Pharmacology of inflammation and immunosuppression

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

Dolor – Pain sensitisation,
Rubor and Calor – increased blood flow (vasodilation),
Tumor- increased vascular permeability

Inflammation is a response to: tissue injury, infection and autoimmunity

Immune response:

- Innate responses (relatively non specific) include blood borne chemical mediators, e.g. complement and kinins, are ready for immediate action. Recognition of PAMPS by PRR's, e.g. TLR (Seed BOD)

- Adaptive responses (highly specific – to a particular antigen) involves the action of lymphocytes, and are slower to activate.

HISTAMINE

Generation: Released from mast cell, basophil, Histaminergic neurones and ECL cells.

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, Unusual signalling via βγ 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
- Cross-linking of IgE.
- Substance P

cAMP inhibits histamine release – hence use of adrenaline in anaphylaxis
Methylxanthines, β2 agonists and sodium cromoglycate interfere with release from mast cells.

Degradtion: cell uptake and metabolism via histaminase (diamine oxidase) producing imidazole acetaldehyde (30%) and N-methyl transferase - producing N-methylhistamine (70%)

Receptors: $H_1 \rightarrow G_{q/11}$ - inflammation
$H_2 \rightarrow G_s$ – found in stomach – gastric acid secretion
$H_3 \rightarrow G_{i/0}$ – mainly on CNS nerves, presynaptic inhibitory autoreceptor
$H_4 \rightarrow G_{i/0}$ – chemotaxis/cytokine release

Effects:

INFLAMMATION

- H_1 leads to similar effects to BK – vasodilatation and increased permeability via NO. Also bronchoconstriction, cytokine/chemokine release

ALLERGY

- Endothelial effects (H_1); bronchoconstriction (H_1); stimulation/sensitisation of sensory afferent fibres leading to itch and pain ($H_{1/3}$); vascular smooth muscle relaxation (H_2); exocrine glands e.g lacrimal (H_2)

- *Omalizumab* (anti-IgE can be used to decrease allergic mast cell degranulation)
- *Sodium Cromoglycate* – mast cell stabiliser, inhibiting degranulation ? by reducing Ca influx
- Raising [cAMP] inhibits mast cell degranulation -> salbutamol, theophylline

PHARMACOLOGY

- Main target is H_1 antagonism – three generations:
- *Mepyramine* and *promethazine* – crosses BBB and has CNS effects (sedative)
- *Terfanadine* – no CNS effects. But problem with some mutations in cytochrome P_{450} can lead to increased concentration in blood leading to K channel block in heart (HERG), leading to long QT syndrome. Terfanadine is metabolised to fexofenadine
- *Loratidine and fexofenadine* – no CNS effect or K channel block. Often taken prophylactically for hay-fever

In allergy: Adrenaline – counteract systemic vasodilation and relieve bronchoconstriction; hydrocortisone – anti-inflammatory

- *Dimenhydrinate* – treating motion sickness

ACID SECRETION

- Histamine from enterochromaffin-like cells can lead to acid release from stomach parietal cells via H_2 receptors. Hence *Cimetidine and ranitidine* – H_2 antagonists, can reduce acid secretion
- *Proglumide* – Inhibits CCK_2 receptors (gastrin receptor that would increase histamine release)
- Consider also, proton pump inhibitors, such as *Omeprazole*; and eradication of H.pylori as a causative agent, with antibiotics.

PEPTIDE MEDIATORS: KININS

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

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

- Kininase I e.g. serum carboxypeptidase
- Kininase II a peptidyl dipeptidase(= ACE – hence, ACEi cause increase in BK)

Action: BK acts on GPCR → $G_{q/11}$. B_1 is inducible present in prolonged inflammation. B_2 constiutively expressed on endothelia and smooth muscle (*Icatibant* is B_2 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 TRPVR-1 (vanilloid receptor), sensitising it to noxious stimuli – decreased threshold.
- *Allodynia – previous innocuous stimulus becomes noxious*

LIPID MEDIATORS

Membrane phospholipid → Arachidonic acid (+ Lyso-PAF). [Rate limiting step] 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 A_2 from phospholipid; and via DAG LIPASE from DAG via PLC

LEUKOTRIENES

Produced predominantly by inflammatory cells such as mast cells and neutrophils.

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

5-HPETE → Leukotriene A_4 → Leukotriene C_4 → LTD_4 and LTE_4 (bioactive)
→Leukotriene B_4 (chemotaxin)

LTD_4 and LTE_4 are *cysteinal leukotrienes* and act on the *cysteinyl LT receptors*: $CysLT_1$ and $CysLT_2$. Receptors lead to increased Ca and cause bronchoconstriction (powerful spasmogens) and pro-inflammatory effects

MONTELEUKAST / ZAFIRLEUKAST: $CysLT_1$ antagonist
ZILEUTON: 5-lipoxygenase inhibitor

Both used as maintenance treatment of asthma

PROSTANOIDS (prostaglandins and thromboxane)

These lipid mediators are synthesised and released on demand. AA pool is the rate limiting step in synthesis. Short lived mediators, lasting ~1 minute

$cPLA_2$ 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 $PG\text{-}H_2$.

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 → TXA_2.

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

- PGI_2 and PGD_2 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

- TXA_2 is antagonistic in its actions and leads to vasoconstriction

Fever
- PGE_2 in response to IL-1 in the pre-optic nucleus of hypothalamus, leads to decreased cAMP and modifies the 'set point'.

Pain
- PGE_2 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
- PGE_2 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 TXA_2 action promotes aggregation via inducing a 'morphology change' in platelets which promotes aggregation
- NOTE, PGI_2 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 (PGI_2 vs TXA_2 antagonism). [note salicylate is a reversible COX inhibitor]. Leads to production of 'aspirin triggered lipoxin' which may be responsible for some of aspirins anti-inflammatory effects

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

Etoricoxib – COX-2 selective inhibitor – selectivity is dependent on the width of the mouth of the enzyme → too large to enter COX-1 [Rofecoxib, withdrawn due to increased risk of MI]

Paracetamol – little inflammatory action. Exact mode of action unknown. Some suggest, acts on COX-3. Other theories propose paracetamol alters oxidation state of the COX2>COX1 enzyme essential for activity, but this is overridden at sites of inflammation where peroxide concentrations are high. Hepatotoxic [Some paracetamol converted to NAPQI, conjugated with glutathione. With overdose, insufficient glutathione, so NAPQI oxidises thiol groups on cellular proteins, killing cells]

Side effects of COXi

- Acid secretion can lead to gastric ulceration due to inhibition of PGE_2 protective effects. Several strategies to overcome this include: enteric coating, prodrugs, use of PPI, COX-2 selective drugs, Misoprostol (PG substitute) [*Arthrotec* – combination of misoprostol and diclofenac]
- *Naproxcinod* – naproxen + NO donoe (NO has similar effects to PG)
- 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.

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:

$5\text{-}HT_1 \rightarrow G_{i/0}$ – mainly in brain – mood
$5\text{-}HT_2 \rightarrow G_{Q/11}$ – vascular and other smooth muscle
$5\text{-}HT_3 \rightarrow$ ligand gated ion channel – peripheral and CNS
$5\text{-}HT_{4/6/7} \rightarrow G_S$ – myenteric and submucosal plexus
$5\text{-}HT_{5A} \rightarrow G_{i/0}$

Migrane: 5-10% population, usually unilateral and pulsing, following an aura (visual/auditory). 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 neurogenic inflammation on endothelial cells
BRAIN – wave of cortical spreading depression – neuronal inhibition with ionic imbalances.

Treatment
Sumatriptan – $5\text{-}HT_{1D}$ 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-HT_1.

Antiemetic

5-HT also a mediator of emesis. *Ondansetron* (5-HT_3 receptor antagonist) used as an antiemetic.

Other pathways that can be targeted:

- Histaminergic - Cyclizine
- Dopaminergic – domperidone, metoclopramide
- Muscarinic – scopolamine/hyoscine

GLUCOCORTICOIDS/CORTICOSTEROIDS

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

CNS regulation → corticotrophin releasing factor → adenohypophysis → adrenocorticotrophic hormone → adrenal gland → cortisol from zona fasiculata 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 ANTI-INFLAMMATORY proteins, such as: LIPOCORTIN-1 which acts to inhibit PLA_2 decreasing AA pool thus decreasing inflammatory mediators; IL-1 antagonist, IκB
- Down-regulation of PRO-INFLAMMATORY proteins such as: IL-2 – GC receptor binds to AP-1 preventing it from activating IL-2 transcription; IL-1, TNF-α
- Down-regulation of COX-2 – via upregulation of IF-κBα which inhibits NF-Kb

Gene regulation is effected in four ways:

- Binds to a positive glucocorticoid response element driving transcription
- Binds to negative response elements dismantling the transcription machinery
- Reduced activity of *Fos/Jun*
- Prevention of P65 and P50 binding to NF-Kb site

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, diabetes, buffalo hump 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

Inflammation, airway hyper-sensitivity, reversible bronchoconstriction

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 inervation
- *Budesonide corticosteroid* – short or long term (inhaled – less effective against COPD)

Long term treatments

- *Salmeterol* - β_2 agonist long acting
- *Xanthines* – phosphodiesterase inhibitors → bronchial relaxation e.g. *Theophyline*
- *Sodium Cromoglycate* – mast cell stabilizers – decreases histamine degranulation
- *Zileuton/zafirleukast* – leukotriene inhibitors
- *Omalizumab* is a humanised monoclonal Ab against IgE

CHRONIC INFLAMMATION

Long term reaction associated with organ transplantation and autoimmunity.

AUTOIMMUNITY

Rheumatoid arthritis – incompletely understood aetiology. Autoimmune disease of the joints, affecting ~1% population

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' DMARDS including:

- *gold* - *sodium aurothiomalate* – injected into joint (? Interferes with PMN migration);
- *sulphasalazine* - ? modifies lymphocyte behaviour and prevents migration to joint;
- *methotrexate* – non-selectively targets dividing cells; even
- *hydroxychloroquine* has been suggested to have effects.
- *Penicillinamine* - ? decreasing IL-1 and collagen maturation
- *Leflunomide* – inhibits dihydroorotate dehydrogenase – inhibiting pyrimidine synthesis

Biological DMARDS:

- *Etanercept* – soluble TNF alpha receptor
- *Infliximab and adalimimab* – mAb against TNF alpha
- *Tocilizumab* – anti-IL-6 receptor inhibiting proinflammatory cytokine release
- *Rituximab* – anti-CD20 – anti-B cell
- *Abatacept* – $CTLA_4$ fusion protein – disrupts antigen presentation
- *Anakinra* – recombinant IL-1 receptor antagonist

Diabetes (Type 2)

- *Metformin* – ?activation of AMP kinase – does not cause hypoglycaemia
- *Sulphonylurea* – glibenclamide, glipizide – bind to SUR1 K_{ATP} channel and promote insulin release – can cause hypoglycaemia
- *Repaglinide* – incretin – faster onset but similar action to sulphonylureas
- *Sitagliptin* – inhibitor of DPP-4 – impacts on appetite
- *Dapagliflozin* – inhibitor of sodium-glucose co-transporter (SGLT) – reduce GI absorption sugar and increase urinary release
- *Pioglitazone* – thiazolidinedione – peroxisome proliferator-activated receptor-χ (PPARχ) activator
- *Acarbose* - α-glucosidase inhibitor

TRANSPLANT IMMUNOSUPPRESSION

Corticosteroids

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

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

Inhibitors of immune cell proliferation
Azathioprine and Mycophenolate mofetil – inhibitors of cell proliferation – particularly lymphocytes

Basiliximab – antibodies against IL-2 receptors (CD25 subunit)

Belatacept – $CTLA_4$ fusion protein interfering with co-stimulation

MONOCLONAL ANTIBODIES

Increasingly used against inflammation and other diseases like cancer. High affinity and specificity for targets.

Actions of these antibodies:

- Binds antigens, blocking them from usual function
- Binds antigens, targeting them for destruction

Examples:

- **Infliximab** and **Adalimumab** - TNFα – rheumatoid arthritis
- **Omalizumab** – IgE - asthma
- **Alemtuzumab** (Campath-IH) – CD52 – CLL, transplant, MS
- **Etanercept** – Soluble TNFα receptor

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 permeablility

Serotonin

a) Serotonin is derived from tyrosine
b) Serotonin is implicated in migrane

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 fasiculata 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 PGH_2 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 permeablility **T**

Serotonin

c) Serotonin is derived from tyrosine **F (TRYPTOPHAN)**
d) Serotonin is implicated in migrane **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 fasiculata 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) H_1 antagonists such as loratidine are used for treating allergy

Prostanoids

m) PGE_2 acts to inhibit goblet cell mucin secretion and promote parietal H^+ secretion
n) Antagonism between TXA_2 and PGI_2 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) Ciclosporin 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 PLA_2
i) GC's upregulates a factor that inhibits NF-kB 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-lipooxygenase
b) LTC_4 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) H_1 antagonists such as loratidine are used for treating allergy **T**

Prostanoids

r) PGE_2 acts to inhibit goblet cell mucin secretion and promote parietal H^+ secretion **F (REVERSE)**
s) Antagonism between TXA_2 and PGI_2 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 PLA_2 **T**
n) GC's upregulates a factor that inhibits NF-kB 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-lipooxygenase **T**
d) LTC_4 is a cysteinyl leukotriene **F (D AND E)**