Mediators of the inflammatory process II

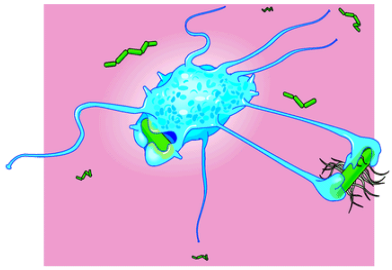
1

The components/mediators of inflammation.

- Cells..
  - Fixed cells such as vascular cells.
  - Migratory cells such as PMNs.
- Mediators..
  - many chemicals released into the body.
- Immune system..
  - Innate.
  - Acquired.

2

Phagocytes.

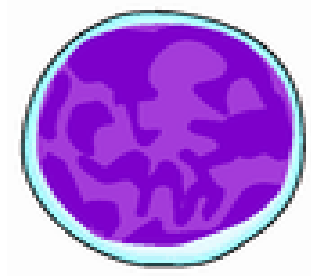


- Uptake of foreign organisms.
- Destruction of micro-organisms etc.
- Many microbiocidal weapons e.g. lytic enzymes, active oxygen etc.

See immunology course..

3

Natural killer (NK) cells.



- Cytotoxic potential.
- Attacks invading, infected or transformed cells.
- Differs from T-cells in the way in which they ‘recognise’ their targets.
- Secrete toxic proteins.
- Sometimes involved in acute rejection.

See immunology course..

4

T-cell mediated immunity.

- The primary immune response.
- Immunological ‘memory’.
- Some effector functions.

See immunology course..

5

Non-immune mediators.

- Soluble chemicals released by injured, activated or dying cells.
- Regulate, activate and terminate the inflammatory response.
- Some are fairly ‘insult specific’, others more generally found in lesions.

6

A ‘chemical mediator‘ ….

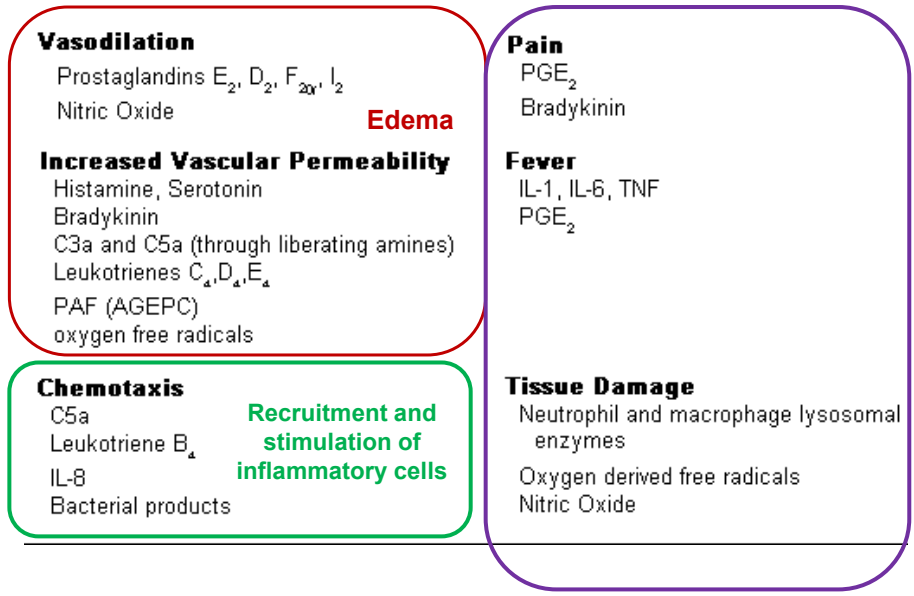
- .. is found in tissues in concentrations that can explain the observed symptoms or effects.
- .. is released by the endogenous trigger which produces the response.
- .. has the same action in all species where the phenomenon occurs.
- .. Is destroyed locally or systemically to avoid undue accumulation.
- .. is blocked (directly or indirectly) by inhibitors of inflammation.

7

The mediators of inflammation.

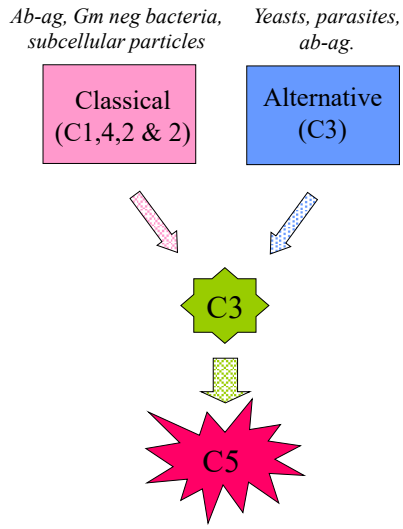
- Plasma proteins such as complement and antibodies.
- Cytokines and chemokines.
- Lipids such as prostaglandins and PAF.
- Amines such as histamine.
- ‘Gasses’ such as NO and $O2^{-}$ (superoxide).
- Kinins such as bradykinin.
- Neuropeptides such as substance P.

8



9

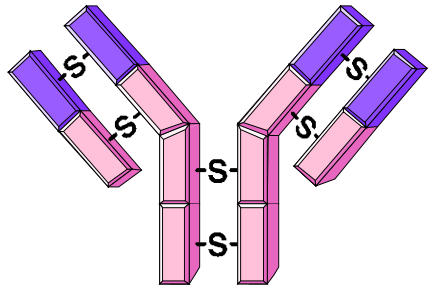
Complement.



- A complex series of about 20 proteolytic enzymes in the blood.
- 'Classical' and 'alternate' pathways act in a cascade fashion.
- Accelerated in the presence of IgGs
- Lytic to many micro-organisms.
- 'Opsonise' others.

10

Antibody mediated effects.

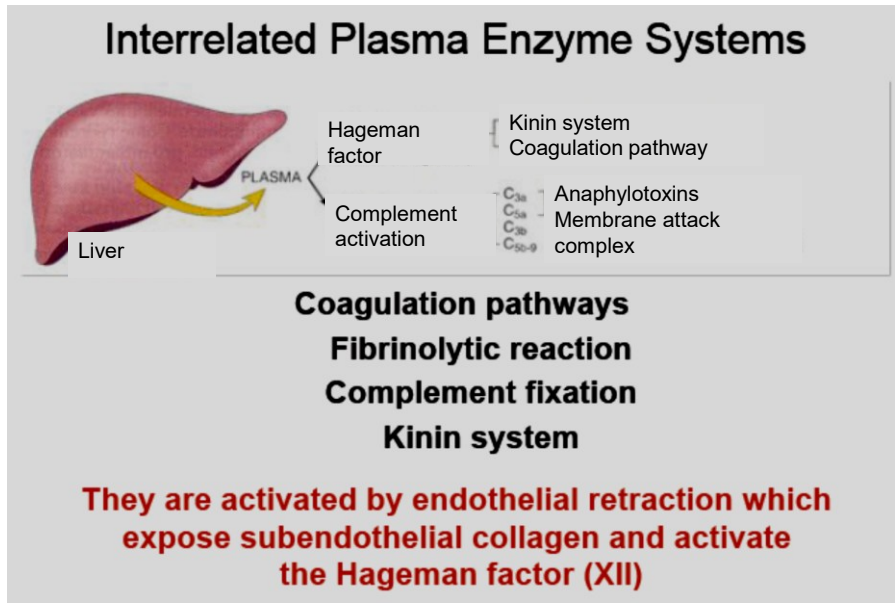


- IgG, IgA, IgM, IgD, IgE subtypes.
- Fab region recognises antigen.
- Fc region important for host defence functions
- Responsible for antibody mediated immunity and some ‘innate’ immunity.

See immunology course..

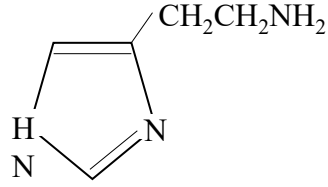
11

Plasmatic mediators



12

Histamine.



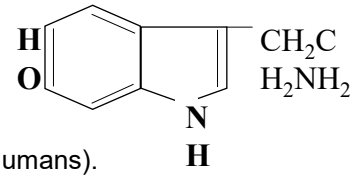
- Formed from *histidine*.
- Stored in high concentrations in mast cells and basophils together with heparin and ATP.
- Three main receptor subtypes (H_1 etc).
- Important in allergies, itch, inflammatory response.

- Assumed to be responsible for anaphylaxis by Dale and Laidlaw (1911, 1960) as synthetic material had the same effects.
- The development of anti-histamine in the 1940's led to the realisation that histamine was not the only inflammatory mediator.

13

serotonin (5-hydroxytryptamine)

- Monoamine neurotransmitter: Found in platelets (8%), neurones and in CNS, enterochromaffin cells (gastrointestinal tract).
- Inactivated by MAO (monoamine oxidase).



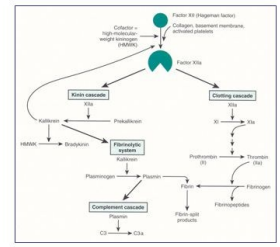
- Multiple receptors (14 receptors in humans).
- Affects mood, anxiety, sleep, appetite, body temperature, bowel movements
- When platelets bind a cloth, they release serotonin, Involved in vasoconstriction (high concentration) or vasodilation.

14

HAGEMAN FACTOR

Dependent Factors

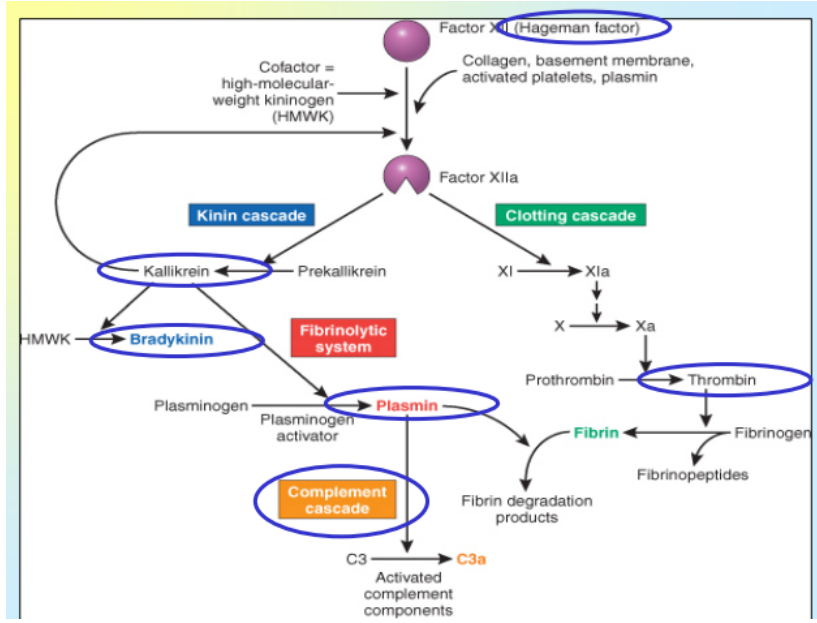
- Factor XII of intrinsic coagulation cascade
- Activated by
  - Negatively charged surfaces
  - Platelets
  - Proteases from inflammatory cells
- Causes
  - Coagulation
  - Activation of fibrinolytic system
  - Produces bradykinin
  - Activates complement
  - Provides an amplification system



In vivo, factor XII is activated by contact to polyanions. Activated platelets secrete inorganic polymers, polyphosphates. Contact to polyphosphates activates factor XII

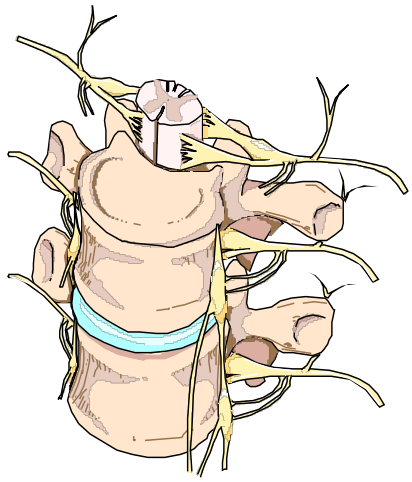
15

Hageman factor plays a central role in the activation of plasmatic mediators



16

Neuropeptides

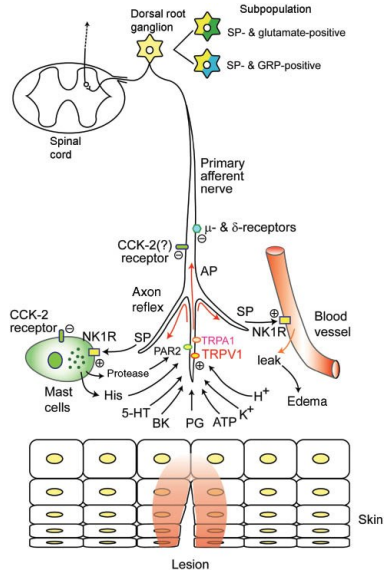


- Tachykinins
  - substance P
  - neurokinin A
  - neurokinin B
  - CGRP
- Kinins:
  - bradykinin
  - kallidin

17

Tachykinins

- Substance P (SP).
- Neurokins A & B.



- Mainly located in sensory neurones.
- Released on nerve stimulation.
- Act on 7TM 'NK' receptors (3 subtypes; NK_1 etc).
- Cause vasodilatation, vascular permeability, smooth muscle contraction, mucus secretion, pain.

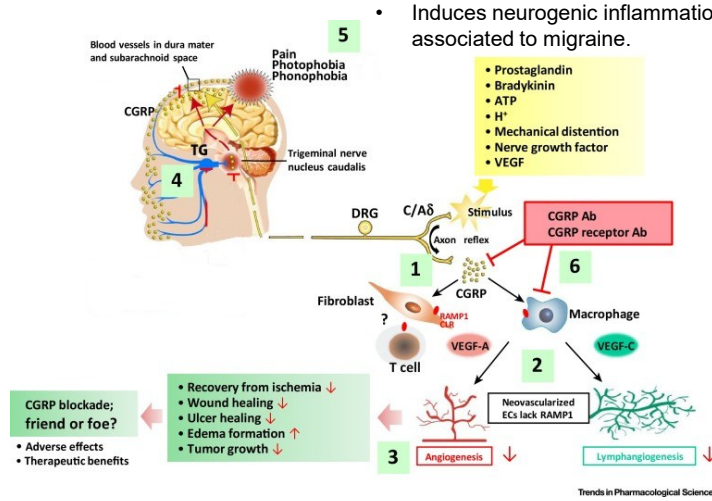
SP triggers degranulation of mast cells releasing histamine and protease and increasing vascular permeability through NK1R.
Activation of both μ- and δ-receptors attenuate peripheral pain, and activation of CCK receptors attenuate itch and degranulation of mast cells

18

Tachykinins.

- CGRP *Calcitonin Gene Related Peptide*.

- A product of the calcitonin gene generated through differential splicing.
- Found in sensory neurons.
- Induces neurogenic inflammation, and associated to migraine.



19

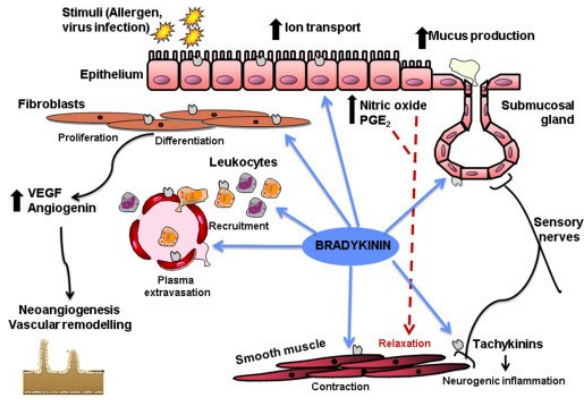
A. Kinin system

- The kinins are vasoactive peptides derived from plasma protein **(kininogens)** by the action of specific proteases called Kallikreins.
- Bradykinin is the most important Kinin .

20

Kinins.

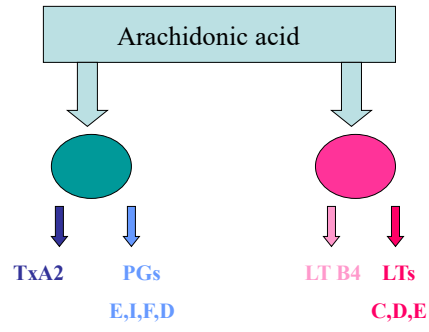
- Bradykinin (9 aa)
- Kallidin (10 aa).



- Formed from *kininogens* (2 forms) by *kallikreins* (also 2 forms).
- Inactivated by *kininases* (2 forms).
- Two receptors B_1 (inducible) and B_2 (constitutive).
- Produce; vasodilation, smooth muscle contraction, pain and inflammation.
- Anti-proteases and receptor antagonists are occasionally useful.

21

Eicosanoids



- Arachidonic acid from cellular phospholipids.
- At least 2 different pathways:
  - cycloxygenase forms *prostaglandins* and *thromboxanes*.
  - lipoxygenase forms *leukotrienes*.

22

The prostaglandin (PG) system.

- PGs discovered in seminal vesicles and in human plasma (1930s).
- Synthesis from essential fatty acids demonstrated (1960s).
- Aspirin like drugs prevent PG synthesis and this explains mechanism of action (1970s).
- Multiple forms of cyclo-oxygenase discovered (1990s).

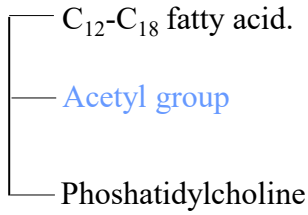
23

PAF (platelet activating factor).

- Modified *phospholipid*.
- Synthesised by many cells including PMN, monocytes, mast cells and eosinophils.
- Acts through specific G-protein linked receptors.
- Sometimes acts intracellularly.
- Causes increased vascular permeability, PMN migration, brochoconstriction and many other signs and symptoms of inflammation.
- PAF *receptor antagonists* useful treatment in experimental models.

24

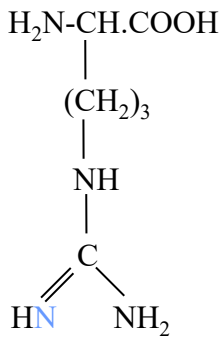
Synthesis of PAF



- PAF formed from phoshatidyl choline by an acetylase.
- Key role of phospholipase A_2

25

Nitric oxide (NO; EDRF Endothelium-derived relaxing factor)



- Formed in many tissues from *arginine*.
- Three enzymes (NOS) described; *iNOS, ncNOS & ecNOS.*
- Responsible for Non-Adrenergic-Non-Colinergic transmission.
- Potent *vasodilator* and microbiocidal.
- Physiological effects dependent of *guanylate cyclase* activation.

26

iNOS (Inducible nitric oxide synthase).

- Induced in cells by *cytokines*, TNFα, IL1β or LPS.
- iNOS does not require Ca^{2+} for activation, only a supply of *arginine*.
- GCs, IL10 and some other factors can inhibit iNOS or its induction.
- With active oxygen, NO can form *peroxynitrite* which is a potent cytotoxic agent.
- Can be blocked in (e.g.septic shock) by arginine analogues such as *L-NMMA*.
- NO is scavenged by haemoglobin and reacts with *thiols*.

27

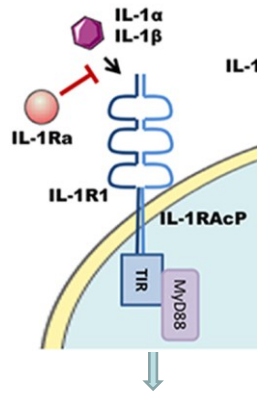
Cytokines.

- All are proteins.
- Mainly synthesised by immune cells.
- Regulate differentiation and activation of immune cells.
- Partly responsible for coordination of the inflammatory response.
- Act through high affinity receptors on target cells.

28

Key cytokines which activate the inflammatory response

- **IL1**



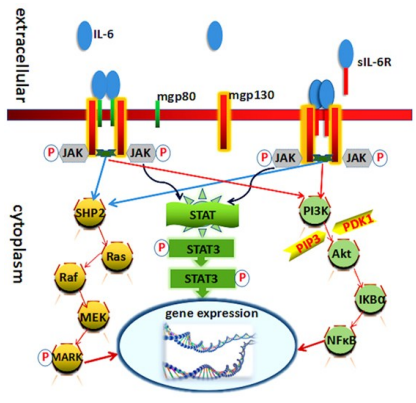
NF-kB, JNK, ERK, p38

- Two forms: IL1α & IL1 β
- 17Kd mw
- Produced by monocytes and many other cells.
- Soluble IL1 receptor regulates their activity.
- Activate lymphocytes and many inflammatory cells.

29

Key cytokines which activate the inflammatory response

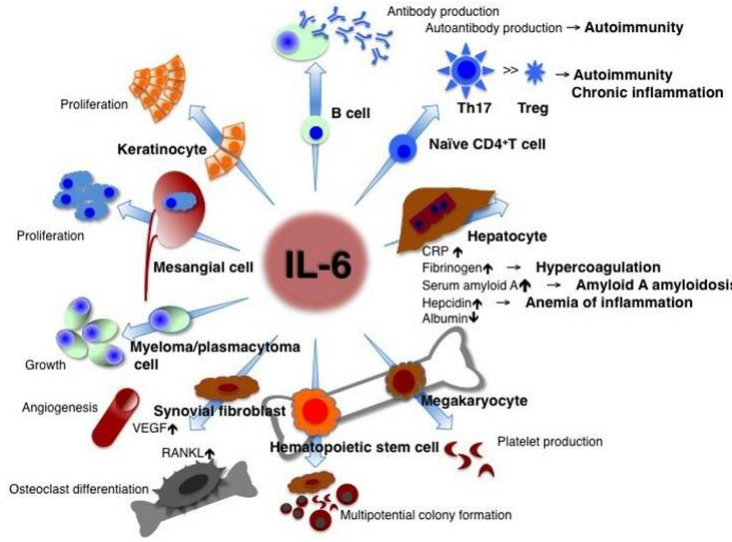
- **IL6**



Survival, proliferation, migration

- 26Kd mw.
- Produced by T-cells but also by many other cells too.
- Activates B & T-cells and other cell type.
- Promotes Survival, proliferation, migration, etc.
- IL-6 may have also inhibitory functions

30

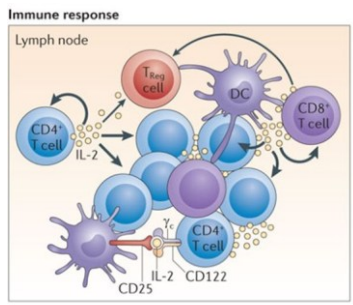


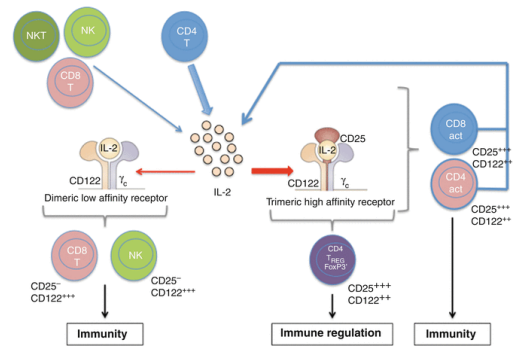
31

Key cytokines which activate the inflammatory response

- IL2

- 15Kd mw.
- Produced by T-cells.
- Activates T-cells, monocytes and NK cells.

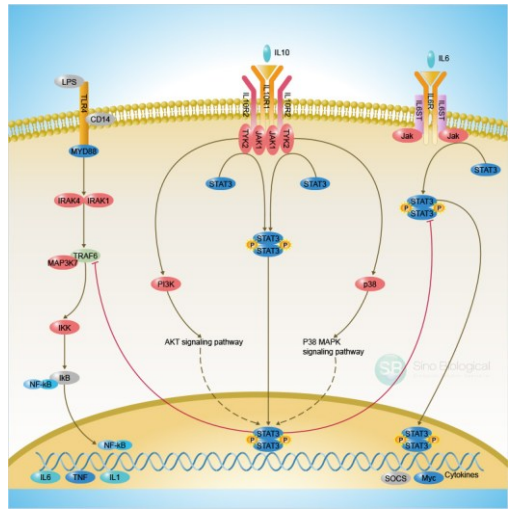




32

Key cytokines which regulate the inflammatory response

- IL10.

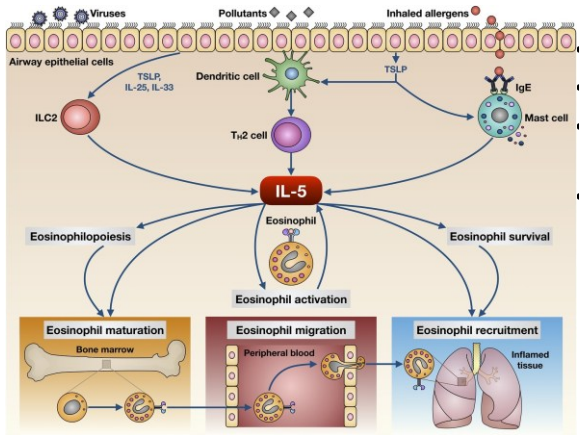


- Produced by T-cells.
- Stimulation of mast cell replication.
- Inhibits cellular immune reactions.

33

Key cytokines which activate the inflammatory response

- IL5

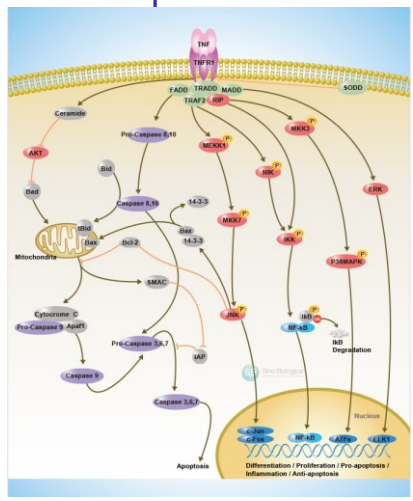


- 45-60Kd mw.
- Produced by T-cells.
- Increases B-cell proliferation.
- Promotes eosinophil maturation and inhibits macrophage activation.

34

Key cytokines which activate the inflammatory response

- TNF-alpha

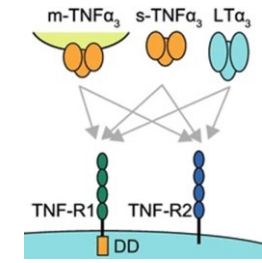


- Two forms found, TNFα and TNFβ (lymphotoxin α).
- 17Kd mw.
- Produced by many cells including monocytes (TNFα) .
- Widespread activation of cells; apoptosis, shock, cachexia etc.

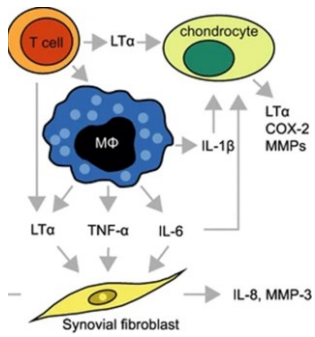
35

TNF-β (lymphotoxinα, LTα)

Produced mainly by T-cells.



Binding of LTα and TNFα to their receptors (TNF-R1 and TNF-R2).
Membrane-bound trimeric TNFα (m-TNFα$_3$) binds TNF-R2 with higher affinity than TNF-R1.
Soluble trimeric TNFα (s-TNFα$_3$) binds TNFR1 with higher affinity than TNF-R2.
LTα$_3$ binds TNF-R1 and TNF-R2 with equal affinity.
TNFR1 mediates proinflammatory signaling
TNFR2 mediates immunomodulatory signaling
DD death domain,
TNF-R tumor necrosis factor receptor

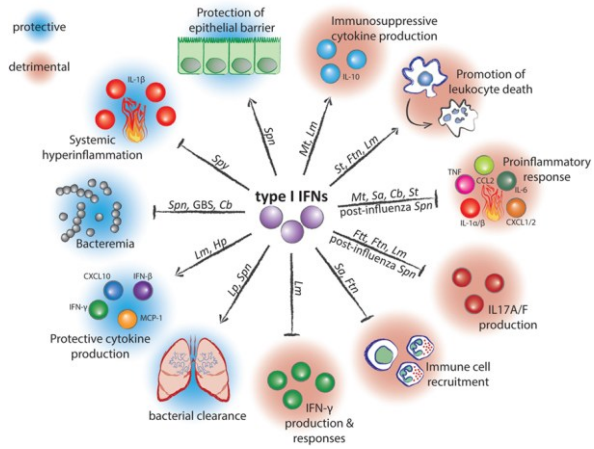


LTα activates NF-κB and other cytokines in synovial fibroblasts, resulting in their proliferation and proinflammatory cytokine secretion.
LTα can also activate NF-κB and the proinflammatory cascade in chondrocytes, resulting in loss of the cartilaginous matrix component characteristics of RA joints.

36

Key cytokines which modulate the inflammatory response.

- Interferons (IFNs).



- 3 forms found: α,β & γ.
- Many different subtypes.
- Generally 19-26 Kd mw.
- Produced by monocytes (α), fibroblasts (β) and T-cells (γ).
- Antiviral, cell activating and tumour suppressant effects.

37

Strategies for inhibiting inflammatory cytokines.

- Reduce cytokine producing cells (e.g. with cytostatics).
- Production of Inhibitory cytokines (e.g. IL 10).
- Inhibitors of signal transduction (e.g.cyclosporin).
- Regulation of gene expression (e.g. glucocorticoids)
- Inhibitors of release (e.g. ICE inhibitors)
- Reduction in circulating cytokines(e.g. monoclonals, soluble receptors)
- Receptor blockade (e.g. antagonists or monoclonals).

38

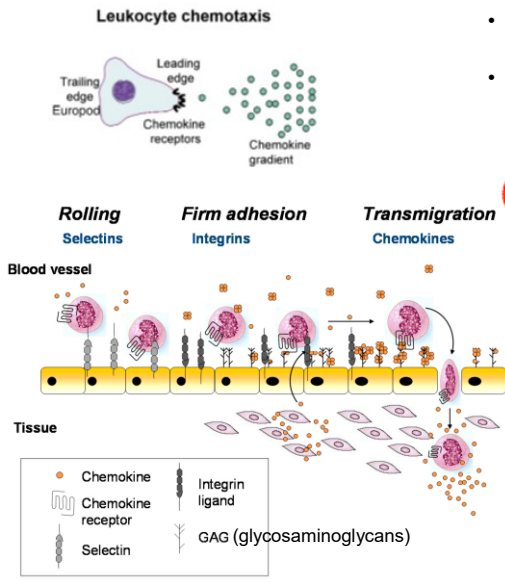
Chemokines.

- The word chemokine is a combination of the words chemotactic and cytokine, in other words cytokines that promote chemotaxis.

- However, chemokines can modulate additional leukocyte functions, (e.g. CCR7 which also regulates survival, migratory speed, endocytosis, differentiation and cytoarchitecture).
- Based on the activities that they regulate, chemokine receptors can be classified into inflammatory (which control both inflammatory and homeostatic functions) and homeostatic families.
- The non-chemotactic functions controlled by chemokine receptors may contribute to optimizing leukocyte functioning under normal physiological conditions and during inflammation

39

Chemokines



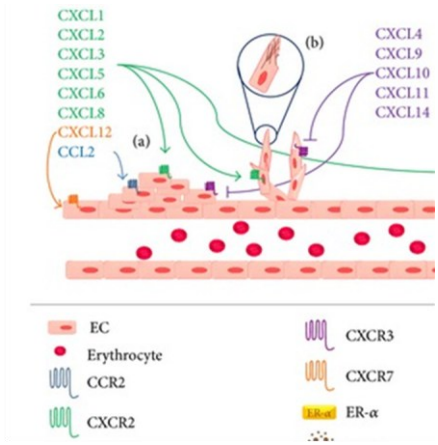
- At least 3 families of small proteins (mw usually 7-15Kd).
- Relative position of *Cys* residue determines nomenclature e.g. C, CC or CXC.

Act through 7TM receptors (which also function as co-receptors for HIV entry into immune cells).

40

CXC Chemokines

- IL8 (CXCL8) known as neutrophil chemotactic factor.
- Platelet factor IV (CXCL4).
- Granulocyte chemotactic protein 2 (CXCL6).
- Platelet basic protein (CXCL7).



- Cytokines CXC have a pivotal role in the control of inflammation and angiogenesis, as a result of the shared expression of their specific receptors by leukocytes and endothelial cells.
- Utilise CXC Receptors 1-5.
- Main targets PMN and endothelial cells.

41

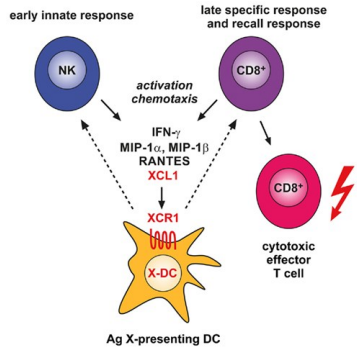
C-C Chemokines

- MCP-1 (CCL2),2 (CCL8),3,&4.
- RANTES (CCL5)
- MIP1α (CCL3) & β (CCL4).
- Eotaxin (CCL11)

- The CC class of chemokines consists of at least 28 members (CCL1-28).
- They utilise CCR1-10 receptors.
- CC chemokine receptors are expressed predominantly by eosinophils, T cells and monocyte-macrophages.

42

C Chemokines



- The C subfamily lacks the first and third cysteine residues.
- Lymphotactin (also known as SCM-1 alpha) and SCM-1 beta are currently the only two family members.
- Lymphotactin produced by T, NK, and NKT cells during infectious and inflammatory responses
- Target are dendritic cells

43

Subfamily	Act on	Sub-group	Nomenclature name	Common name
CC Chemokines	Monocytes Eosinophils Basophils T lymphocytes NK cells Dendritic cells	MCP/Eotaxin (allergenic)	CCL1 CCL2 CCL7 CCL8 CCL11 CCL12 CCL13 CCL24 CCL26	I-309 MCP-1 MCP-3 MCP-2 Eotaxin MCP-5# MCP-4* Eotaxin-2 Eotaxin-3*
		Inflammatory	CCL3 CCL4 CCL5 CCL6 CCL9 CCL10 CCL18	MIP-1α MIP-1β RANTES C10# MIP-1γ# CCL10# MIP-4*
		HCC	CCL14 CCL15 CCL16 CCL23	CC-1* Leukotactin-1* LEC MPIF-1*
		Devlop-mental	CCL17 CCL22 CCL25	TARC MDC TECK
		Homeo-static	CCL19 CCL20 CCL21	ELC LARC SLC
		Others	CCL27 CCL28	ESkine MEC
CXC Chemokines	Neutrophils Lymphocytes Monocytes	ELR+	CXCL1 CXCL2 CXCL3 CXCL5 CXCL6 CXCL7 CXCL8 CXCL15	GROα GROβ GROγ ENA-78 GCP-2 NAP-2 IL-8 Lungkine
		ELR-	CXCL4 CXCL9 CXCL10 CXCL11 CXCL12 CXCL13 CXCL14 CXCL16	PF-4 MIG IP-10 I-TAC SDF-1 BCA-1 BRAK CXCL16
C Chemokines	Lymphocytes		XCL1 XCL2	Lymphotactin α Lymphotactin β*
CX3C Chemokines	T lymphocytes Monocytes NK cells		CX3CL1	Fractalkine

* human only, # mouse only

44

Other Mediators which suppress inflammation

- ACTH, GlucoCorticoids and products of the hypothalamic–pituitary–adrenal axis.
- Some cytokines such as IL-10.
- Some induced proteins such as anti-proteases and lipocortin 1(annexin 1).

45

Mediator	Principal Sources	Actions
Cell-Derived		
Histamines	Mast cells, basophils, platelets	Vasodilation, increased vascular permeability, endothelial activation
Serotonin	Platelets	Vasodilation, increased vascular permeability
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased vascular permeability, chemotaxis, leukocyte adhesion and activation
Platelet-activating factor	Leukocytes, mast cells	Vasodilation, increased vascular permeability, leukocyte adhesion, chemotaxis, degranulation, oxidative burst
Reactive oxygen species	Leukocytes	Killing of microbes, tissue damage
Nitric oxide	Endothelium, macrophages	Vascular smooth muscle relaxation, killing of microbes
Cytokines (tumor necrosis factor [TNF], interleukin 1 [IL-1])	Macrophages, endothelial cells, mast cells	Local endothelial activation (expression of adhesion molecules), fever/pain/anorexia/hypotension, decreased vascular resistance (shock)
Chemokines	Leukocytes, activated macrophages	Chemotaxis, leukocyte activation
Plasma Protein-Derived		
Complement products (C5a, C3a, C4a)	Plasma (produced in liver)	Leukocyte chemotaxis and activation, vasodilation (mast cell stimulation)
Kinins	Plasma (produced in liver)	Increased vascular permeability, smooth muscle contraction, vasodilation, pain
Proteases activated during coagulation	Plasma (produced in liver)	Endothelial activation, leukocyte recruitment

46

What we do know about the plurality of mediators?

- Different mediators are required to produce different aspects of the inflammatory response.
- Sequential release is necessary throughout to co-ordinate the process.
- Synergism between mediators is required to produce the full response.

47