

Pharmacology of inflammation and immunosuppression

Inflammation = local reaction to injury or invasion associated with swelling and pain

- Innate responses (relatively non specific) include blood borne chemical mediators, e.g. complement and kinins, are ready for immediate action.
- Adaptive responses (highly specific – to a particular antigen) involves the action of lymphocytes, and are slower to activate.

KININS

Hageman factor activated by negative charge of basement membrane → converts prekallikrein (620aa) → kallikrein → which converts kininogen (640aa) → bradykinin (9aa)

Degradation = kininases (membrane bound) active site pointing into blood stream. Several including kininase II (= ACE)

Action: BK acts on GPCR → G_{q/11}. B₁ is inducible present in prolonged inflammation. B₂ constitutively expressed on endothelia and smooth muscle (Icatibant is B₂ selective antagonist)

Effects:

ENDOTHELIAL CELLS:

- NO production: Ca → Calmodulin → eNOS → NO. NO → cGMP → PKG → (MLCK decrease) → relaxation
- Weakens tight junctions increasing the vascular permeability
- Activation of *non-muscular myosin*. Myosin phosphorylation causes contraction of cells → in conjunction with weakened tight junctions allows cells to be drawn apart
- Activation of phospholipase A2 → increases availability of arachidonic acid

NEURONES

- Involves polymodal C fibres (heat, pressure, capsaicin, H⁺)
- BK activates PKC via its receptors at the site of inflammation, which can phosphorylate TRPV1 (vanilloid receptor), sensitising it to noxious stimuli – decreased threshold.
- *Allodynia – previous innocuous stimulus becomes noxious*

TNF α

- Mediator important in initiating inflammation
- Membrane anchored precursor protein cleaved
- TNFR1 – constitutive, TNFR2 – inducible
- Activates NF- κ B: activates endothelial cells, priming of leukocytes
- Infliximab – antibody against TNF α used to treat severe inflammatory conditions e.g. rheumatoid

HISTAMINE

Generation: Released from mast cell, basophils etc. Histidine → histamine, via histidine decarboxylase. Occurs in cytosol and the histamine is packaged in granules

Release: released in response to many factors including C3a and C5a, Cross-linking of IgE. Unusual signalling via $\beta\gamma$ units → Ca leads to COMPOUND EXOCYTOSIS – mass degranulation due to fact that granules can fuse with each other. Leads to, rapid, total loss of histamine. Resynthesis takes ~ 10 days

Degradation: cell uptake and metabolism via diamine oxidase (30%) and N-methyl transferase (70%)

Receptors:

- H₁ → G_q
- H₂ → G_s – found in stomach
- H₃ → G_{1/0} – mainly on CNS nerves
- H₄ → G_{1/0} – found on eosinophils, mast cells, T cells & dendritic cells

Effects:

INFLAMMATION

- H₁ leads to similar effects to BK – vasodilatation and increased permeability. Also bronchoconstriction, cytokine/chemokine release

ALLERGY

- Endothelial effects (H₁); bronchoconstriction (H₁); stimulation of sensory afferent fibres leading to itch and pain (H_{1/3}); vascular smooth muscle relaxation (H₂); exocrine glands e.g lacrimal (H₂)

PHARMACOLOGY

- Main target is H₁ antagonism – three generations:
- *Mepyramine* and *promethazine* – crosses BBB and has CNS effects (sedative)
- *Terfanadine* – no CNS effects. But problem with some mutations in cytochrome P₄₅₀ can lead to increased concentration in blood leading to K channel block in heart, leading to long QT syndrome.
- *Loratidine* and *fexofenadine* – no CNS effect or K channel block. Often taken prophylactically

ACID SECRETION

- Histamine from enterochromaffin-like cells can lead to acid release from stomach parietal cells via H₂ receptors. Hence *Cimetidine* and *ranitidine* – H₂ antagonists, can reduce acid secretion

SEROTONIN (5-HT)

Tryptophan → 5-hydroxy tryptophan (via tryptophan hydroxylase) → serotonin/5-HT (via DOPA decarboxylase)

Stored in granules for release. Selective reuptake mechanisms and degradation by MAO-A to 5-HIAA

Main actions include: increased gut motility, platelet aggregation, enhanced nociception, vasodilation/constriction, bronchial smooth muscle contraction.

Receptors:

- 5-HT₁ → G_{i/o} – mainly in brain – mood
- 5-HT₂ → G_{Q/11} – vascular and other smooth muscle
- 5-HT₃ → ligand gated ion channel – peripheral and CNS
- 5-HT₄ → G_{i/o} – myenteric and submucosal plexus

Migrane: 5% population, usually unilateral. No clear understanding of its development, initiating factors include stress, food and changes in sleep pattern. 3 theories:

VASCULAR – constriction of intracerebral arteries causes aura, rebound extracerebral dilatation produced headache

NEURONAL – CGRP release in meninges and vessels, by activation of trigeminal nerve, causing inflammation on endothelial cells

BRAIN – wave of cortical spreading depression – neuronal inhibition with ionic imbalances.

Treatment

Sumatriptan – 5-HT₁ agonist (inhibitory receptor). Present on neuronal afferents and inhibit stimulation. Also induce smooth muscle contraction

Ergot alkaloids, e.g. ergotamine – exact mode of action unknown, but ? partial agonists at 5-HT₁.

LIPID MEDIATORS

Membrane phospholipid → Arachidonic acid (+ Lyso-PAF). This is then converted to many derivatives depending on the enzyme involved: e.g. COX → prostanoids; 15-lipoxygenase → lipoxins; 5-lipoxygenase → Leukotrienes

The pool of arachidonic acid is limiting. AA is produced by phospholipase A₂ from phospholipid; and via DAG LIPASE from DAG via PLC

LEUKOTRIENES

Arachidonic acid → HPETE (via 5 lipoxygenase which requires FLAP)

HPETE → Leukotriene A₄ → Leukotriene C₄ → LTD₄ and LTE₄ (bioactive)

LTD₄ and LTE₄ are *cysteinyl leukotrienes* and act on the *cysteinyl LT receptors*: CysLT₁ and CysLT₂. Receptors lead to increased Ca and cause bronchoconstriction and pro-inflammatory effects

ZAFIRLEUKAST: CysLT₁ antagonist

ZILEUTON: 5-lipoxygenase inhibitor

PROSTANOIDS

These lipid mediators are synthesised and released on demand. AA pool is the rate limiting step in synthesis

cPLA₂ is Ca dependent – Ca translocates enzyme from cytosol to ER/nuclear membrane where it can act.

COX also membrane bound bifunctional enzymes – cyclo-oxygenase reaction producing PG-G, then a peroxidase reaction resulting in PG-H.

There is then selective production of the various prostanoids due to specific localisation of enzymes in the specific cells. E.g. platelets have thromboxane synthase: cyclic endoperoxides → TXA₂.

At least 3 isoforms of COX. COX-1 – constitutive; COX-2 – upregulated in inflammatory disease (but is constitutive in some cell types) – hence aim to selectively block COX-2 to reduce inflammatory effects but retain endogenous functions. COX-3 CNS specific ? body temperature involvement

Degradation – prostanoids are short lived $t_{1/2}$ in order of minutes/seconds. They are taken up and/or consequently degraded, hence they are locally acting mediators – only paracrine effects

Actions:

Swelling and Oedema

- PGI₂ and PGD₂ lead to increased cAMP and vasorelaxation. Leads to redness. The swelling is dependent on increased vascular permeability which would be contributed by other mediators such as BK or histamine
- TXA₂ is antagonistic in its actions and leads to vasoconstriction

Fever

- PGE₂ in response to IL-1 in the pre-optic nucleus of hypothalamus, leads to decreased cAMP and modifies the ‘set point’.

Pain

- PGE₂ via alternative receptors in tissue can lead to increased cAMP which leads to phosphorylation of TTX resistant Na channels and sensitises the receptors leading to hyperalgaesia and allodynia

Acid secretion

- PGE₂ is protective. It is able to act to inhibit parietal H⁺ secretion as well as promote goblet mucin secretion. Hence non-specific COX inhibitors can disrupt this system leading to gastric ulceration.

Platelet aggregation

- Autocrine TXA₂ action promotes aggregation via inducing a ‘morphology change’ in platelets which promotes aggregation

- NOTE, PGI₂ has the opposite effect – declumping of platelets and vasodilation

Pharmacology of prostanoids

COX inhibitors are all analgesic and antipyretic, most are anti-inflammatory. 1/5 chronic users of NSAID have gastric damage

Aspirin – irreversible acylation of serine 530 leading to steric blockade of catalytic site. Also anticoagulant effect (since platelets with no nucleus have no regenerative capacity unlike endothelia (PGI₂ vs TXA₂ antagonism). [note salicylate is a reversible COX inhibitor]

Ibuprofen – reversible steric blockade of the catalytic pocket interacting with arginine 120

Celecoxib – COX-2 selective inhibitor – selectivity is dependent on the width of the mouth of the enzyme → too large to enter COX-1

Paracetamol – little inflammatory action. Exact mode of action unknown. Some suggest, acts on COX-3. Other theories propose paracetamol alters oxidation state of the enzyme essential for activity, but this is overridden at sites of inflammation where peroxide concentrations are high. Hepatotoxic.

Side effects of COXi

- Acid secretion can lead to gastric ulceration due to inhibition of PGE₂ protective effects. Several strategies to overcome this include: enteric coating, prodrugs, use of PPI, COX-2 selective drugs, Misoprostol (PG substitute)
- PGI regulates renal blood flow, and so drugs can lead to kidney failure
- Asthma - AA is diverted along other pathways, including leukotrienes implicated in genesis of asthma.
- Aspirin specific – Reyes syndrome in children, and salicylate poisoning.

CHRONIC INFLAMMATION

Long term reaction associated with organ transplantation and autoimmunity.

IMMUNOSUPPRESSION

Calcineurin inhibitors

A calcium dependent serine-threonine phosphatase. In T cells normally bound to FKBP12. It acts to dephosphorylate NF-AT, which translocates to the nucleus to regulate the expression of cytokines like IL-2. The constitutive phosphorylation of NF-AT prevents its passage into the nucleus

Ciclosporin – binds to cytosolic cyclophilin which as a complex inhibits calcineurin

Tacrolimus/FK506 – binds to FKBP12, which in turn inhibits calcineurin.

By reducing NF-AT transcriptional activity, reduces T-cell proliferation

Others

Sirolimus/Rapamycin – blocks mTOR signalling pathway – preventing maturation of APC and inflammatory cell proliferation

Azathioprine and Mycophenolate mofetil – inhibitors of cell proliferation

Transplantation – typical drug regimen would include calcineurin inhibitors, such as ciclosporin or tacrolimus; glucocorticoids, such as cortisol, dexamethasone; and inhibitors of T cell proliferation such as azathioprine or mycophenolate mofetil.

Autoimmunity, e.g. rheumatoid arthritis – incompletely understood aetiology. Treatments aimed at reducing immune response.

Treatment includes: NSAID's – analgesic as well as anti-inflammatory; glucocorticoids; calcineurin inhibitors.

In addition there are a series of 'disease modifiers' including: *gold* – injected into joint (? Interferes with PMN migration); *sulphasalazine* - ? modifies lymphocyte behaviour and prevents migration to joint; *methotrexate* – non-selectively targets dividing cells; even chloroquine has been suggested to have effects.

GLUCOCORTICOIDS

These have many physiological functions, part of the bodies endogenous system to dampen the immune response.

CNS regulation → corticotrophin releasing factor → adenohipophysis → adrenocorticotrophic hormone → adrenal gland → cortisol from zona fasciculata and reticulate → GC cytoplasmic receptors (with HSP-90), which dimerise and translocate to the nucleus, leading to transcriptional regulation of many genes leading to dampening of immune response.

- Up-regulation of LIPOCORTIN-1 which acts to inhibit PLA₂ decreasing AA pool thus decreasing inflammatory mediators
- Down-regulation of IL-2 – GC receptor binds to AP-1 preventing it from activating IL-2 transcription
- Down-regulation of COX-2 – via upregulation of IF-κBα which inhibits NF-Kb

Drugs involved – glucocorticoid receptor agonist which have anti-inflammatory effects:

Short acting - hydrocortisone and prednisolone

Medium acting – triamcinolone

Long acting - dexamethasone

BUT when taken systemically, they can interfere with the negative feedback pathway inhibiting the ACTH production. Thus the patient must be weaned off of the drug to allow time for the feedback loop to recover, and endogenous ACTH production to increase. In addition, during treatment there may be reduced innate immunity.

Side effects: glucose intolerance, myopathy, thinning of skin, osteoporosis, gastritis, cushings syndrome etc.

ASTHMA

Increasing prevalence, reversible obstruction of the lungs triggered by allergens, irritants, exercise, cold air and NSAID (shunting of AA → leukotrienes). There are early (5-HT / histamine) and late (leukotrienes) phases of the attack

Treatments are divided into short term treatments during attack, and long term prophylactic treatments:

Short term treatments

- *Salbutamol* – β_2 agonist → bronchodilatation
- *Ipratropium* – muscarinic antagonist interferes with vagal innervation
- *Budesonide corticosteroid* – short or long term

Long term treatments

- *Salmeterol* - β_2 agonist long acting
- *Xanthines* – phosphodiesterase inhibitors → bronchial relaxation
- *Cromolyn* – mast cell stabilizers – decreases histamine degranulation
- *Zileuton/zafirleukast* – leukotriene inhibitors

True / False MCQ – Negatively marked

Kinins

- a) Kallikrein catalyses the conversion of kininogen to bradykinin
- b) Bradykinin is a nonomeric peptide
- c) Kininase I is also known as angiotensin converting enzyme

Histamine

- a) Small quantities of Histamine is released from mast cells
- b) All histamine receptors are G protein coupled

Prostanoids

- a) Generation of arachidonic acid is the rate limiting step
- b) Cyclooxygenase generates PGH_2 precursor in a one step mechanism
- c) COX-3 is upregulated in inflammatory disease
- d) Generation of the different prostanoids in different tissues is dependent upon neural signals
- e) Prostanoids are involved with endocrine, as well as paracrine signalling
- f) Prostanoids have no role in increasing vascular permeability

Serotonin

- a) Serotonin is derived from tyrosine
- b) Serotonin is implicated in migraine

Calcineurin

- a) Calcineurin is a calcium dependent tyrosine phosphatase
- b) NF-AT is a primary target for calcineurin

Glucocorticoids

- a) Glucocorticoids have many physiological functions
- b) Glucocorticoids form an endogenous system to dampen the immune response
- c) Cortisol is released from the zona fasciculata of the adrenal cortex

Asthma

- a) There are early and late phases to an asthma attack
- b) Cromolyn can be used as a prophylactic for asthma

True / False MCQ – Negatively marked

Kinins

- d) Kallikrein catalyses the conversion of kininogen to bradykinin **T**
- e) Bradykinin is a nonomeric peptide **T**
- f) Kininase I is also known as angiotensin converting enzyme **F (KININASE II)**

Histamine

- c) Small quantities of Histamine is released from mast cells **F (COMPOUND EXOCYTOSIS)**
- d) All histamine receptors are G protein coupled **T**

Prostanoids

- g) Generation of arachidonic acid is the rate limiting step **T**
- h) Cyclooxygenase generates PGH₂ precursor in a one step mechanism **F (2 STEP)**
- i) COX-3 is upregulated in inflammatory disease **F (COX-2)**
- j) Generation of the different prostanoids in different tissues is dependent upon neural signals **F (TISSUE SPECIFIC ENZYME EXPRESSION)**
- k) Prostanoids are involved with endocrine, as well as paracrine signalling **F (SHORT LIVED)**
- l) Prostanoids have no role in increasing vascular permeability **T**

Serotonin

- c) Serotonin is derived from tyrosine **F (TRYPTOPHAN)**
- d) Serotonin is implicated in migraine **T**

Calcineurin

- c) Calcineurin is a calcium dependent tyrosine phosphatase **F (SERINE/THREONINE)**
- d) NF-AT is a primary target for calcineurin **T**

Glucocorticoids

- d) Glucocorticoids have many physiological functions **T**
- e) Glucocorticoids form an endogenous system to dampen the immune response **T**
- f) Cortisol is released from the zona fasciculata of the adrenal cortex **T**

Asthma

- c) There are early and late phases to an asthma attack **T**
- d) Cromolyn can be used as a prophylactic for asthma **T**

True / False MCQ – Negatively marked

Kinins

- a) One effect of bradykinin is to decrease vascular permeability
- g) Allodynia is also an observed effect of bradykinin

Histamine

- e) Histamine is a primary component of the allergic response
- f) Loratidine is known to cause long QT syndrome
- g) H₁ antagonists such as loratidine are used for treating allergy

Prostanoids

- m) PGE₂ acts to inhibit goblet cell mucin secretion and promote parietal H⁺ secretion
- n) Antagonism between TXA₂ and PGI₂ regulates platelet aggregation
- o) Aspirin causes reversible steric blockade by interacting with serine 530
- p) Celecoxib is COX-2 specific due to width of the enzyme mouth
- q) Asthma may be promoted by prostanoid antagonism due to diverting arachidonic acid towards lipoxins

Calcineurin

- e) Cyclosporin binds to FKBP-12 which inhibits calcineurin
- f) Tacrolimus/FK506 binds to cyclophilin which in turn inhibits calcineurin
- g) Blocking calcineurin has the effect of reducing proliferation of T cells

Glucocorticoids

- g) GC's have cytosolic receptors
- h) GC's upregulate Lipocortin-1 which inhibits PLA₂
- i) GC's upregulate a factor that inhibits NF-κB which leads to downregulation of many anti-inflammatory mediators
- j) Patients must be weaned off exogenous GC's due to the interference with the negative feedback pathway
- k) Patients taking prednisone systemically can have problems if they stop taking the drug immediately without weaning off.

Leukotrienes

- a) Zileuton acts to inhibit 5-lipoxygenase
- b) LTC₄ is a cysteinyl leukotriene

True / False MCQ – Negatively marked

Kinins

- a) One effect of bradykinin is to decrease vascular permeability **F (INCREASE)**
- h) Allodynia is also an observed effect of bradykinin **T**

Histamine

- h) Histamine is a primary component of the allergic response **T**
- i) Loratidine is known to cause long QT syndrome **F (TERFANIDINE)**
- j) H₁ antagonists such as loratidine are used for treating allergy **T**

Prostanoids

- r) PGE₂ acts to inhibit goblet cell mucin secretion and promote parietal H⁺ secretion **F (REVERSE)**
- s) Antagonism between TXA₂ and PGI₂ regulates platelet aggregation **T**
- t) Aspirin causes reversible steric blockade by interacting with serine 530 **F (IRREVERSIBLE ACETYLATION)**
- u) Celecoxib is COX-2 specific due to width of the enzyme mouth **T**
- v) Asthma may be promoted by prostanoid antagonism due to diverting arachidonic acid towards lipoxins **F (HPETE)**

Calcineurin

- h) Ciclosporin binds to FKBP-12 which inhibits calcineurin **F**
- i) Tacrolimus/FK506 binds to cyclophilin which in turn inhibits calcineurin **F**
- j) Blocking calcineurin has the effect of reducing proliferation of T cells **T**

Glucocorticoids

- l) GC's have cytosolic receptors **T**
- m) GC's upregulate Lipocortin-1 which inhibits PLA₂ **T**
- n) GC's upregulate a factor that inhibits NF-κB which leads to downregulation of many anti-inflammatory mediators **F (PROINFLAMMATORY)**
- o) Patients must be weaned off exogenous GC's due to the interference with the negative feedback pathway **T**
- p) Patients taking prednisone systemically can have problems if they stop taking the drug immediately without weaning off **T**

Leukotrienes

- c) Zileuton acts to inhibit 5-lipoxygenase **T**
- d) LTC₄ is a cysteinyl leukotriene **F (D AND E)**