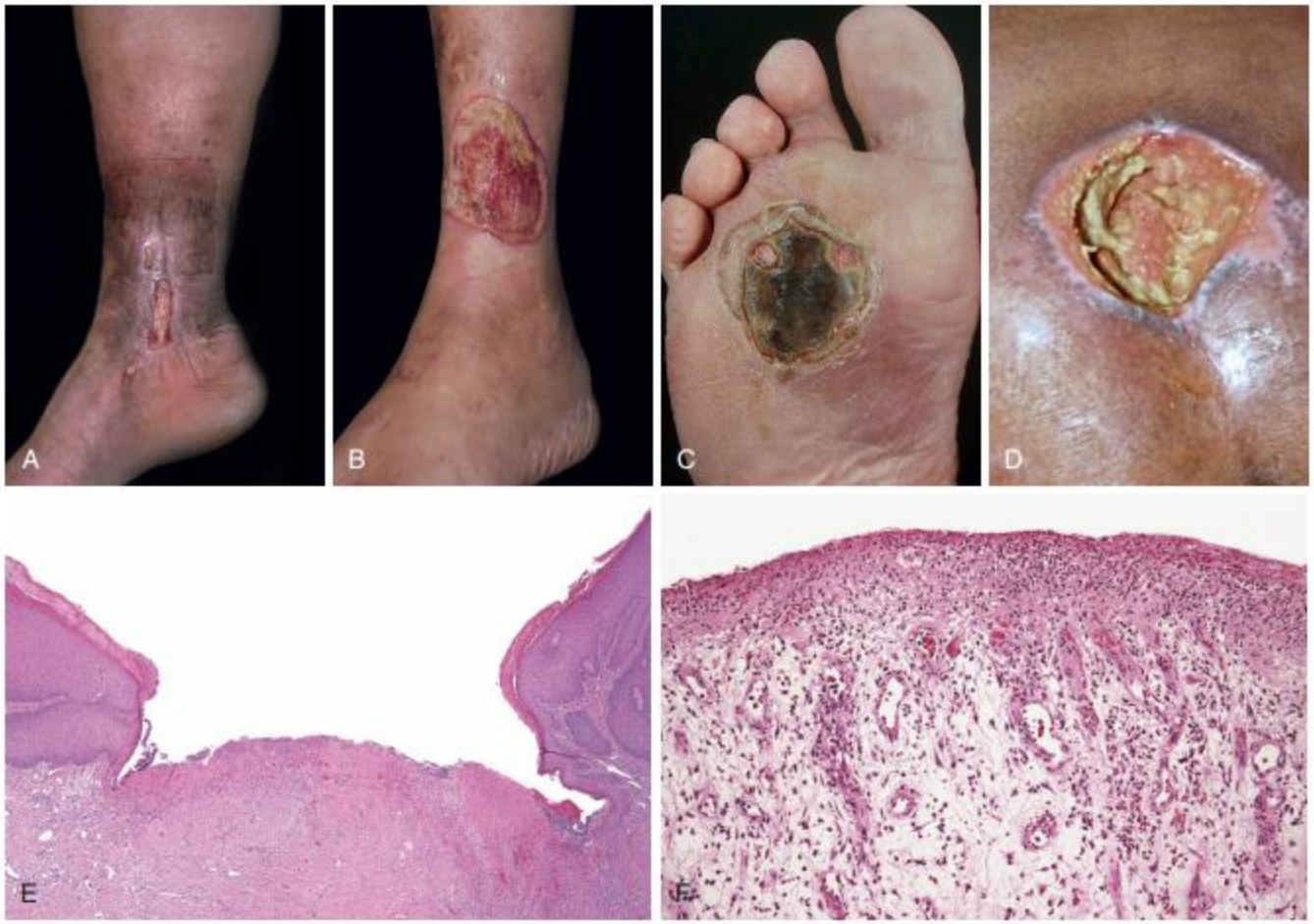
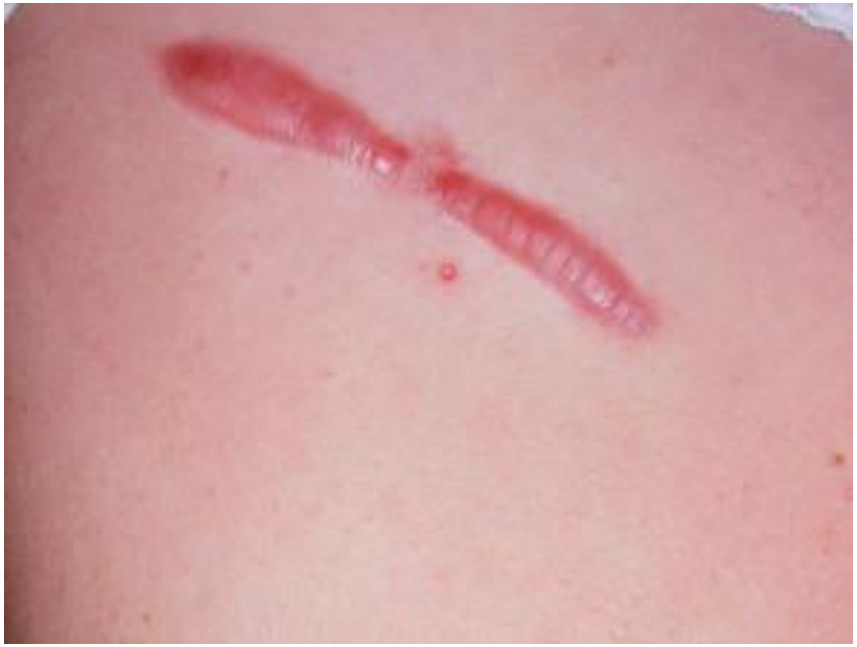


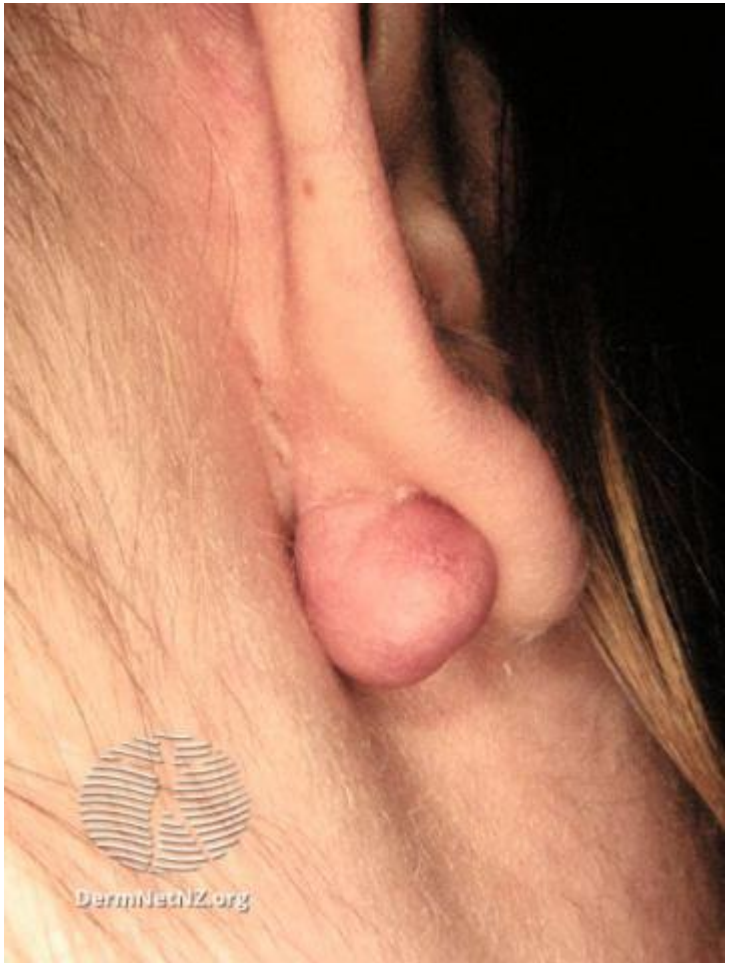
Figure 3.30 Chronic wounds illustrating defects in wound healing. (A–D) External appearance of skin ulcers. (A) Venous leg ulcer; (B) arterial ulcer, with more extensive tissue necrosis; (C) diabetic ulcer; (D) pressure sore. (E, F) Histologic appearance of a diabetic ulcer. (E) Ulcer crater; (F) chronic inflammation and granulation tissue. (A–D, From Eming SA, Margin P, Tomic-Canic M: Wound repair and regeneration: mechanisms, signaling, and translation, *Sci Transl Med* 6:265, 2014.)

Skin wound healing
Secondary intention

- Excessive ECM gives rise to a raised scar (hypertrophic)
- If the scar grows belong the boundaries of the original wound and does not regress, is a keloid
- Common in those of Mediterranean littoral as well as African ancestry
- Exuberant production of granulation tissues is “proud flesh”
- Occasionally may see disordered repair (fibromatosis)

Keloid





https://dermnetnz.org/topics/keloid-images/
Accessed 12/10/2019

Skin wound healing
Secondary intention

- Inadequate formation of granulation tissue or scar may lead to dehiscence (wound rupture)
- Usual cause is suture technique
- Subacute infection, COPD, anemia, obesity, poor nutrition, and diabetes mellitus are comorbidities
- If wounded area in region of neuropathic change, it is unlikely to heal (e.g., diabetics)

Wound closure

- Purse string closure
- The mechanism employs actomyosin ring contractions along the leading edge of the wound.
- The distribution of mechanical force generated by this contractile ring should decrease radially towards the centre.
- Likely to scar

Wound closure

- The use of subcutaneous/fascial tensile reduction sutures places the tension on the deep fascia and superficial fascia layer
- The wound edges may thus be joined naturally under very small tension, avoiding dermal sutures.
- The consequences of this suturing strategy are that wound edges are elevated, smooth, with minimal tension on the dermis.
- Less likely to scar.

Table 2 | Tissue strength during healing[24]	
Time after incision	**% of pre-incision breaking strength**
1 week	3
3 weeks	30
3 months	80

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

Table 3 | Proportion of dehisced wounds that are infected

Type of dehiscence	Proportion of dehisced wounds that are infected
Abdominal dehiscence[4,16,36]	52%–61%
Dehiscence following colorectal surgery[5]	36.7%
Sternal incision dehiscence[3]	49%
Episiotomy dehiscence[37]	Up to 80%

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

- Mortality rates may be as high as 40% following dehiscence of an abdominal wound
- Facial sutures are generally removed at 6 days because of infection risk

Table 8 | Signs of progressing and impaired incision healing[8,14,91]			
Parameter	**Relationship to TIME framework***	**Signs that incisional healing is progressing well**	**Signs that healing is impaired**
Incision colour	**T**issue	■ Days 1–4: red ■ Days 5–14: bright pink ■ Day 15–1 year: pale pink, progressing to white or silver in light-skinned patients or to darker than usual skin colour in patients with darkly-pigmented skin	■ Days 1–4: may be red, tension in the incision line ■ Days 5–9: edges may be well-approximated and the tension remains ■ Days 10–14: if SWD does not occur, colour may remain red or progress to pink and may be followed ultimately by hypertrophic scarring
Healing ridge		■ Days 5–9: a healing ridge of thickened tissue indicating newly formed collagen can be felt about 1cm either side of the incision along its length, and persists into the remodelling phase	■ Lack of healing ridge
Peri-incisional area	**I**nfection/ inflammation	■ Signs of inflammation: - Mild oedema, erythema, warmth or skin discolouration that resolves by day 5 - Pain	■ Signs of inflammation may be absent in the first few days after surgery ■ Signs of inflammation and ongoing pain may be present for extended periods
Exudate	**M**oisture	■ Days 1–4: decreasing in volume from moderate to minimal and changing from sanguineous (blood) to serosanguineous (mixture of blood and serum) to serous (clear, amber serum) ■ Resolves by day 5	■ Exudate persists beyond days 1–4 ■ Exudate may be serosanguineous, serous or purulent (e.g. cloudy, green, yellow or brown)
Wound margins	**E**dge	■ Epithelial closure should be seen by day 4 along the entire incision ■ Approximated	■ Epithelial resurfacing may be only partially present or entirely absent ■ Area(s) of separation (SWD) may be present by day 14

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

Table 9 | Assessment of SWD using the TIME framework (adapted from[93,96,97,99])		
Parameter	**Assess**	**Specifics**
Tissue	Location and extent of dehiscence	■ Location of the incision ■ Proportion of the incision affected ■ Number of areas of dehiscence ■ Presence of sutures/clips and condition (intact/broken)
	Depth of dehiscence	■ Partial or full-thickness dehiscence and tissue layers affected (see Figure 8, page 18); WUWHS SWD Grade (see Table 10, page 18) ■ Extension to or exposure of organs/bone/implant ■ Presence of undermining/tunnelling ■ For abdominal SWD, presence of evisceration
	Tissue viability	■ Condition of exposed tissues ■ Wound bed tissue types and proportions – e.g. of necrotic/devitalised tissue, slough and granulation tissue
	Dimensions	■ Dimensions of the dehisced area(s): maximum length, width, depth
Infection (or inflammation)	For local indicators of infection or inflammation	■ Clinical signs and symptoms ■ See Box 5 and Box 6, page 13, and Box 8, page 17, for signs and symptoms of acute and chronic infection ■ N.B. In patients who are immunosuppressed, signs and symptoms may be less obvious
Moisture	Exudate/drainage colour, consistency, type and odour	■ Purulent (cream, yellow or green) or haemopurulent (red, brown) may indicate infection ■ Yellow or brown exudate may indicate a urinary or enteric fistula ■ Malodour may indicate infection or fistula
	Exudate/drainage level	■ Indications of the level of exudate production can be gained from the condition of the current dressing (i.e. a dry dressing indicates low exudate levels; a saturated or leaking dressing indicates higher levels) and the appearance of the wound bed
Edge	Edges of dehisced area	■ In long-standing areas of dehiscence, the edges may become undermined
	Colour and condition of the surrounding skin	■ Signs of dermatological conditions that may affect healing – e.g. radiation dermatitis ■ Signs of spreading infection – e.g. spreading erythema, warmth and oedema ■ Periwound maceration may indicate high exudate/drainage levels and/or inadequate absorbency of the dressing

World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
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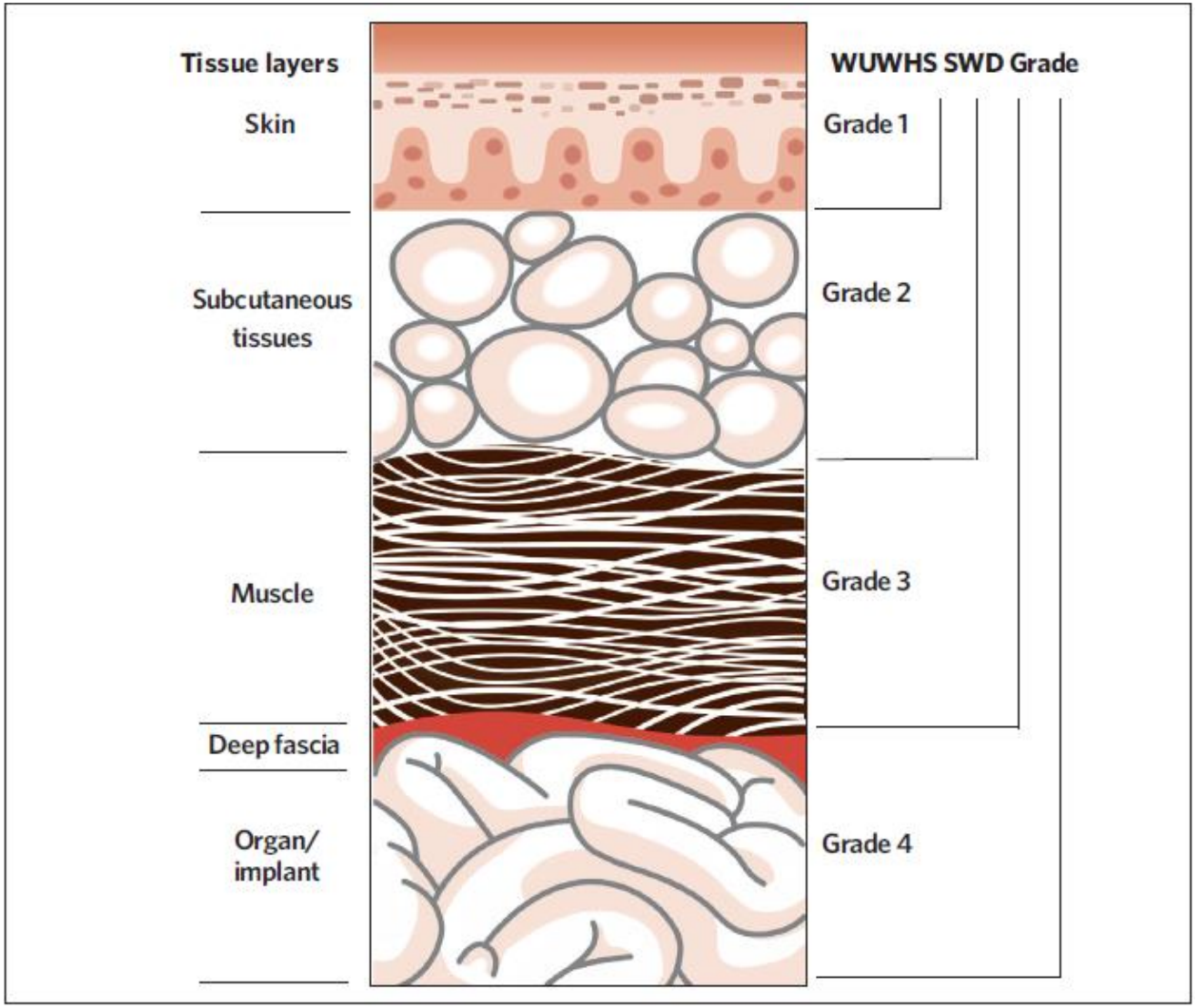
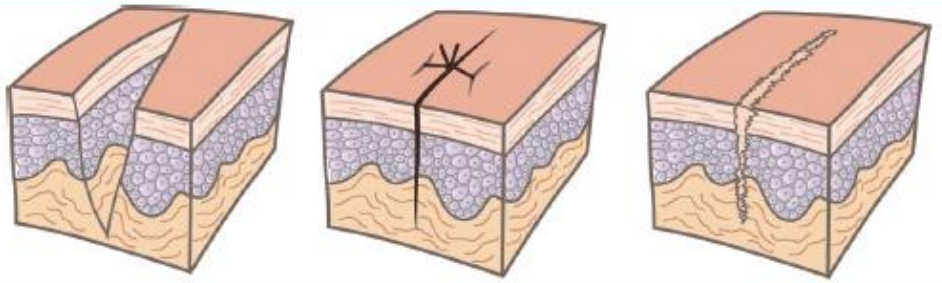
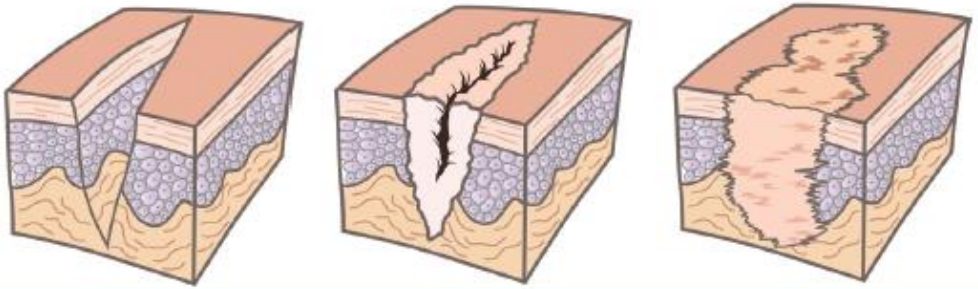


World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

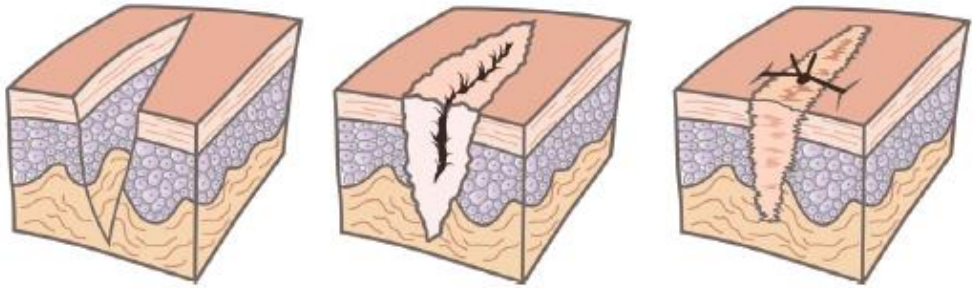
Primary closure
The edges of the incision are closely opposed, e.g. by suturing, stapling or taping, to allow healing by primary intention



Secondary closure
The incision is left open and heals by secondary intention as new tissue infills from the base and sides of the wound

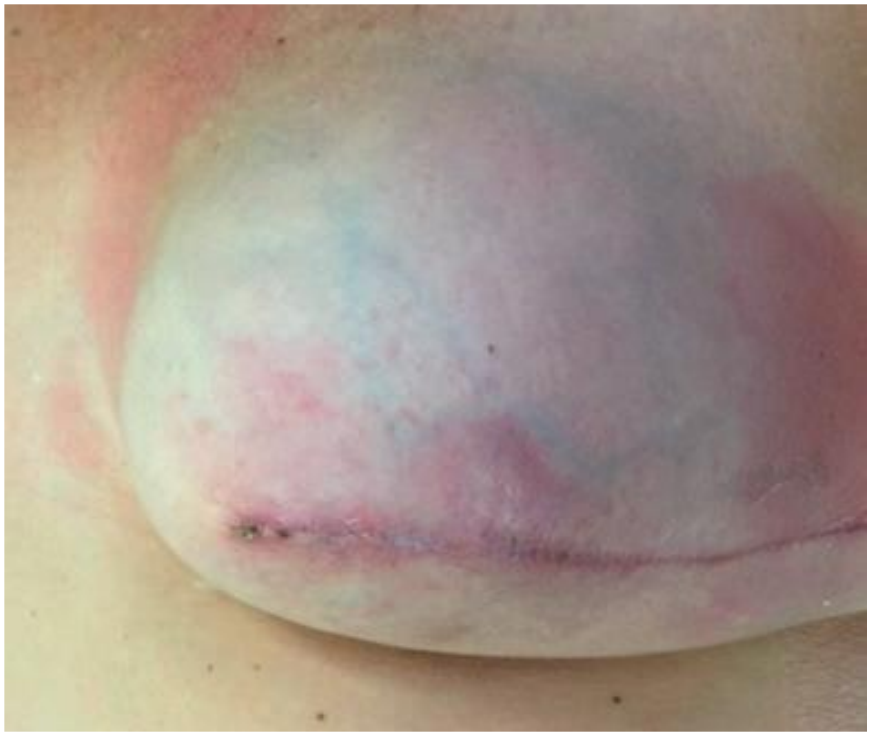


Delayed primary closure
The incision is left open for up to several days or sometimes weeks, to allow for treatment of infection/contamination, removal (sequentially if necessary) of non-viable tissue, and/or for resolution of swelling, before proceeding to primary closure or closure with a flap/graft

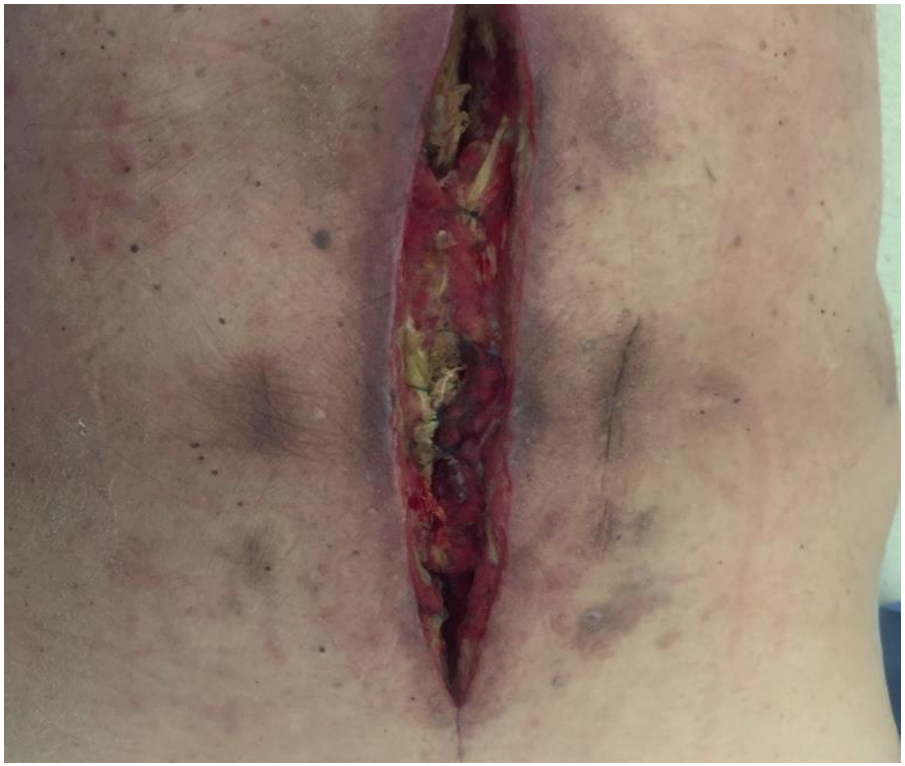


World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

Grade 2 dehiscence



Grade 3 dehiscence



World Union of Wound Healing Societies (WUWHS) Consensus Document. *Surgical wound dehiscence: improving prevention and outcomes*. Wounds International, 2018
Accessed 12/10/2019

Superficial fibromatoses

- Characterized by nodular or poorly defined broad fascicles of mature-appearing myofibroblasts surrounded by abundant dense collagen.
- Trisomy 3 and trisomy 18 common abnormalities.
- The palmar variant (Dupuytren contracture), there is irregular or nodular thickening of the palmar fascia (bilaterally in 50%).
- Attachment to the overlying skin causes puckering and dimpling.
- At the same time, a slowly progressive flexion contracture develops, mainly of the fourth and fifth fingers of the hand.

Fibromatoses

- With the plantar variant flexion contractures are uncommon and bilateral involvement is infrequent.
- In penile fibromatosis (Peyronie disease), a palpable induration or mass appears usually on the dorsolateral aspect of the penis.
- It may cause eventually abnormal curvature of the shaft or constriction of the urethra, or both.
- 10% of all fibromatoses.

Deep seated fibromatoses

- Deep-seated fibromatoses (desmoid tumors) lie in the borderland between nonaggressive fibrous tumors and low-grade fibrosarcomas.
- 2^{nd}-4^{th} decades
- No sex predilection
- Commonly present as large, infiltrative masses that frequently recur after incomplete excision.

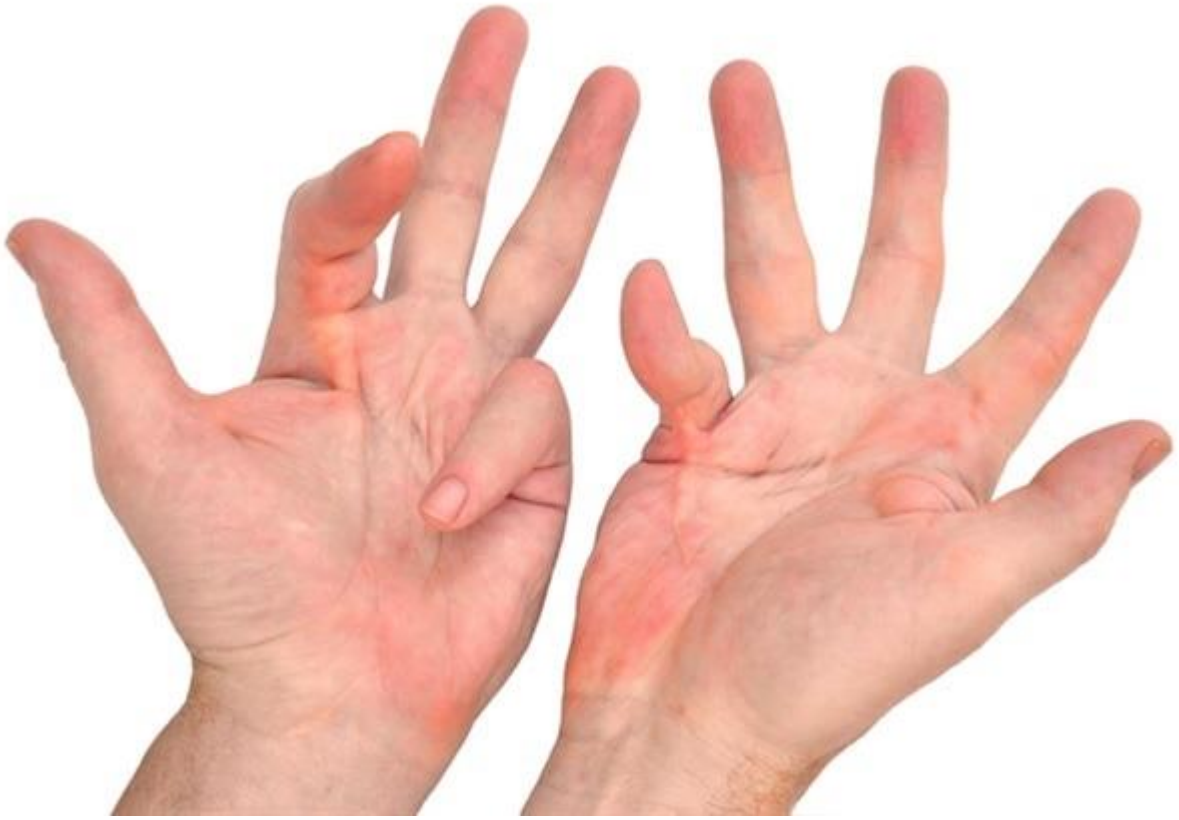
Deep seated fibromatoses

- Commonly present as large, infiltrative masses that frequently recur after Composed of plump fibroblasts arranged in broad sweeping fascicles that infiltrate to the adjacent tissue.
- Mitoses are usually infrequent.
- Trapped regenerating muscle cells may be mistaken for multi-nucleated giant cells

Desmoid tumor

- Extra-abdominal arise principally in the musculature of the shoulder, chest wall, back, and thigh.
- Abdominal desmoids generally arise in the musculo-aponeurotic structures of the anterior abdominal wall in women during or after pregnancy.
- Intra-abdominal desmoids tend to occur in the mesentery or pelvic walls, often in patients having familial adenomatous polyposis (Gardner syndrome).
- Mutations in the APC gene at 5q22.2 or β-catenin gene at 3p22.1 (both in the WNT pathway).
- <u>Do not metastasize.</u>

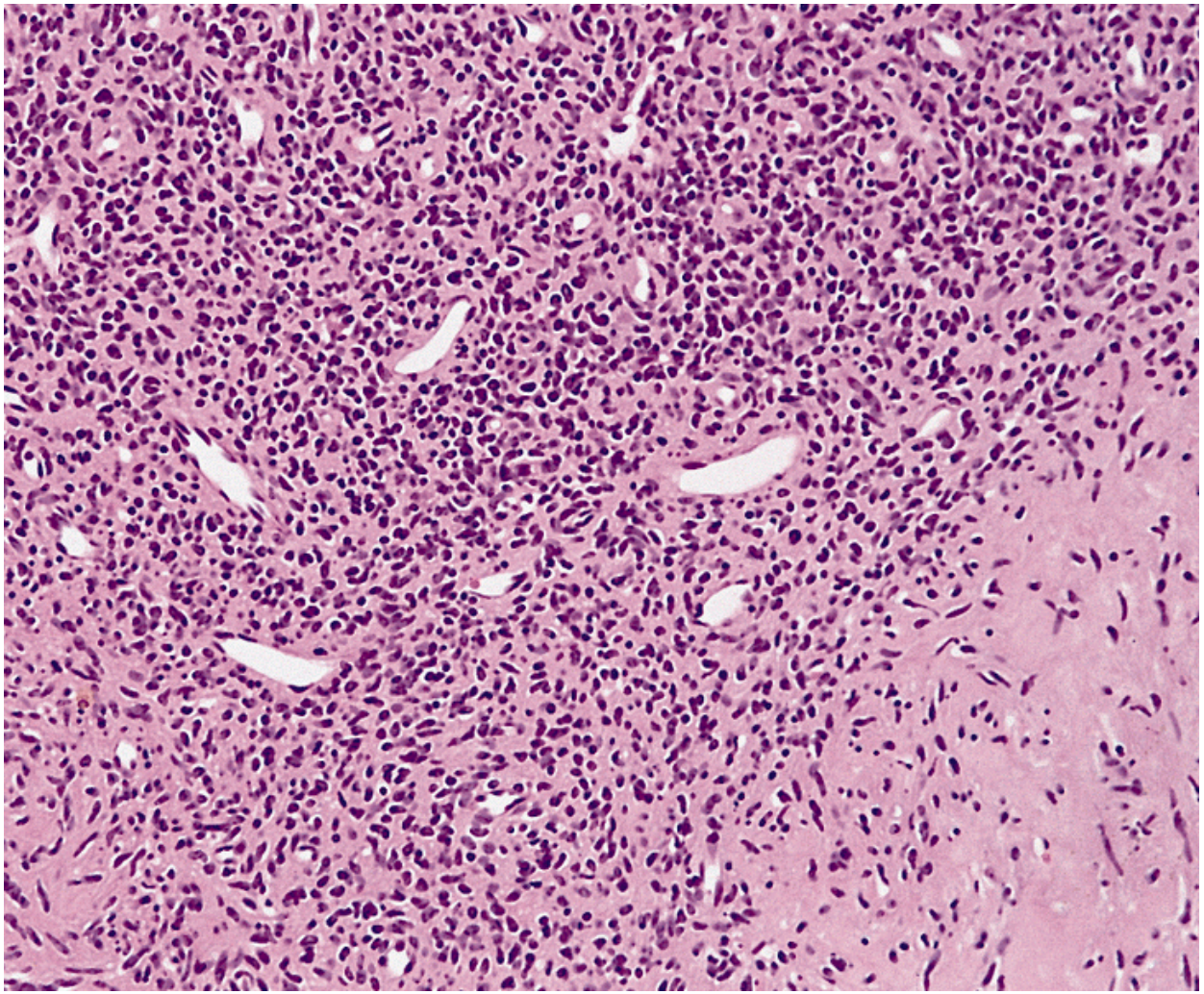
Palmar fibromatosis



Excessive scar formation with contraction may lead to deformity (e.g., palm)

https://www.news-medical.net/health/Dupuytrens-Contracture-(Palmar-Fibromatosis).aspx
Accessed 12/10/2019

Palmar fibromatosis



Excessive proliferation of fibroblasts adjacent to area of fibrosis

Fig. 2-45R Kempson, Richard L., Fletcher, Christophr DM, Evans, Harry L, Hendrickson, Michael R, and Sibley, Richard K, Tumors of the Soft Tissues, Fascicle 30, Atlas of Tumor Pathology. 3rd Series. Armed Forces Institute of Pathology, Washington, DC (2001).

Desmoid

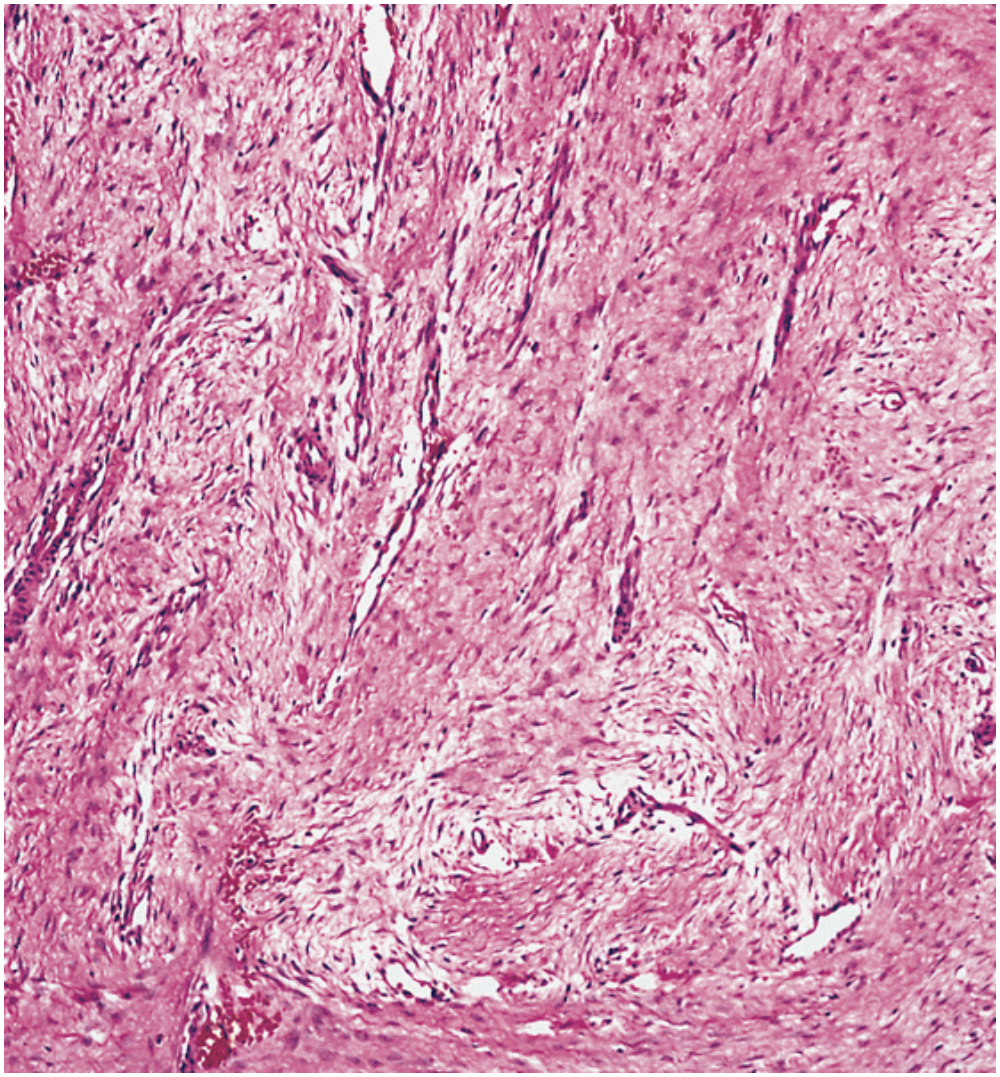


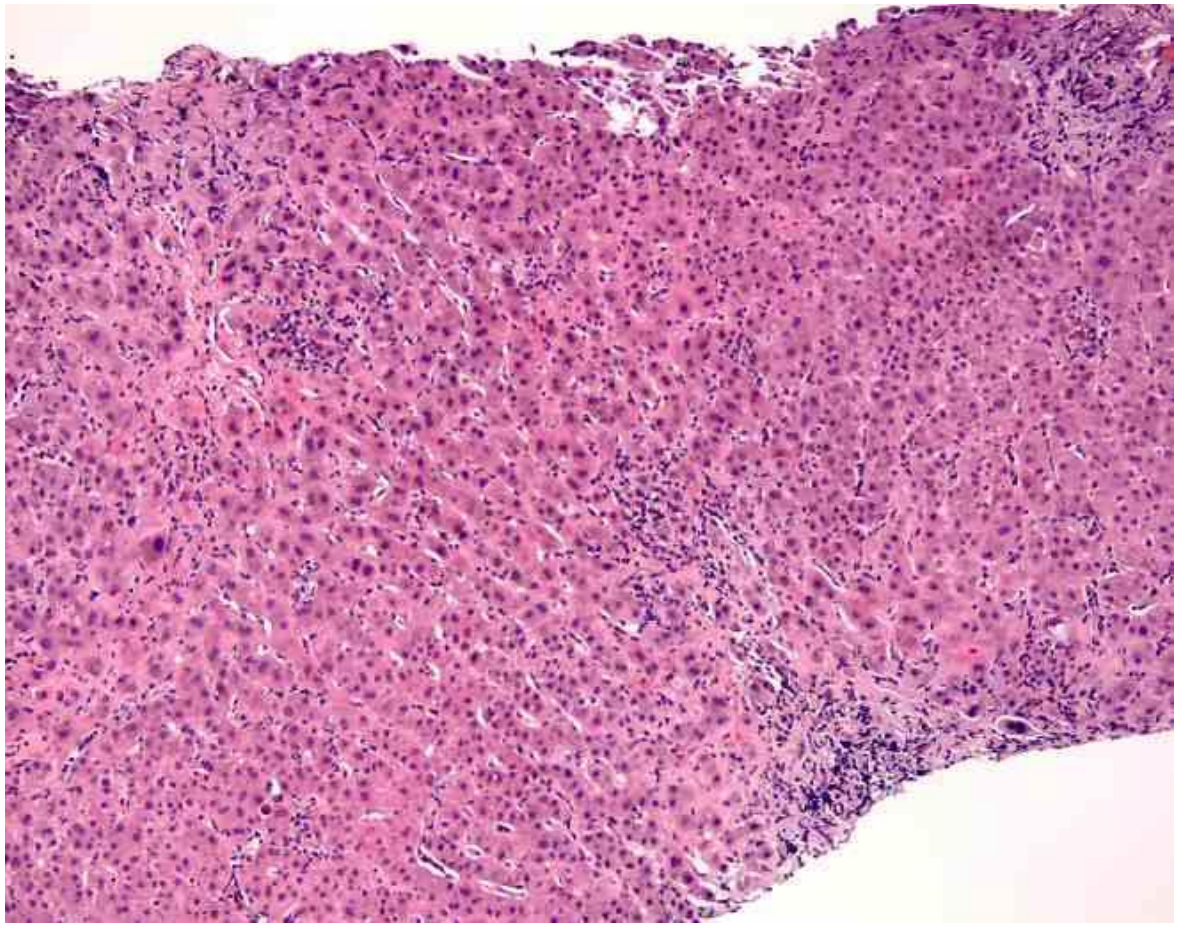
Fig. 2-49L Kempson, Richard L., Fletcher, Christophr DM, Evans, Harry L, Hendrickson, Michael R, and Sibley, Richard K, Tumors of the Soft Tissues, Fascicle 30, Atlas of Tumor Pathology. 3rd Series. Armed Forces Institute of Pathology, Washington, DC (2001).

PARENCHYMAL REPAIR

Parenchymal repair

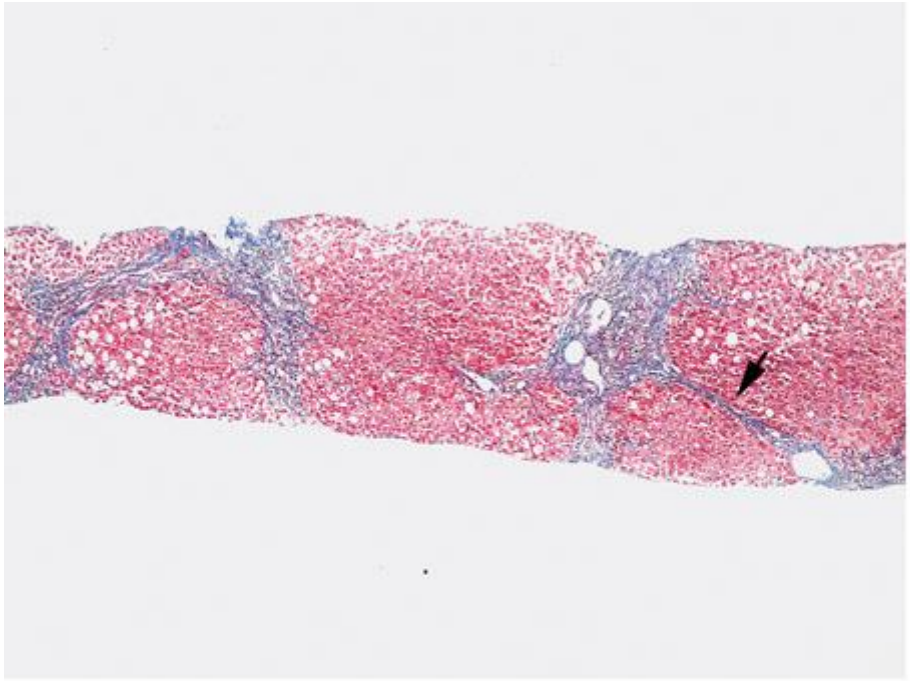
- Liver
- Regeneration of hepatocytes
- IL 6 produced and released by Kuppfer cells make hepatocytes competent to receive growth factor signals
- In severe injury, nodular regeneration
- Lack sinusoids and portal triads
- Fibrosis around portal triads with bridging between portal triads or to or between central veins
- Potential for cirrhosis

Chronic hepatitis



https://tpis.upmc.com/tpislibrary/images/H00190dmod.jpg
Accessed 12/04/2019

Chronic persistent hepatitis with bridging fibrosis



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

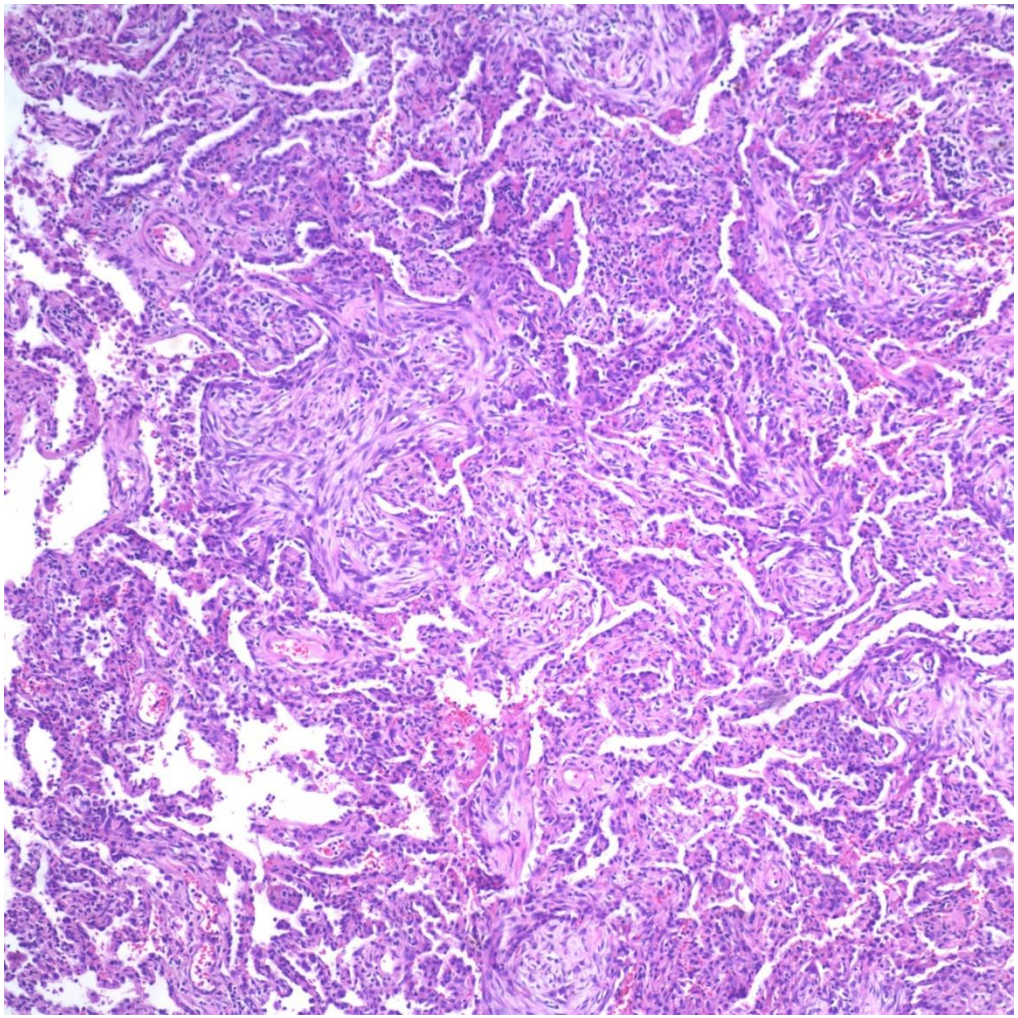
Fibrosis extends from portal area to portal area without loss of the limiting plate and with minimal inflammation. Masson Trichrome 10x

Fig. e26-12 Accessed 03/01/2010

Parenchymal repair

- Lung
- Type II pneumocytes replace damaged Type I and Type II pneumocytes
- Produce surfactant
- Fibrosis along alveolar septae

Organizing pneumonia



https://media.clinicaladvisor.com/images/dsm/ch4359.fig5.jpg
Accessed 12/04/2019

Parenchymal repair

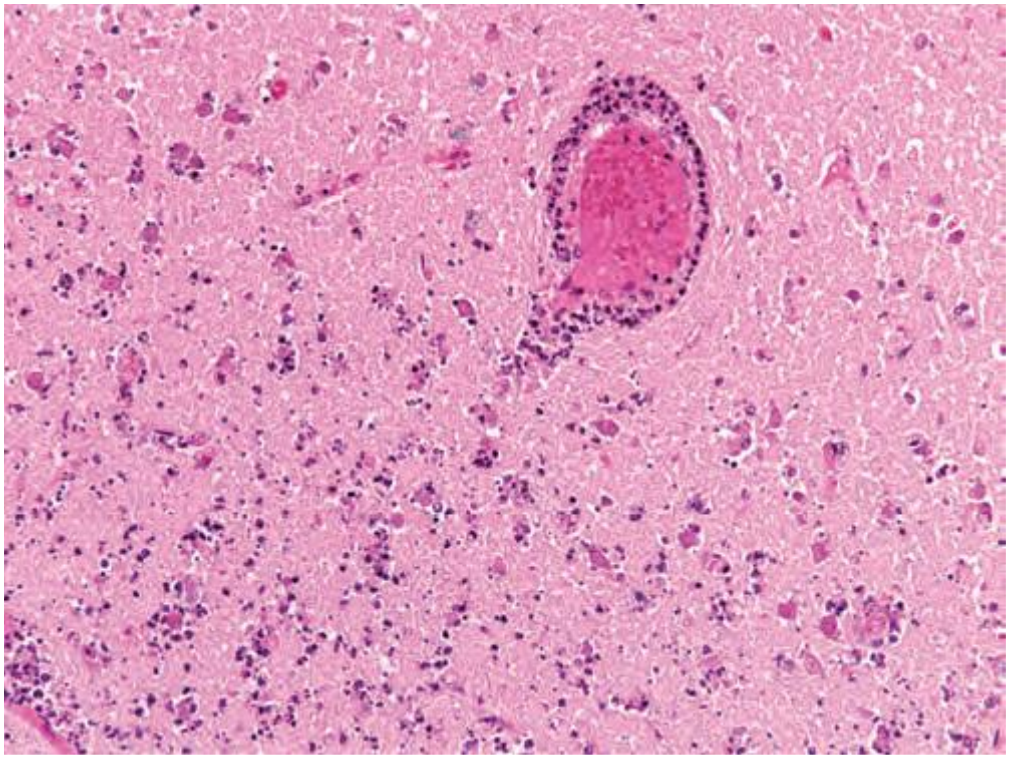
- Brain
- Microvacuolization of neurons followed by eosinophilia of neuronal cytoplasm (“Red neurons”)
- Neuronal bodies shrink; pyknosis and loss of nucleolus follow
- Occur later in astrocytes and oligodendrocytes.
- Myelinated fibers (axons) begin to disintegrate.
- Tau proteins from ruptured microtubules may aggregate, migrate, and further injure
-

Parenchymal repair

- Microglial cells (macrophages) prominent by 48 hours and become the predominant cell type over time.
- Mesoderm derived cells
- React by elongating nuclei (rod cells as in neurosyphillis)
- Aggregate around foci of necrosis
- Phagocytize neurons
- May persist in the lesion for months or years

Parenchymal repair

Neutrophil infiltration of injury site begins at edges of site where vascular supply has remained intact.



Frosch, MP, Anthony, DC, De Girolami, U, “The Central Nervous System,” in Kumar, V, Abbas, AK, Aster, JC, (eds), Robbins and Cotran Pathologic Basis of Disease (9th ed.), Elsevier. Philadelphia. (2015) Fig. 28-13C Accessed 10/25/2019

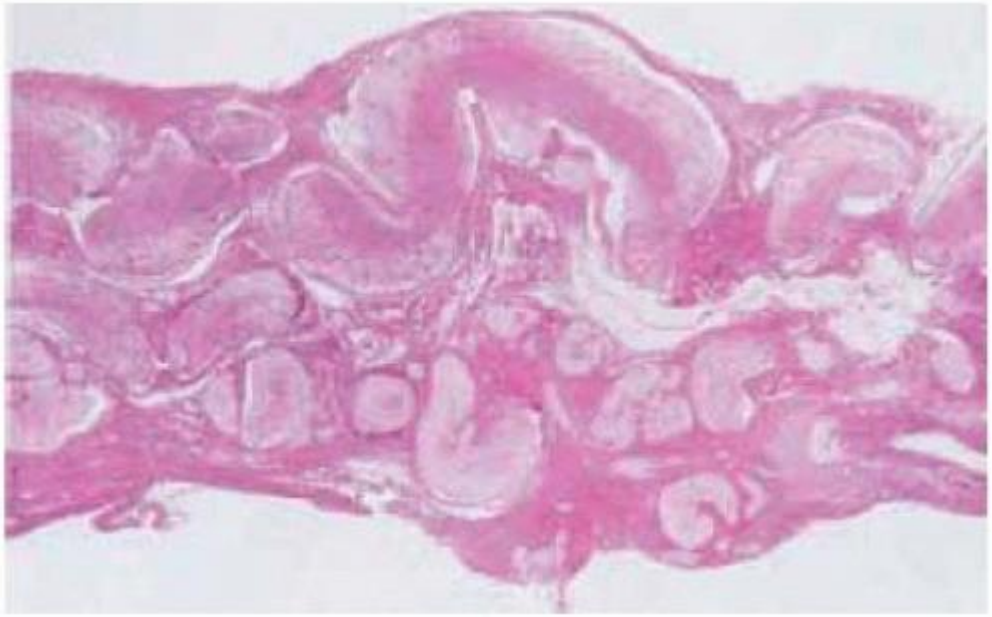
Parenchymal repair

- Peripheral nerve
- Distal degeneration of axon and myelin sheath (Wallerian degeneration)
- Proximal degeneration to node of Ranvier
- Schwann cells and macrophages phagocytize debris
- Muscle atrophy at 15 days
- Nerve cell body swells; Nissl bodies disappear centrally; nucleus to periphery

Parenchymal repair

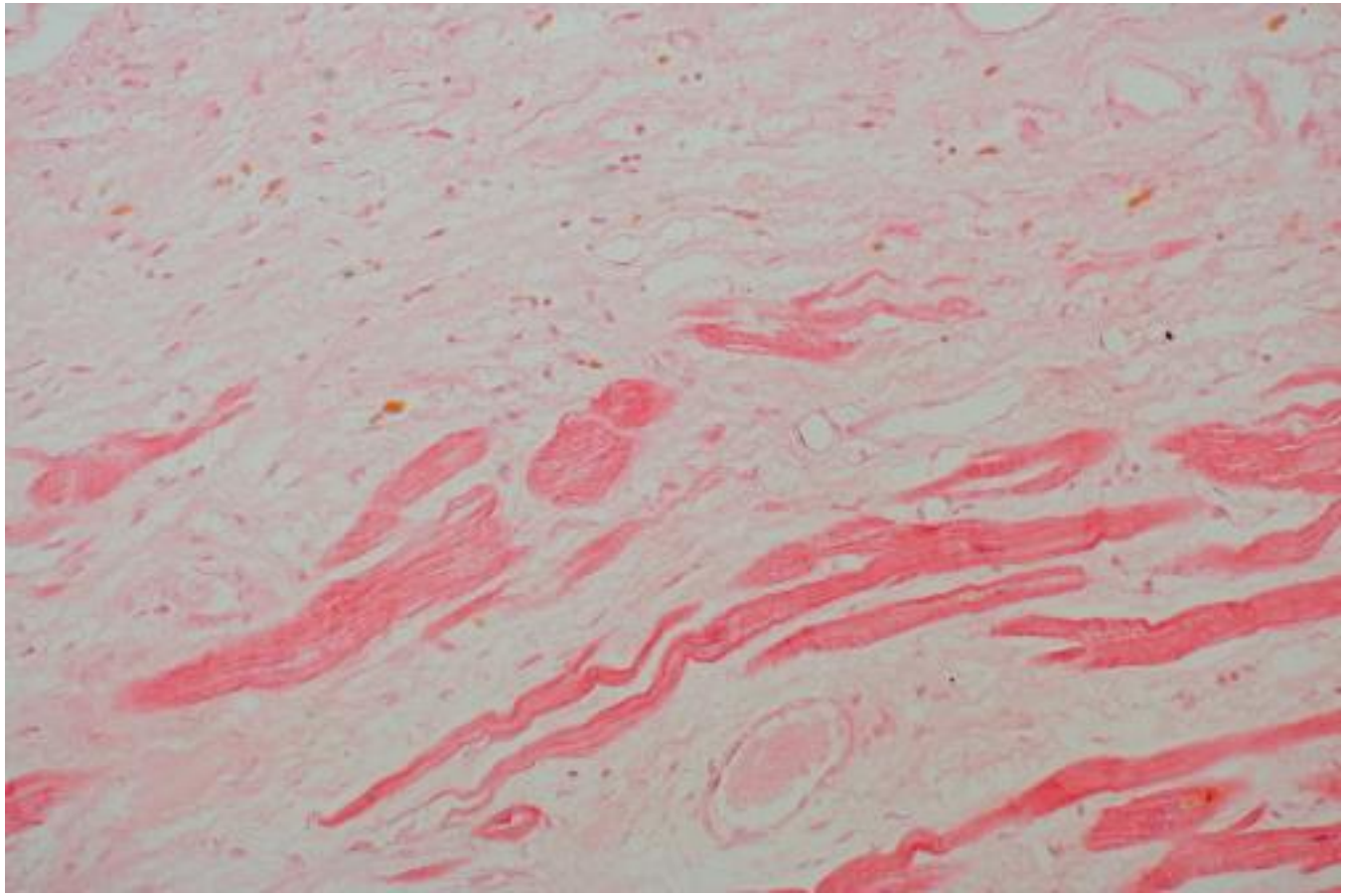
- Then, Schwann cells proliferate in distal stump
- Axonal sprouts from proximal stump extend distally to distal stump (under Schwann cell guidance)
- Axon growth 2-3 mm/day
- Demyelination
- Nerve eventually re-innervated
- Disordered organization gives rise to plexiform neurofibroma
- The heart heals only by scarring

Plexiform neurofibroma



https://entokey.com/wp-content/uploads/2016/08/DA1-C7-FF11.gif
Accessed 12/04/2019

Myocardium

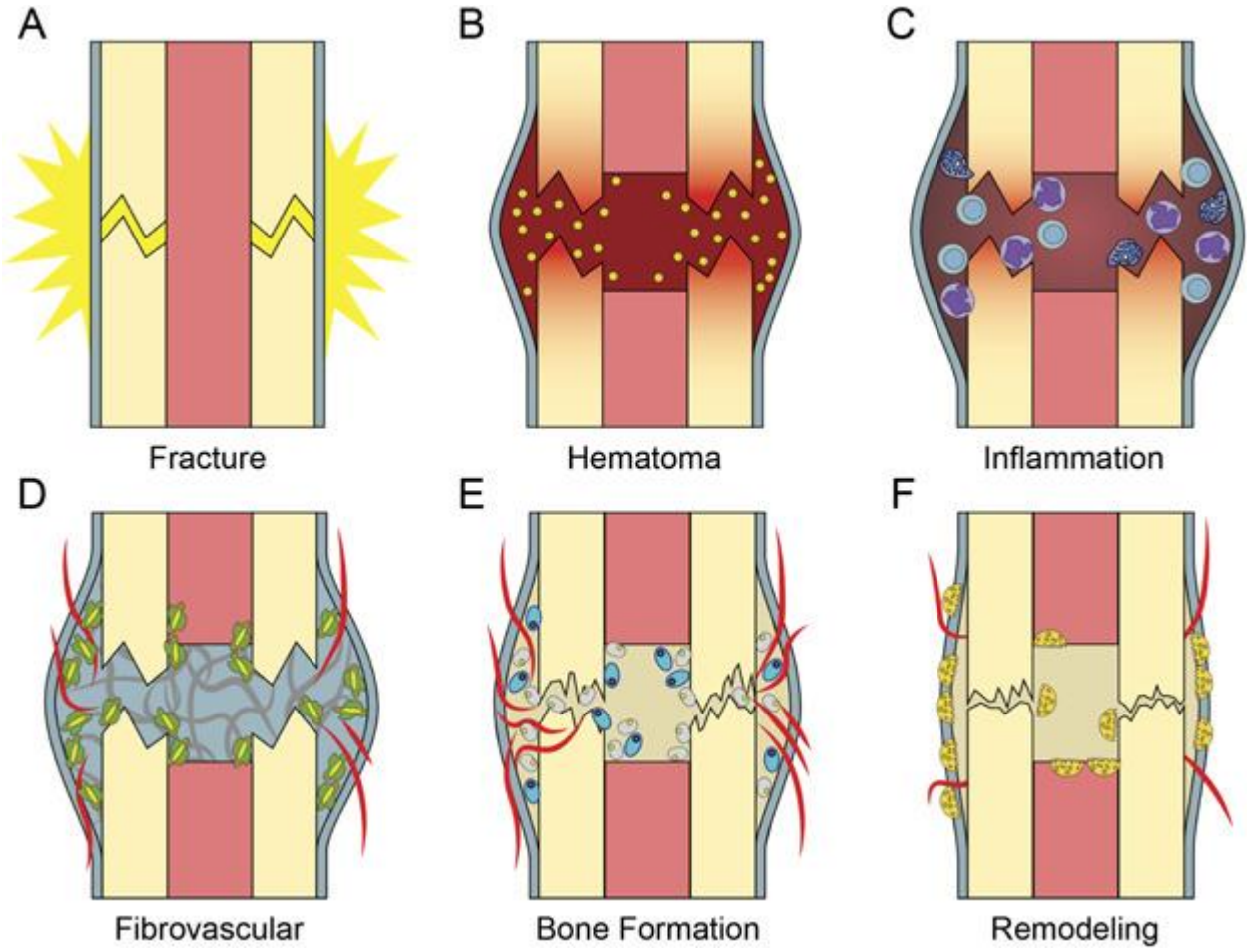


Two months post infarct. Little muscle remaining. Fibrous tissue deposition marked.

https://webpath.med.utah.edu/CVHTML/CV326.html
Accessed 12/10/2019

BONE HEALING

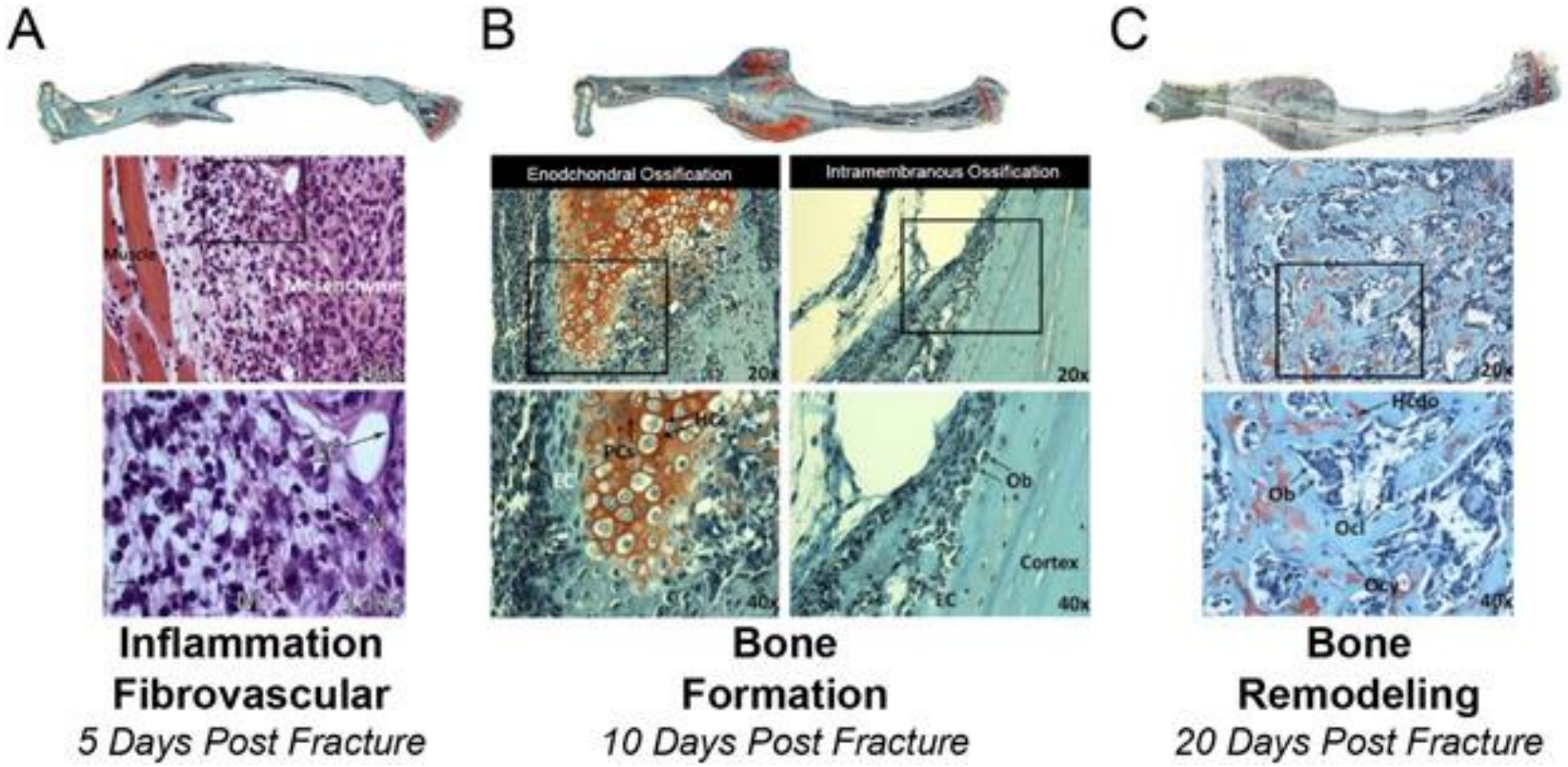
Cellular biology of fracture healing



Fracture healing is temporally-defined process.

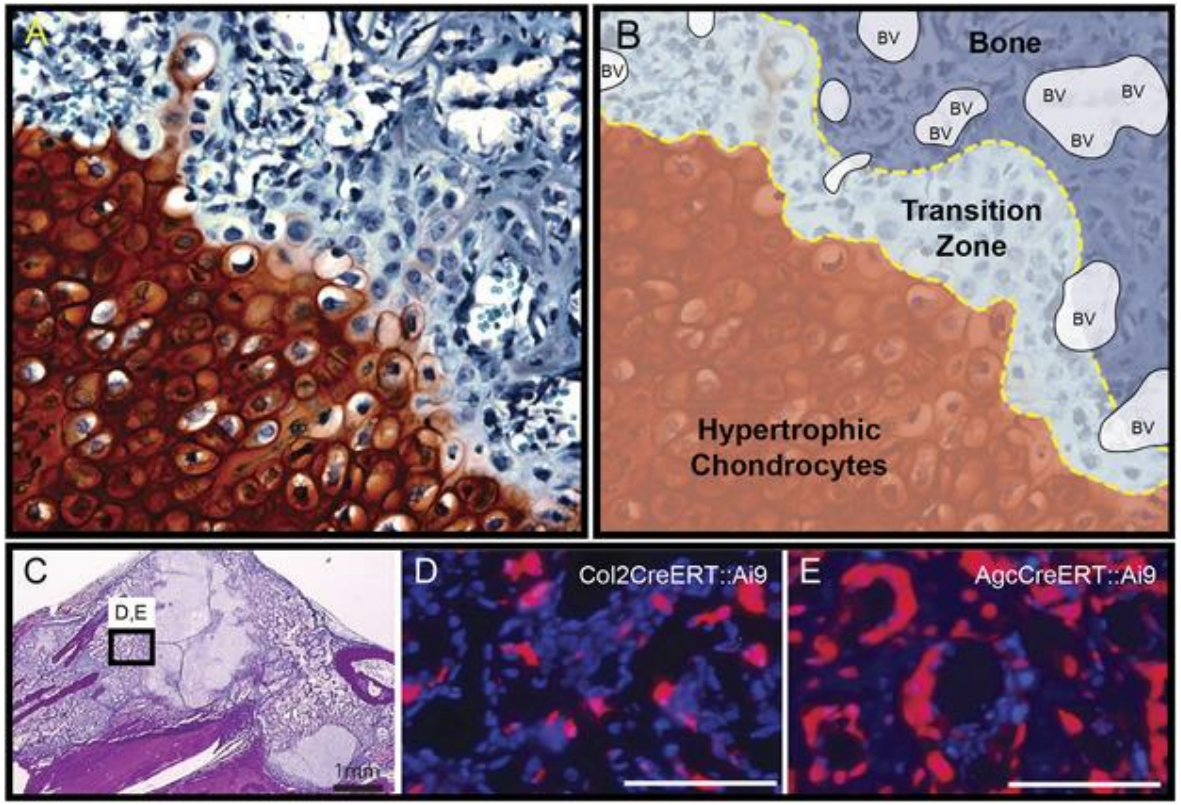
Journal of Orthopaedic Research, Volume: 37, Issue: 1, Pages: 35-50, First published: 28 October 2018, DOI: (10.1002/jor.24170)

Cellular biology of fracture healing



(A) undifferentiated mesenchymal cells are present in the callus and areas of inflammation remain (B) 10 days post-fracture there is both endochondral ossification (red staining, safranin-o stains cartilage) and intramembranous bone formation occurring. (C). An extensive network of primary bone has formed and endochondral ossification is complete.

Journal of Orthopaedic Research, Volume: 37, Issue: 1, Pages: 35-50, First published: 28 October 2018, DOI: (10.1002/jor.24170)



Hypertrophic chondrocytes develop into osteoblasts and osteocytes

Journal of Orthopaedic Research, Volume: 37, Issue: 1, Pages: 35-50, First published: 28 October 2018, DOI: (10.1002/jor.24170)

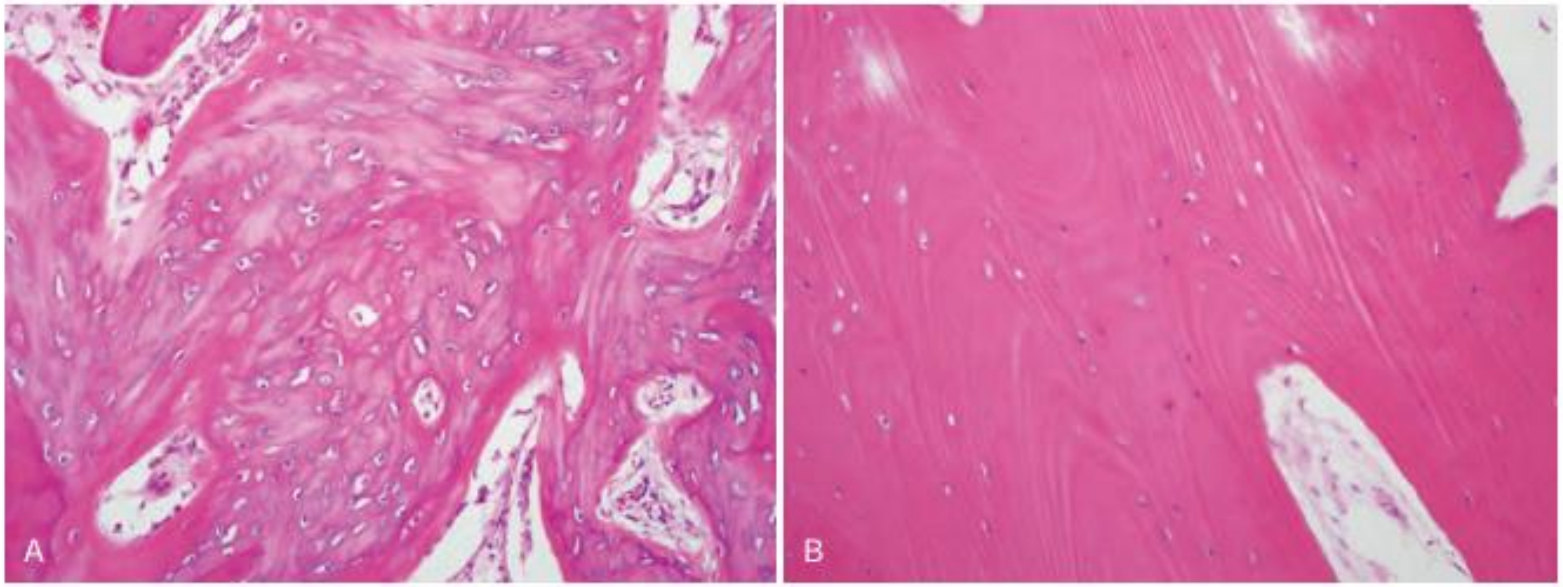
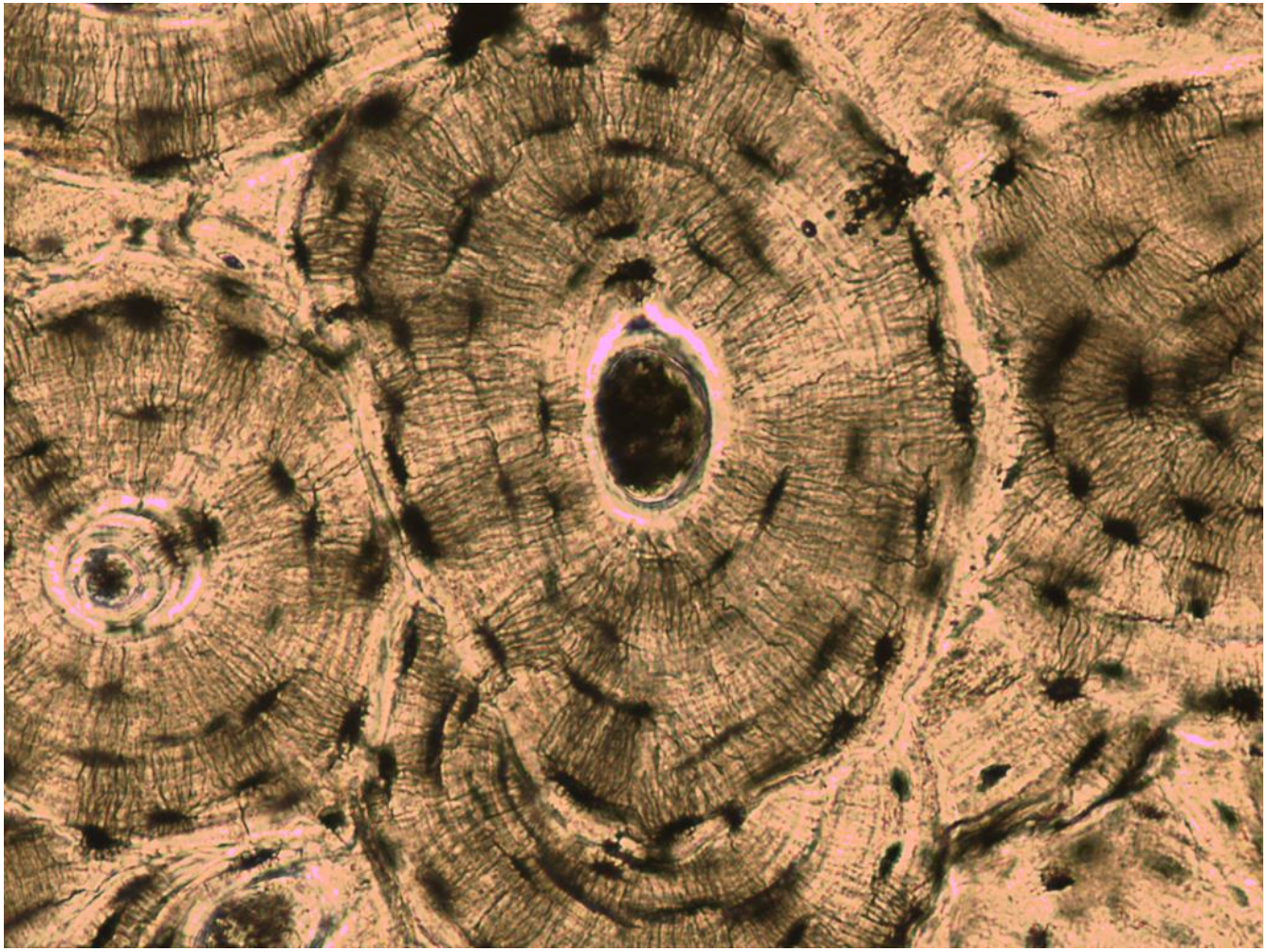


Figure 26-1 Woven bone **(A)** is more cellular and disorganized than lamellar bone **(B)**.



Osteon with Haversian canal in center

http://medcell.med.yale.edu/histology/bone_lab/images/haversian_system.jpg

Accessed 05/10/2020

Healing of bone fracture

- Following the initial trauma, bone heals by either direct intramembranous or indirect fracture healing, which consists of both intramembranous and endochondral bone formation.
- The most common pathway is indirect healing as direct bone healing requires an anatomical reduction and rigidly stable conditions.
- When such conditions are achieved, the direct healing cascade allows the bone structure to immediately regenerate anatomical lamellar bone and the Haversian systems without any remodeling steps necessary.

Contact healing

- Bone on one side of the cortex must unite with bone on the other side of the cortex to re-establish mechanical continuity.
- Under these conditions, osteoclasts which cross the fracture line, generate longitudinal cavities.
- These cavities are later filled by bone produced by osteoblasts residing at the rear of the juncture.
- This results in the simultaneous generation of a bony union and the restoration of Haversian systems formed in an axial direction.

Contact healing

- The re-established Haversian systems allow for penetration of blood vessels carrying osteoblastic precursors.
- The bridging osteons later mature by direct remodeling into lamellar bone resulting in fracture healing without the formation of periosteal callus.

Gap healing

- Bony union and Haversian remodeling do not occur simultaneously.
- It occurs if stable conditions and an anatomical reduction are achieved, although the gap must be less than 1 mm.
- In this process the fracture site is primarily filled by lamellar bone oriented perpendicular to the long axis, requiring a secondary osteonal reconstruction.

Gap healing

- The primary bone structure is then gradually replaced by longitudinal revascularized osteons carrying osteoprogenitor cells which differentiate into osteoblasts and produce lamellar bone on each surface of the gap.
- This lamellar bone, however, is laid down perpendicular to the long axis and is mechanically weak.

Gap healing

- This initial process takes approximately 3 and 8 weeks, after which a secondary remodeling resembling the contact healing cascade takes place.
- Although not as extensive as endochondral remodeling, this phase is necessary in order to fully restore the anatomical and biomechanical properties of the bone.

Healing of bone fracture

- Indirect healing
- Involves an acute inflammatory response and the recruitment of mesenchymal stem cells in order to generate a primary cartilaginous callus.
- This primary callus later undergoes revascularization and calcification, and is finally remodeled to fully restore a normal bone structure.

Healing of bone fracture

- Immediately following the trauma, a hematoma is generated and consists of cells from both peripheral and intramedullary blood, as well as bone marrow cells.
- The injury initiates an inflammatory response which is necessary for the healing to progress.
- The response causes the hematoma to coagulate in between and around the fracture ends, and within the medulla forming a template for callus formation.

Acute response

- TNF-α concentration has been shown to peak at 24h and to return to baseline within 72h post trauma.
- During this time-frame TNF-α is expressed by macrophages and other inflammatory cells, and it is believed to mediate an effect by inducing secondary inflammatory signals, and act as a chemotactic agent to recruit necessary cells.
- TNF-α also induces osteogenic differentiation of mesenchymal stem cells (MSC).

Acute response

- These effects are mediated by activation of the two receptors TNFR1 and TNFR2 which are expressed on both osteoblasts and osteoclasts.
- However, TNFR1 is always expressed in bone whereas TNFR2 is only expressed following injury

Acute response

- IL-1 and IL-6 are believed to be most important cytokines for fracture healing.
- IL-1 expression overlaps with that of TNF-α with a biphasic mode
- It is produced by macrophages in the acute phase of inflammation and induces production of IL-6 in osteoblasts
- Promotes the production of the primary cartilaginous callus, and also promotes angiogenesis at the injured site by activating either of its two receptors

Acute response

- IL-6 is only produced during the acute phase and stimulates angiogenesis, vascular endothelial growth factor (VEGF) production, and the differentiation of osteoblasts and osteoclasts.

- BMP-2 is essential for bone repair
- BMP-7 may plays a more important role in the recruitment of progenitor cells.

Acute response

- Stromal cell-derived factor-1(SDF-1) and its G-protein-coupled receptor CXCR-4 form an axis (SDF-1/CXCR-4) that is a key regulator of recruiting and homing specific MSCs to the site of trauma.
- SDF-1 expression is increased at the fracture site, especially in the periosteum at the edges of the fracture.
- SDF-1 has a specific role in recruiting CXCR-4 expressing MSCs to the injured site during endochondral fracture healing

Acute response

- Hypoxia inducible factor-1α (HIF-1α) induces the production of VEGF in the revascularization process
- Hypoxic gradients regulate MSC progenitor cell trafficking by HIF-1

Healing phase

- Following the formation of the primary hematoma, a fibrin-rich granulation tissue forms.
- Within this tissue, endochondral formation occurs in between the fracture ends, and external to periosteal sites.
- These regions are mechanically less stable
- The cartilaginous tissue forms a soft callus which gives the fracture a stable structure.

Healing phase

- Peak of soft callus formation occurs 7–9 days post trauma with a peak in both type II procollagen and proteoglycan core protein extracellular markers.
- At the same time, an intramembranous ossification response occurs subperiostally directly adjacent to the distal and proximal ends of the fracture, generating a hard callus.
- It is the final bridging of this central hard callus that ultimately provides the fracture with a semi-rigid structure which allows weight bearing

Healing phase

- The Wnt-family is thought to regulate the differentiation of pluripotent MSCs into the osteoblastic lineage, and, at later stages of development, to positively regulate osteoblastic bone formation.
- As fracture callus chondrocytes proliferate, they become hypertrophic and the extracellular matrix becomes calcified.
- Both osteoblasts and hypertrophic chondrocytes express high levels of VEGF
- Promote the invasion of blood vessels and transforming the avascular cartilaginous matrix into a vascularized osseous tissue.

Healing phase

- A cascade orchestrated primarily by macrophage colony-stimulating factor (M-CSF), RANKL, osteoprotegerin (OPG) and TNF-α initiates the resorption of this mineralized cartilage.
- During this process M-CSF, RANKL and OPG are also thought to help recruit bone cells and osteoclasts to form woven bone.
- TNF-α further promotes the recruitment MSC with osteogenic potnetial
- Initiates chondrocyte apoptosis.

Healing phase

- Mitochondria accumulate calcium-containing granules created in the hypoxic fracture environment.
- After elaboration into the cytoplasm of fracture callus chondrocytes, calcium granules are transported into the extracellular matrix where they precipitate with phosphate and form initial mineral deposits.
- These deposits of calcium and phosphate become the nidus for homogeneous nucleation and the formation of apatite crystals.
- The peak of the hard callus formation is usually reached by day 14

Remodeling

- The remodeling process is carried out by a balance of hard callus resorption by osteoclasts, and lamellar bone deposition by osteoblasts.
- Although the process is initiated as early as 3–4 weeks in the remodeling may take years to be completed to achieve a fully regenerated bone

Remodeling

- Bone remodeling has been shown to be a result of production of electrical polarity created when pressure is applied in a crystalline environment.
- This is achieved when axial loading of long bones occurs, creating one electropositive convex surface, and one electronegative concave surface, activating osteoclastic and osteoblastic activity respectively.
- By these actions the external callus is gradually replaced by a lamellar bone structure, whereas the internal callus remodelling re-establishes a medullar cavity characteristic of a diaphyseal bone.

IMMUNE RESPONSES

Hypersensitivity reactions

Type	Mediator	Reaction
Anaphylaxis I	IgE	Degranulation of mast cells and basophils by allergen sensitized bound IgE. Histamine, leukotrienes, tryptase released. Eosinophil chemotaxis occurs. Uriticaria.
Antibody mediated Cytotoxic II	IgG	Complement mediated lysis of antigen-antibody coated cells in circulation or in extracellular matrix. Autoimmune hemolysis (methyldopa, penicillin, sulfonamide); ITP.
Immune Complex III	IgG	Antigen-antibody complexes deposited in basement membranes and capillary venules, activating complement. Neutrophils attracted to site. Lysozymal injury. Serum sickness; SLE.
Delayed IV	T Cell	Antigen sensitized CD4 cells release lymphokines, inducing inflammation and activating macrophages. (May see CD8 mediation in contact dermatitis.)

Transplantation

- Recipients of HLA identical allografts from a monozygotic twin usually receive azathioprine and steroids only for three months. They are subsequently maintained without immunosuppressive medication given that the allograft and recipient are immunologically identical.

GVHD

- BAF, CD86, IL-4 from the recipient, GARP, IL-10, IL-13 from the donor, and CD22, CD80, NOD2/CARD15, TACI from both involved in GVHD.
- B-cell activation: BAF, TACI, CD22
- T_{H2} response: IL-4.IL-13, IL-10
- T-cell regulation: GARP
- Innate immunity: NOD2/CARD15
- HSV+ T cells administered to haploidentical recipient in bone marrow transplant associated with reconstitution of thymus with HSV- T cells and with production of IL-17; suicide HSV+ T cells then controls GVHD

Secondary amyloidosis

- Reactive
- AA
- Proteolyzed form of liver produced serum amyloid associated (SAA) protein in response to inflammation
- Not an immunoglobulin homologue
- Bound to HDL (high density lipoprotein)
- IL-1, IL-6 stimulate
- Cardiac involvement uncommon (5% of patients)
- Associated with inflammation and chronic infection
- Rheumatoid arthritis (3% of patients)
- Inflammatory bowel disease
- Ankylosing spondylitis

amyloidosis foundation

Common AA Amyloidosis Signs/Symptoms

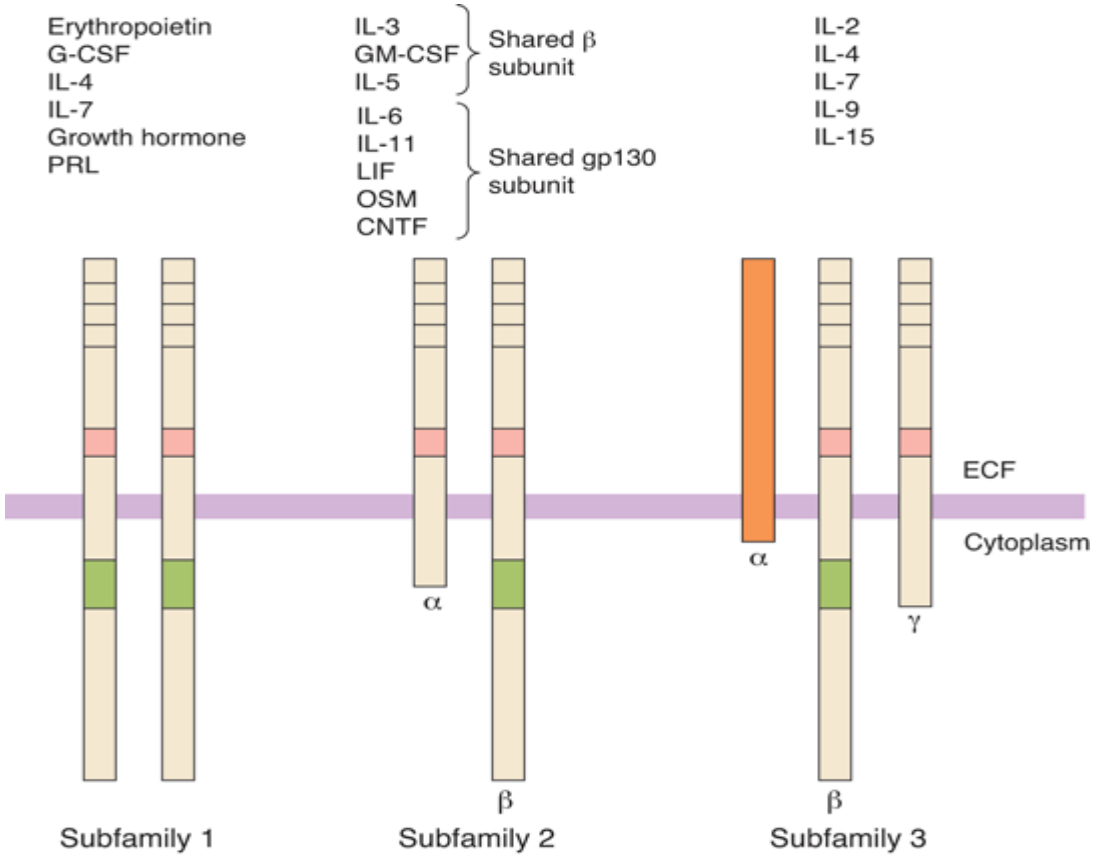
- Swelling of ankles & legs
- Enlarged spleen, liver or thyroid
- Protein in the urine
- High cholesterol
- Renal failure
- Weight loss/Weakness
- Low blood pressure upon standing
- Diarrhea/Constipation

www.amyloidosis.org

Activated in GVHD

B-cell	T-cell	T_{reg}	T_{H17}	Innate Immunity	Autoimmunity
BAFF APRIL BCMA	IL-2 IL-4	FOXP3 GARP INDO	IL-17A IL-17B	NOD2/CARD15 TNF PAI-1	IRF5 STAT4 CTGF
CD80 CD86 CTLA4	IL-13 IL-10			IFN-γ MPO CD31	CCL2 CCL9
CD40 CD22	CD28				
FCRL3	TGFβ				
TACI IL6 CD19	CD40L				

Cytokine receptor superfamilies



(Modified from D'Andrea AD: Cytokine receptors in congenital hematopoietic disease. *N Engl J Med* 1994;330:839.)

Fig. 3-4 Accessed 07/01/2010

Important cytokines

Cytokine	Major Source	Major Function
IL-1	Macrophages	Proinflammatory cytokine; activates CD4 and endothelial cells
IL-2	T_{H1}subset of T cells	Activates B, CD4 and CD8 cells.
IL-4	T_{H2} subset of T cells	Stimulates B-cell growth Increases T_{H2} subset of CD4 cells Increases isotype switching and IgE
IL-5	T_{H2} subset of T cells	Stimulates B-cell differentiation Increases eosinophils and IgA
IFN-γ	T_{H1} subset of T cells	Increases class I and II MHC expression Stimulate phagocytosis and killing by macrophages and NK cells
TNF-α	Macrophages	Proinflammatory cytokine Activates neutrophils and endothelial cell adhesion

Important cytokines

Type	Major Source	Function
IL-6	CD4 cells and macrophages	Stimulates B-cell differentiation Acute phase reactant
IL-8	Activated mononuclear cells	Attracts neutrophils (Cysteine-amino acid- cysteine characterizes polypeptide; α-class chemokine)
IL-10	T_{H2} subset	Regulate T_{H1} production by limiting IFN-γ production
Il-12	T_{H1} subset	Promotes development of T_{H1} subset
TGF-β	CD4 T cells	IgA switch; stimulate B cells, macrophages, neutrophils; diminish T cell function

Other important cytokines

Type	Major Source	Function	
IL-13	T_{H2} subset	Binds to receptor that shares chain with IL-4 receptor Induces IgE production	Airway hyperresponsive
RANTES MCAF	Activated T cells	Attracts monocytes	Cysteine-- cysteine characterizes polypeptide β-class chemokine
IL-3	Activated CD4 cells	Stimulates stem cell growth and differentiation	
GM-CSF	T lymphocytes and macrophages	Stimulates neutrophil and macrophage growth	
IFN-α IFN-β	Leukocytes Fibroblasts	Block viral protein synthesis (inactivating elongation factor; activate RNA endonuclease)	May be induced by double stranded RNA as well

Immunosuppression

- Prednisone is metabolized in the liver to prednisolone.
- Blocks release of lymphocytes into the circulation.
- Inhibits glucose transport into the cell as well as blocks phosphorylation upstream of NF-κB transcription (regulates genes involved in immune expression).
- Induction of cell death in immature lymphocytes.
- ABC cassette system (P-glycoprotien) resistance leads to increased drug efflux from cell.

Immunosuppression

- Azathioprine is an antimetabolite that antagonizes purine metabolism and may inhibit synthesis of DNA, RNA, and proteins.
- Metabolized in the liver to 6-mercaptopurine (6-MP), by glutathione S-transferase.
- Three major pathways for further metabolism of 6-MP in the liver and GI tract:
- (1) Hypoxanthine guanine phosphoribosyl-transferase (to 6-thioguanine-nucleotides, or 6-TGN, the major metabolite). Incorporates into DNA.

Immunosuppression

- (2) Xanthine oxidase (to 6-thiouric acid, inactive). Allopurinol increases toxic metabolites by blocking this pathway.
- (3) Thiopurine methyltransferase (TPMT), which forms 6-methylmercapotpurine (6-MMP). Blocks de novo purine synthesis.
- Mycophenolate mofetil blocks purine biosynthesis (noncompetitive inhibitor of inosine monophosphate dehydrogenase).
- May see Crohn like enterocolitis. Contraindicated in pregnancy.

Immunosuppression

- Cyclosporine inhibits a nuclear transcription factor that leads to a block of production and release of IL-2 and inhibits IL-2 induced activation of resting T-lymphocytes.
- Widely distributed in the body; crosses the placenta.
- ABC cassette system (P-glycoprotein) resistance leads to increased drug efflux from cell.
- Long latency period (time to which drug adequate levels are achieved).

Immunosuppressive agents

- Rapamycin binds to mTOR. Inhibits T-cell response to IL-2. Used if calcineurin toxicity.
- OKT3 is a murine antibody that binds to CD3 (ε-chain), blocking cellular interaction with CD3 protein responsible for T-cell signal transduction.

MICROBIOME AND IMMUNITY

Overview

- Bacterial metabolites, anti-microbial peptides and bacteria can prime dendritic cells (DCs) which migrate to the lymph nodes to stimulate T and B cells.
- Pathogen associated molecular patterns (PAMPs) from the gut may also interact directly with immune cells, inducing activation and maturation of antigen-presenting cells (APCs) including DCs.
- These mature APCs may then translocate into mesenteric lymph nodes and mediate priming of lymphocytes, and disseminate systemically and induce the differentiation of naïve T cells into CD4+ T cells which function at distant sites.

Short chain fatty acids

- Short chain fatty acids (SCFAs) and polyamines are the most abundant microbial metabolites in the gut.
- SCFAs include acetate, propionate and butyrate.
- Produced by bacterial fermentation in the gut from dietary fibers that remain undigested or partially digested.
- Serve as the major energy sources of intestinal epithelial cells and maintain gut barrier integrity.
- SCFA may bind to G-coupled protein receptors (GPR43 and GPR41) of intestinal epithelial cells, altering host gene expression and inducing autophagy and stimulating production of anti-inflammatory cytokines.

Short chain fatty acids

- SCFAs deactivate NF-κB and abrogate the expression of the pro-inflammatory cytokine TNF in mononuclear cells and neutrophils
- Also mediate immune cell phenotype through epigenetic mechanism, by inhibiting histone deacetylases (HDACs)
- This mechanism can promote CD8+ memory cell differentiation.
- SCFAs also influence antigen-specific adaptive immunity by stimulating the synthesis of IgA by B cells

Short chain fatty acids

- The acetate producing bacterium Bifidobacterium longum prevents the translocation of toxins from enteropathogenic Escherichia coli O157:H7 into the systemic compartment.
- Segmented filamentous bacteria (SFB) can induce secretion of IL-22 from ILC3 cells.
- Drive TH17 polarization in lamina propria DCs via production of serum amyloid A

Other metabolites

- Compounds such as tryptophan, indole, and bile acids may interact with aryl hydrocarbon receptor (AhR) and pregnane X receptor (PXR).
- Upregulate the expression of tight junctions
- Induce cytokine production by AhR expressing lymphoid cells.
- In contrast, AhR expression on Tregs affects their homing and suppressive activity

Other metabolites

- Lactobacillus spp., produce metabolites by metabolizing dietary tryptophan. These metabolites bind to the aryl hydrocarbon receptor (AHR) on a variety of cells, regulating colonic inflammation.
- IL-22 producing IL C3 cells activated.
- Inosine significantly promotes the differentiation of Th1 cells in the presence of exogenous IFN-γ by acting on the A2A receptor on T cells.
- IFN-γ inhibits the function of M2 macrophages induced by IL-4/IL-13.

Polyamines

- Polyamines such as putrescine, spermidine and spermine induce the secretion of IgA in the gut as well as maintain barrier integrity.
- Elevated levels of polyamine in breastmilk promote the maturation of gut CD8+ and CD4+ T cells.
- The polyamine-producing bacterial strain Bifidobacterium animalis subsp. lactis LKM512 along with arginine diminishes levels of intestinal inflammatory cells.

Gut microbiota

- Gut microbes can stimulate the body to produce CD47 antibodies by activating STING signaling
- Bifidobacteria may affect activating DC cells, thereby improving the activity of antigen-specific $CD8^+$ T cells.

Gut microbiota

- Translation of gram-positive bacteria into secondary lymphoid tissues stimulate Th17 and Th1 immune response.
- IL-12 dependent.
- Bacteroides fragilis inhibits CTLA-4, restoring Th1 immune responses
- Supplementation with inulin and fructo-oligosaccharide may select for such beneficial bacteria as Lactobacillus and Bifidobacterium

Gut microbiota and tumor response

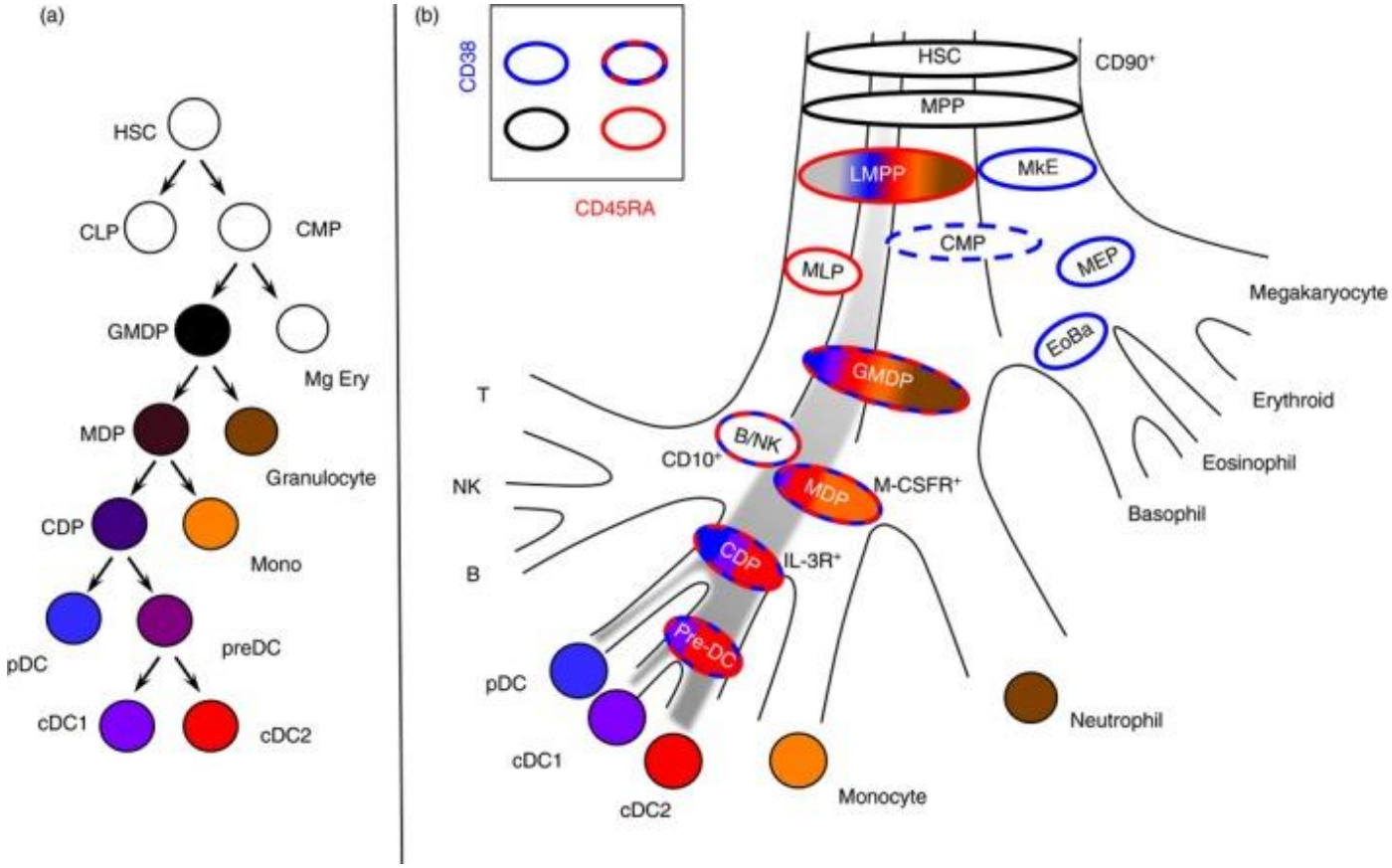
Tumor microenvironment

- The main cells of the tumor microenvironment (TME) in cancer immunity are NK cells, DC cells, CD8 + T cells, Treg cells, fibroblasts, tumor associated macrophages (TAMs), and myeloid derived suppressor cells (MDSCs).
- NK cells induce the death of tumor cells by the ways of releasing perforin and granzyme, secreting tumor necrosis factor-a, and mediating cytotoxicity by TRAIL and FAS1 receptors.

Tumor microenvironment

- Myeloid cDC1 cells are able to promote the differentiation and maturation of $CD8^+$ T cells, and cDC1 cells can recruit CCL5 and XCL1, which induce the accumulation of cDC1 cells in the TME, thereby improving the immune control of tumors.
- IL-2 contributes to enhancing the antitumor activity of NK cells.
- When CD4 + T cells migrate to lymph nodes, cDC2 can activate $CD4^+$ T cell responses. cDC2 resistant $CD4^+$ T cells can be inhibited by Treg cells.

Classic and revised models of hematopoiesis



Common myeloid progenitors are mixtures of mega-erythroid and myeloid precursors and the most significant early partitioning of cell fate occurs when megakaryocyte and erythroid potentials separate from lympho-myeloid potentials.

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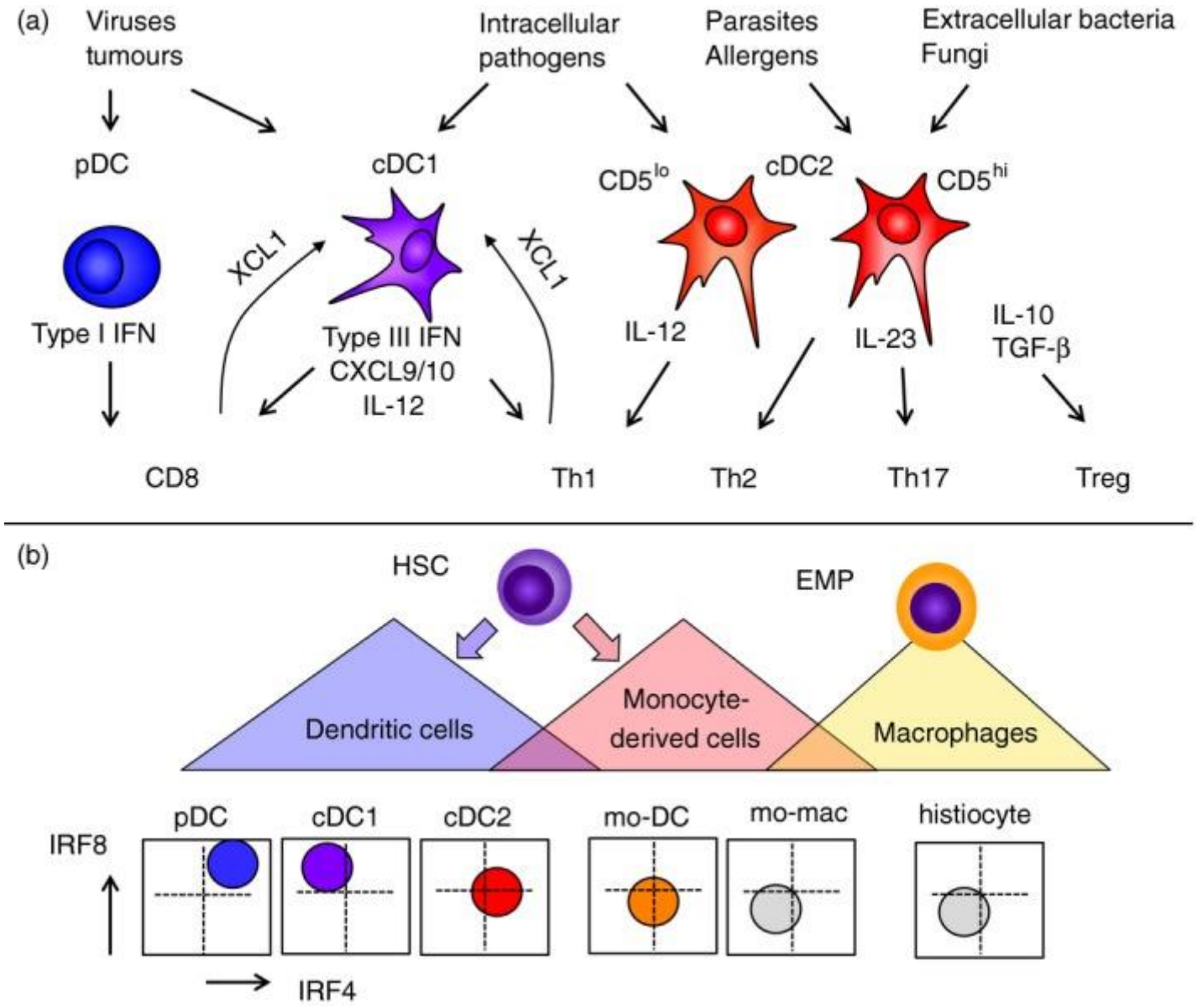

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Tumor microenvironment

- VEGFA activates cancer associated fibroblasts, which secrete FSP1.
- TAMs can promote the growth and metastasis of tumor cells through multiple pathways. They are one of the dominant cells in the tumor microenvironment.
- Lactate produced by cancer and acidification of the microenvironment increase ARG1 expression in TAMs.
- TAMs play an M2 role to produce high levels of reactive oxygen free radicals, promote DNA damage and genomic instability, tumor infiltration and metastasis, as well as participate in the digestion and reconstruction of extracellular matrix (ECM), inhibit

Tumor microenvironment

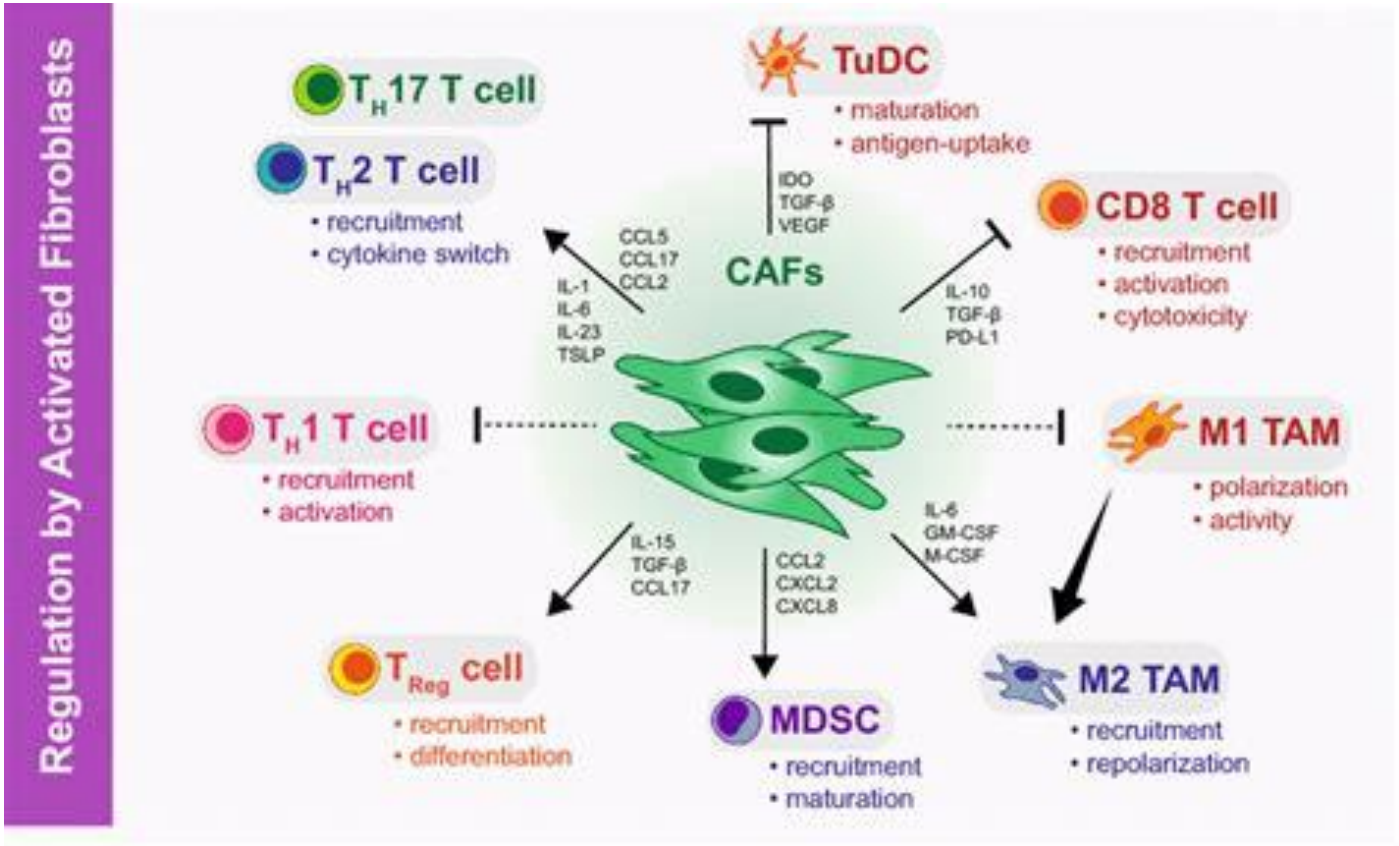
- MDSCs induce the nitration of the T-cell receptor/CD8 complex through the excessive production of reactive oxygen species and peroxynitrite in the process of cell-cell direct contact
- Leads to the inability of $CD8^+$ T cells to bind to peptide-MHC (pMHC) and affect the ability to respond to non-specific stimulation
- This $CD8^+$ T cell tolerance is one of the major mechanisms of tumor escape.
- MDSCs also increase the metabolism of L-arginine by producing arginase I, which inhibits T cell-lymphocyte reaction and blocks T-cell activation by consuming cysteine

Tumor microenvironment

- The stimulating factor 1 receptor (CSF1R) is significantly expressed in MDSCs and TAMs, which cause functionally reprogram the response of macrophage and enhance antigen presentation and anti-tumor T cell response.
- T cell checkpoint molecules, including PDL1 and CTLA4, are upregulated by CSF1R

Tumor microenvironment

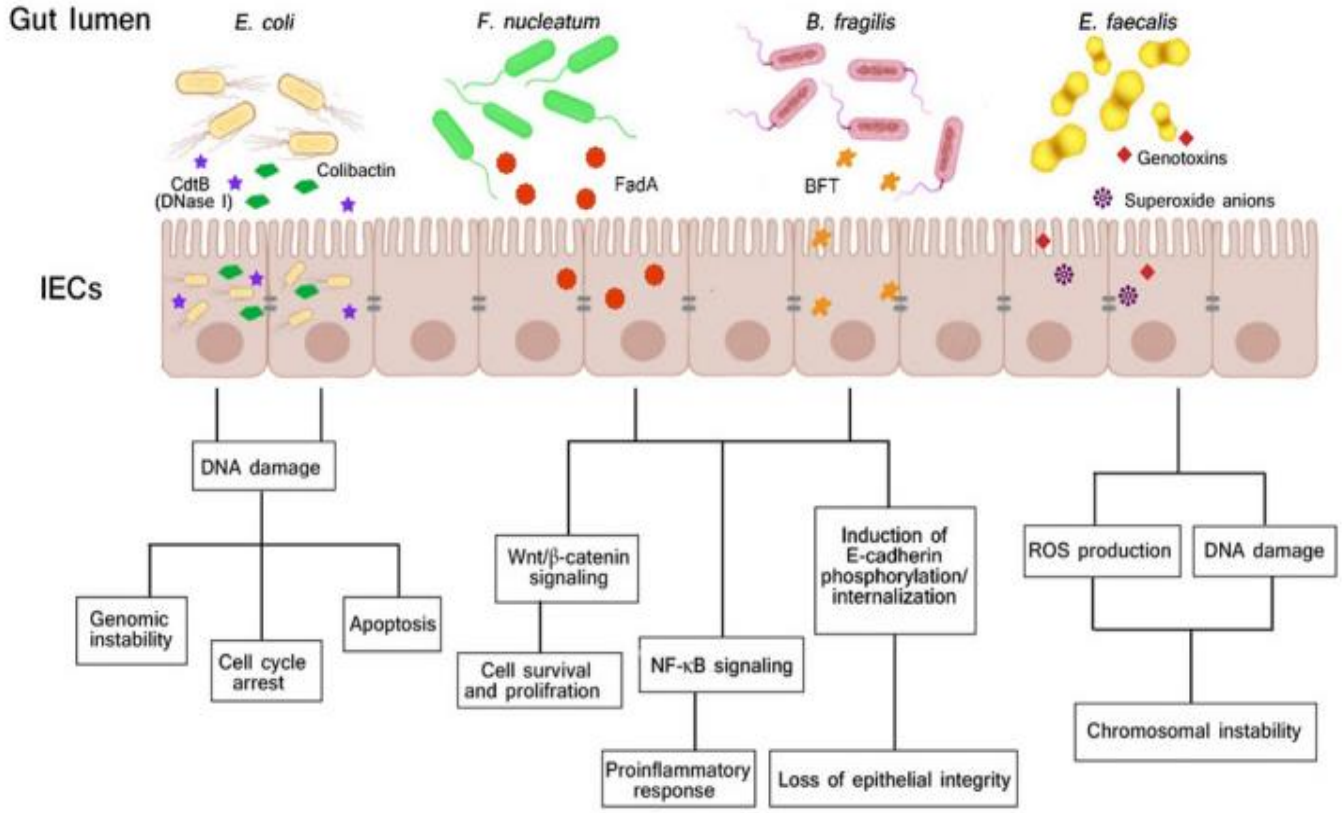
- Cancer associated fibroblasts (CAFs) inhibit the activity of cytotoxic T lymphocytes and recruit lymphocytes that produce inflammatory signals to promote cancer progression
- CAFs can direct or coordinate the infiltration of immune cells directly or through secreted cytokines and surface proteins, or indirectly and coordinate the infiltration of immune cells by depositing various ECM substrates and remodeling matrices, thereby promote cancer.



DOI:10.1007/s00262-017-2003-1

Tumor microenvironment

- TME promotes the occurrence of hepatocellular carcinoma (HCC), for example.
- NK cells and DCs participate in immune escape mechanisms
- 25% of NK cells are PD-1 positive
- Macrophages are involved in promotion of angiogenesis and tissue remodeling, and the production of cytokines and chemokines leading to persistent inflammation-related damage
- TLR4 signaling in tumor cells is able to recruit neutrophils, while TNF released by neutrophils is able to induce metastasis of tumor cells.



https://dx.doi.org/10.1002%2Fcam4.3694

Microbiota	Main effects on immunity	Potential effect on immunotherapy
Beneficial microbiota	Enhanced the antitumor efficacy of PD-L1 blockade, enhancement of DC maturation, improving activity of the tumor-specific $CD8^+$ T cells, increased IFN-γ production	Effective
Bifidobacterium		
Bacteroides fragilis, *Bacteroides thetaiotaomicron*, *Burkholderia cepacia*	Increased the efficacy of anti-CTLA-4 therapy by inducing Th1 response and promoting DC maturation, an increase in $CD8^+$ T cells and a decrease in Tregs in the tumor environment	Effective
Akkermansia muciniphila	Enhanced the infiltration of immune cells in tumor site, as $CCR9^+CXCR3^+CD4^+$ T cells were recruited to the tumor microenvironment and the ratio of $CD4^+$ T cells to $CD4^+FoxP3^+$ T cells (Tregs) was enhanced	Effective
Enterococcus hirae	Enhanced IL-12 secretion by DCs	Effective
Harmful microbiota	Increased host PD-1 and PD-L1 expression, higher level of pro-inflammatory cytokines (TNF-α), suppressed the proliferation of CD4+ T cells, the inhibitory effect can be blocked using antibodies PD-L1	Ineffective
Helicobacter pylori		
HBV, HCV, HPV, EBV	Established chronic infections in humans and increased host PD-1 or PD-L1 expression	Ineffective

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7897953/

Modification of immune response

- Elimination of gut microbiota enhances effect of TP53 mutations
- Accumulation of Bifidobacteria in the TME can significantly improve the antitumor efficacy of anti-CD47 immunotherapy
- Vancomycin enhances the blocking effect of CTLA-4 by increasing the proportion of Gram-negative Burkholderia and Bacteroides in the intestines

Modification of immune response

- The cross-priming of antigen-specific T cells of tumor-resident DCs can be enhanced by anti-CD47 therapy.
- In addition, type I IFN plays an important role in enhancing the adaptive immune response to anti-CD47 antibody therapy in tumor-resident DCs
- Patients with more Faecalibacterium have a significantly prolonged progression-free survival with a higher level of effector T cells and a stabilized cytokine response to PD-1 blockade.
- Simultaneously, systemic and anti-tumor immunity are also enhanced

Modification of immune response

- Elimination of gut microbiota enhances effect of TP53 mutations
- Accumulation of Bifidobacteria in the TME can significantly improve the antitumor efficacy of anti-CD47 immunotherapy
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Modification of immune response

- After taking probiotics, the abundance of butyrate-producing bacteria helps maintain the intact intestinal barrier to avoid the activation of inflammation-related factors in TME, in colon cancer, for example
- Lactobacillus casei, Rhamnose and Bifidobacterium have been shown to reduce radiotherapy-associated diarrhea by inhibiting the expression of TNF, IL1b, and IL6mRNA
- Fermented Lactobacillus is considered as weakening the response to immunotherapy

Modification of immune response

- The inhibition of the production of lactic acid in cancer cells (Warburg effect) helps to recover active oxygen homeostasis of physiological mitochondrial and restore normal function of cells
- Cyclophosphamide (CTX) induces immunogenic cancer cell death and immunomodulatory effects.
- Oral administration with Enterococcus hirae restores CTX anti-tumor efficacy by inducing differentiation of TH17 and pathogenic TH17 cells, promoting tumor-specific Th1 and CTL activity.

Modification of immune response

- Abiraterone acetate is both an inhibitor of androgen biosynthesis and a highly effective drug of prostate cancer
- Reduces harmful microorganisms through altering gut microbiota
- Lenalidomide can enhance the cytotoxicity mediated by NK cell and ADCC
- IFN-γ and celecoxib inhibit M2 differentiation