Hormone therapy:

Hormones are secreted in the body by several glands that are essential for the growth, development, reproduction, etc. They are the chemical substances which coordinate the activities of living organisms and also their growth. They are secreted by special tissues in our body through endocrine glands. Different hormones have different effects on the shape of the body. They help in body growth, development, metabolism, reproduction etc.

1) Insulin: Insulin is called as Humulin. This hormone is released by the pancreas, located in the abdominal cavity behind the stomach. It allows the body to use glucose from carbohydrates in the food for energy or to store glucose for future use. It helps in keeping blood sugar level from getting too high i.e. hyperglycaemia or too low i.e. hypoglycaemia.

Therapeutic applications:

- **a)** Insulin is used to treat a diabetes. Insulin is used to treat a diabetes and. It is also used along with glucose to treat Insulin lowers **blood** glucose by stimulating peripheral glucose uptake primarily by skeletal muscle cells and fat, and by inhibiting glucose production and release by the liver.
- **b)** therapy for acute hyperkalaemia (high blood potassium levels.) in patients. It is typically used in conjunction with dextrose to prevent hypoglycaemia, and is often combined with other therapies.
- **c)** hyperglycaemic states: patients with insulin-resistant type 2 diabetes with hyperosmolar hyperglycemic state require larger insulin doses. Once plasma glucose reaches 300 mg/dL (16.7 mmol/L), insulin infusion should be reduced to basal levels (1 to 2 units/h) until rehydration is complete and the patient is able to eat.
- **d)** diabetic ketoacidosis: A diagnosis of diabetic ketoacidosis requires the patient's plasma glucose concentration to be above 250 mg per dL. When the blood sugar level falls to about 200 mg/dL and the blood is no longer acidic, may be able to stop intravenous insulin therapy and resume your normal subcutaneous insulin therapy

2) **Insulin like growth factor**

Insulin-like growth factor (**IGF 1**), formerly called somatomedin, any of several peptide hormones that function primarily to stimulate **growth** but that also possess some ability to decrease blood glucose levels. **The liver** is the main source of circulating insulin-like growth factor I, accounting for ~75% of circulation. IGF-1 also stimulates **brain** development and supports **brain** function, and children with higher levels of IGF-1 generally have higher IQs. After childhood, IGF-1 continues to support the **brain** by repairing damage and aiding our neurons

Therapeutic applications:

a) Used in the treatment of hypoglycaemia(low blood glucose): IGF is capable of suppressing type 1 diabetes the body's immune system turns on itself, attacking beta cells in the pancreas that produces insulin. IGF 1 may be able to defend against the body's own attack help control diabetes.
b) Used to treat in the stunned growth: Growth delay due to insulin-like growth factor I deficiency. by recombinant growth hormone (GH) therapy. Recombinant IGF-I therapy can be used in patients with complete IGF-I deficiency or those showing an insufficient response to recombinant GH treatment.
c) Treatment of Acromegaly is a hormonal disorder that develops when your pituitary gland produces too much growth hormone during adulthood. Sometimes, although GH levels have been reduced by the operation, drug therapy and/or radiotherapy. So that Levels of GH in the blood can change throughout the day.

3) Human growth factor

Growth hormone (GH), also called somatotropin or **human growth hormone**, peptide **hormone** secreted by the anterior lobe of the pituitary gland. It stimulates the **growth** of essentially all tissues of the body, including bone.

Therapeutic applications:

a) **Turner syndrome**: Treatment of growth hormone deficiency involves regular injections of synthetic human growth hormone. If Children receive daily injections which usually lasts several years.
b) **Overproduction of growth hormone causes** excessive growth. In children, the condition is called gigantism. Admistrating this GH stop the release of growth hormone. They are often effective for the long-term control of gigantism.

4) Erythropoietin

Erythropoietin is a **hormone** that is produced predominantly by specialised cells in the kidney it acts on red blood cells to protect them against destruction. it stimulates stem cells of the bone marrow to increase the production of red blood cells. The hormone is active for a short period of time and then eliminated from the body in the urine.

Therapeutic applications:

a) **Anemia treatment:** Healthy kidneys produce a hormone called **EPO**. **EPO** prompts the bone marrow to make red blood cells, which then carry oxygen throughout the body. When the kidneys are diseased or damaged, they do not produce enough **EPO**. As a result, the bone marrow makes fewer red blood cells, **causing anemia. Erythropoietin** can be used to treat anemia by stimulating red blood cell production in the bone marrow in these conditions. The medication is known as epoetin alfa (Epogen, Procrit)
b) low **levels** may be because of polycythaemia . This is a bone marrow disorder that causes your body to make too many red blood cells. Low **EPO levels** may also mean you have kidney disease.In people with **low erythropoietin** levels due to kidney disease

5) calcitonin

Calcitonin is involved in helping to regulate levels of calcium and phosphate in the blood. Calcitonin is a 32 amino acid hormone secreted by the C-cells of the thyroid gland.

Therapeutic applications:

a) If your calcitonin levels were high, it may mean you have C-cell hyperplasia or medullary thyroid cancer. If person are already being treated for this thyroid cancer, high levels may mean the treatment is not working or that cancer has returned after treatment.
b) When given to patients with osteoporosis, calcitonin produces modest increases in bone mass because it slows the rate at which osteoclasts absorb bone. Only women who are at least five years past menopause can take calcitonin. Patients with severe osteoporosis seem to do the best with this drug

6) Lipocortin

Lipocortin (and possibly other related proteins) also called as Annexin A-I then is a sort of 'second messenger' of the glucocorticoids. It is only one of many such regulatory proteins but it is an important one, controlling as it does the mediators which promote development of the symptoms of the inflammatory response. it is secreted by anterior pituitary gland.

Therapeutic applications:

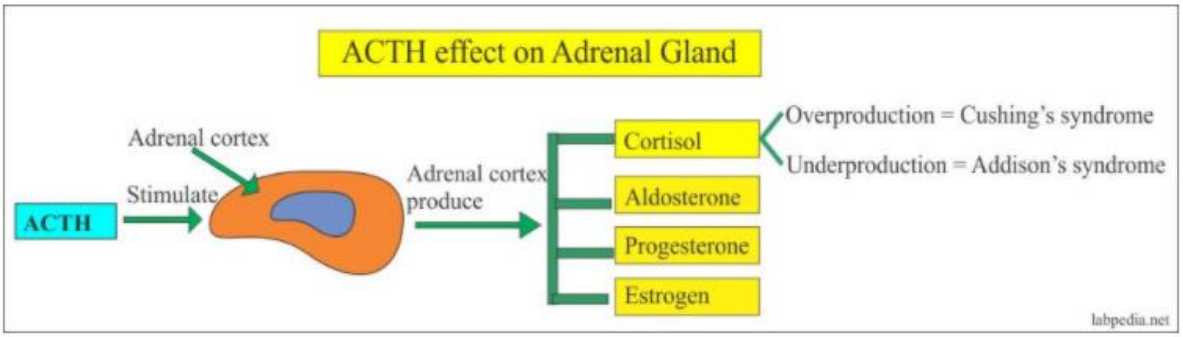
Lipocortin suppress phospholipase A2. Increased expression of the gene coding for annexin-1 is one of the mechanisms by which glucocorticoids (such as cortisol) inhibit inflammation.

Annexin A-I involved annexins in anti-inflammatory responses. Upon infection or damage to tissues, annexin A-I is believed to reduce inflammation of tissues by interacting with annexin A-I receptors on leukocytes

Annexin 1 (ANXA1) was originally identified as a mediator of the anti-inflammatory actions of glucocorticoids (GCs) in the host defence system. ANXA1 thus contributes to the regulation of processes as diverse as cell migration, cell growth and differentiation, apoptosis, vesicle fusion, lipid metabolism, and cytokine expression

7) ACTH:

Adrenocorticotropic hormone (ACTH) is made in the pituitary gland. It is needed for your adrenal glands to work properly and the body react to stress. ACTH stimulates the release of another hormone called cortisol from the cortex (outer part) of the adrenal gland. CRH stimulates the anterior pituitary to release ACTH. ACTH acts on the adrenal cortex to release cortisol and androgens.



Therapeutic applications:

a) **Treating Cushing's syndrome** is a disorder that occurs when your body makes too much of the hormone cortisol over a long period of time. Cortisol is sometimes called the "stress hormone" because it helps your body respond to stress. Cortisol also helps. maintain blood pressure. Medications to control excessive production of cortisol at the adrenal gland include ketoconazole, mitotane (Lysodren) and metyrapone (Metopirone).
b) **Treating Addison's syndrome** is a long-term endocrine disorder in which the adrenal glands do not produce enough steroid hormones. Symptoms generally come on slowly and may include abdominal pain, weakness, and weight loss. hormone replacement therapy by injecting Adrenocorticotropic hormone which comes to normal.

8) SOMATOSTATIN

Somatostatin, also known as growth hormone-inhibiting hormone (GHIH) is a peptide hormone that regulates the endocrine system and affects neurotransmission and cell proliferation. Somatostatin is secreted by scattered cells in the GI epithelium, and by neurons in the enteric nervous system. Somatostatin is often referred to as having neuromodulator activity within the central nervous sytem, and appears to have a variety of complex effects on neural transmission. Injection of somatostatin into the brain of rodents leads to such things as increased arousal and decreased sleep.

Somatostatin acts on tumours which oversecretes the hormones and rapid production of cells also stop the unnatural rapid reproduction of cells resulting in prevention of tumours. due to its ability to inhibit growth hormone secretion.

a) Giantism: Gigantism is a serious condition that is nearly always caused by an adenoma, a tumour of the pituitary gland. Gigantism occurs in patients who had excessive growth hormone in childhood. The pituitary tumour cells secrete too much growth hormone (GH), leading to many changes in the body. hormone somatostatin is injected inhibit growth hormone secretion. They are effective for the long-term control of gigantism.

9) CHOLECYSTOKININ AND BOMBESIN

CHOLECYSTOKININ (**CCK**) A hormone which is secreted by cells in the duodenum and stimulates the release of bile into the intestine and the secretion of enzymes by the pancreas Cholecystokinin plays a key role in facilitating digestion within the small intestine. It is secreted from mucosal epithelial cells in the first segment of the small intestine (duodenum), and stimulates delivery into the small intestine of digestive enzymes from the pancreas and bile from the gallbladder.

Therapeutic applications:

a) Pancreatitis is inflammation of the pancreas. It happens when digestive enzymes start digesting the pancreas itself. Pancreatitis can be acute or chronic. Which is serious and can lead to complications. CCK mediates digestion in the small intestine by inhibiting gastric emptying. It stimulates the acinar cells of the pancreas to release a juice rich in pancreatic digestive enzymes (hence an alternate name, pancreozymin) that catalyze the digestion of fat, protein, and carbohydrates.

b) **Cholecystokinin** (**CCK**) is a gut hormone and a neuropeptide that has the capacity to stimulate insulin secretion. As insulin secretion is impaired in type 2 **diabetes**, administration of this CCK exerts antidiabetogenic action.

BOMBESIN

Bombesin (BBS, BB), is a 14-amino acid neurohormone polypeptide, derived initially from amphibians with a wide range of physiological effects in the brain, lungs, and GI tract. Bombesin regulates gastrointestinal hormone release and gastrointestinal motility. Bombesin is predominantly known to regulate homeostasis within the gastrointestinal tract. Bombesin plays an important role in tumour growth, cellular proliferation, and inflammation. Bombesin is combined homologs of neuromedin B and gastrin-releasing peptide. Bombesin has no known antagonist capability (i.e., receptor blocking effects). The area of the bombesin molecule that controls essential physiological activity also determines the affinity of the peptide toward its receptors.

a) Loss of bombesin receptors correlates with age-dependent obesity, hypertension, glucose intolerance, and high insulin levels. This expanded adipose deposition may, in part, be due to a decline in energy consumption without a shift in eating or movement, which infers that bombesin receptors may signify a plausible target upon which notable advancement can take place in the realm of anti-obesity agents.
b) Bombesin has the theoretical capability to address prostate cancer releasing peptide antagonist might hold promise as a possible new agent for the treatment of cancer.

10. **MSH (Melanocyte Stimulating Hormone)**

MSH is produced by an intermediate lobe of the pituitary gland. Its secretion causes a darkening of the skin.The darkening occurs as granules of melanin spread through the branches of specialized melanocytes. Ultraviolet (UV) rays, increase MSH production by the skin and pituitary gland. It has a vital role in making pigmentation (colouring) of the skin, hair, and eyes. Specialized skin cells known as melanocytes produces melanin.

Melanocyte-stimulating hormone deficiency can cause increased inflammation, pain, and sleeping problems, as well as a reduction in the levels of anti-diuretic hormone, which causes thirst and frequent urination. Melanocyte-stimulating hormone deficiency may also result in increased food intake and obesity.So administrated with MSH hormones.

Side effects of hormones

1) **Digestive system problem:** diarrhoea. Lose of appetite a little. Or you may have an increased appetite, which can lead to weight gain.

2) Muscle and bone changes: Person might develop pains in your joints. This often settles down after a few weeks.

3) Some hormone therapies such as aromatase inhibitors can cause thinning of your bones. Blood clots :(thrombosis) can slightly increase when you take tamoxifen. Some men and women have mood swings and even depression.

4) Some men and women feel that their memory gets worse when they have been having hormone treatment for a while.

5) Headaches, feeling sick.,vaginal bleeding

6) Weight gain: Person might put on weight. You should be able to control this with diet and exercise. But it is often a struggle to keep weight down when you are having hormone treatment. Ask to see a dietician for advice about managing your weight.

Potentials and Problems of hormone therapy

- Help prevent fractures caused by osteoporosis (thinning bones)
- Make some women less likely to have heart disease
- Lower your chances of dementia.
- Relieve hot flashes and night sweats
- Help you sleep better
- Ease vaginal dryness and itching.
- improve muscle function
- reduce the risk of heart failure and heart attacks
- reduce mortality among younger postmenopausal people
- prevent skin aging, in some people.
- Oestrogen also plays a part in controlling other functions, including bone density, skin temperature and keeping the vagina moist. It is a reduction in oestrogen that causes most symptoms associated with the menopause

- The main role of progesterone is to prepare the womb for pregnancy. It also helps to protect the lining of the womb, known as the endometrium.

- A decrease in the level of progesterone does not affect your body in the same way as falling levels of oestrogen. However, taking oestrogen as HRT on its own when you have a womb increases the risk of womb (uterus) cancer, sometimes called endometrial cancer.

Introduction

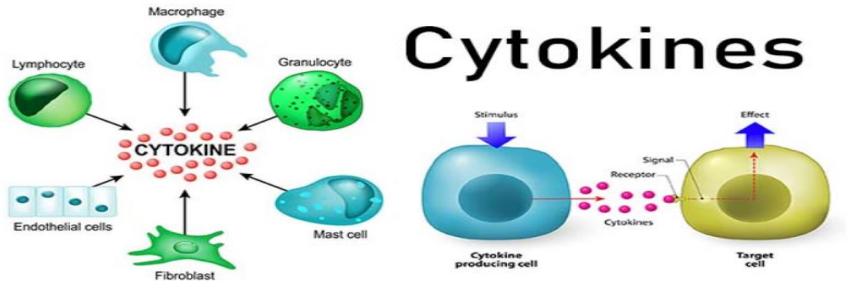
Therapeutic proteins

Proteins which are engineered in the laboratory for pharmaceutical use are referred to as therapeutic proteins" Proteins which are absent or low in individuals with an illness such as Cancer, Infectious diseases, Haemophilia, Anaemia, Multiple sclerosis, Hepatitis B/C, etc. Thus, proteins are artificially synthesized on large scale through genetically modified host cells and delivered.

This therapeutic approach in treating diseases using proteins and peptides is termed protein therapeutics. protein therapy delivers protein to the body in specific amounts (as would be ordinarily present), to help repair illness, treat pain or remake structures.

Cytokines

The word cytokines are derived from the Greek words - "cyto" meaning cell and "kines" meaning movement. Cytokines are low molecular weight protein secreted by white blood cells in response to injury or infection. These regulatory proteins help in regulating the development of immune effector cells. Cytokines are the proteins secreted by the cells of immune system that control the immune responses by interaction between the neighbouring cells. Cytokines are the signalling molecules like hormones and are the end products of interaction among immune cells. Cytokines tend to bind the specific receptors on the target cells but the structure of cytokines and their receptors is very different. Once the cytokines bind to their receptors, transcription factors are produced as a result of changes in the cell behaviour by the process called as signal transduction. Transcription factors stimulate the selected genes for transcription which then secrete new cytokines or signalling molecules. Examples of cytokines include the interleukin and the interferon which are involved in regulating the immune system's response to inflammation and infection



Mechanism action of cytokines

Cytokines bind to the specific receptors on the membrane of target cells, triggering signal transduction pathways that ultimately alter gene expression in the target cells. bind to receptors on the membrane of the same cell that secreted it, exerting autocrine action.Cytokines bind to receptors on a target cell in close proximity to the producer cell, exerting paracrine action. Cytokines bind to target cells in distant parts of the body exerting endocrine action.

Features of cytokines

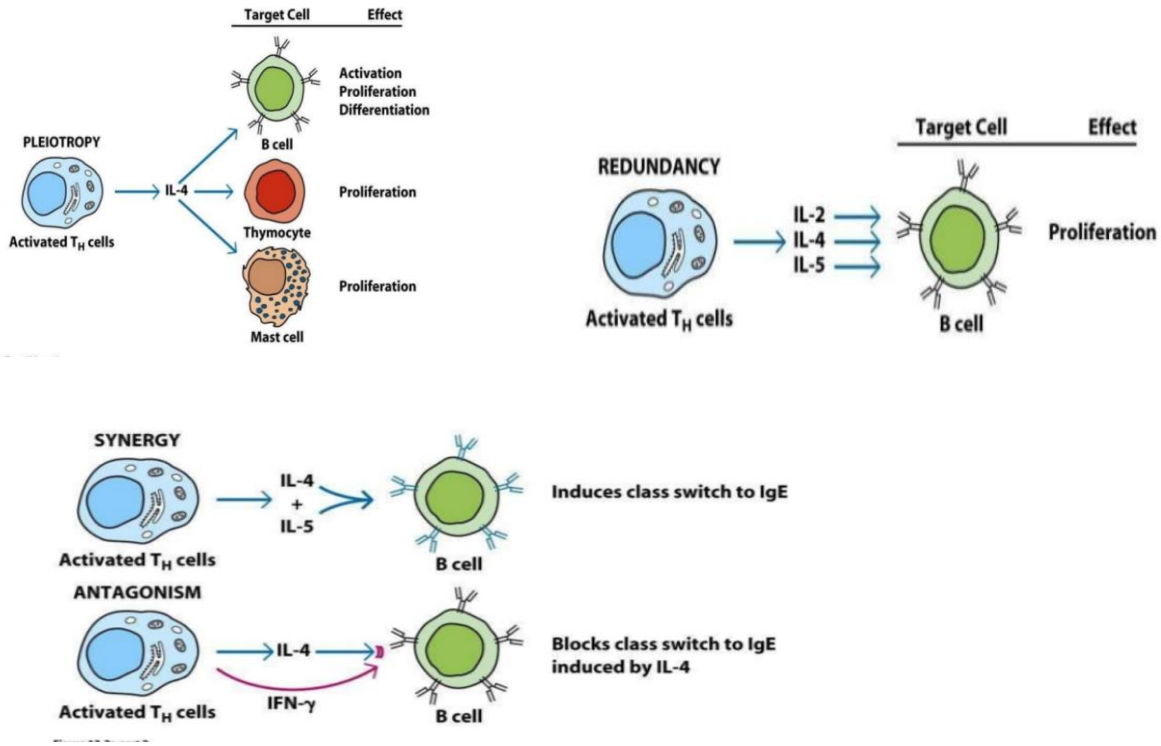
1) **Target specific and induce signal transduction**:

Cytokines binds to specific receptor on the cell membrane of target cell which triggers signal transduction pathway that ultimately alter gene expression in target cell.

2) **High affinity**
The affinity between cytokines ad their receptor is very high. Because of high affinity, cytokines can mediate biological effects at picomolar concentration.

3) **Action:**
i) **Autocrine action:** the cytokine may binds with the membrane receptor of same cell that secrete it.
ii) **Paracrine action:** the cytokine may bind to the receptor on a target cell in close proximity to producer cell.
iii) **Endocrine action:** cytokine may bind to the target cell in distant part of the body.

4) Cytokines are short lived and rapidly degraded
5) Cellular responses to cytokines are generally slow (hours), requires new m RNA and protein synthesis.
6) Share many properties with hormones.
7) Pleiotropic action: A cytokines that has different biological effects on different target cells.
8) Redundancy: Two or more cytokines mediates similar function.
9) Synergy: A combined effects of two cytokines or cellular activity is greater than the additive effects of the individual cytokines.
10) Cascade function: When the action of one cytokine on a targeted cell induce that cell to produce on or more cytokines, which in turn induce other target cells to produce other cytokines.
11) Cytokines need extreme regulation and are toxic in high doses.
12) Cytokines produce their effect on many cell types i.e. they are pleiotropic.
13) They are secreted in multiple numbers, e.g. macrophages secrete more than five interleukins and tumour necrosis factor



Cytokine nomenclature Most of the cytokines are named according to the Interleukin nomenclature subcommittee of the international union of immunological societies. Although the definition of cytokines is quite broad, but together they can be classified as lymphokines, interleukins, interferons, chemokines etc. depending on their function, cell of secretion, or target of action. The name

interleukin was coined initially for those cytokines that mediate signalling between lymphocytes and leukocytes. leukocytes were thought to be the principal target for interleukins but now this definition is no longer in function and the name interleukin is given to any new cytokine discovered routinely. Cytokines are produced mainly in response to viral infection or in response to immune attack. Interferons are the cytokines that interfere with viral RNA and protein synthesis to prevent viral replication. Types of interferon – Interferons are mainly divided into two types.
Type I Interferons --- Interferon-α (IFN- α) and interferon-β (IFN- β).
Type II interferon --- Interferon-γ Tumor necrosis factors (TNFs) can kill tumor cells and colony stimulating factors assist in regulation of stem cell activities. Chemokines play role in leukocyte recruitment and circulation.

Table shows Different cytokines and their examples:

Cytokine types	Examples
Lymphokines	MAF (Macrophage activating factor)
Interleukins	IL-7, IL-13, IL1-7
Interferons	IFN-α, IFN –β, IFN-γ, IFN-ω, IFN -
Tumor necrosis factors	TNF- α (Cachectin), TNF – β (lymphotoxin)
Colony stimulating factors	GSF (Granulocyte colony stimulating factor)
Polypeptide growth factors	EGF (Epidermal growth factor)
α-Chemokines	PF-4 (Platelet factor 4)
β-Chemokines	MCP-1 (Monocyte chemoattractant protein 1)
Transforming growth factors	TGF- α, TGF - β
Stress proteins	Heat shock proteins
RANTES	

Biological functions of cytokines

- Stimulate development of cellular and humeral immune response
- Induction of inflammatory response
- Regulation of haematopoiesis: Cytokines act on hematopoietic stem cells resulting to generation of blood cells.
- Control of cellular proliferation and differentiation
- Healing of wounds
- Take part in innate immunity: Interferons(α,β,∞) are produced in the response to viral infection by the infected cells and act on their target cells to inhibit viral infection.
- Cytokines are involved in the adaptive immunity: Cytokines have effects on activation, proliferation and differentiation of lymphocytes.
- Cytokines have an important role in generation of antibody class diversity.
- Mediate inflammation and Increases the resistance of uninfected cells.
- Activate immune cells such as natural killer cells and macrophages.
- Increase the recognition of infection or tumour cells by up-regulating antigen presentation to T-lymphocytes.

Therapeutic uses of cytokines

1) Used in the treatment of inflammation, infectious diseases and modification of response during organ transplation.
2) Treatment of allergic diseases like asthma.
3) Treatment of viral diseases, cancer.
4) Several cytokines are used to enhance T-cell activation in immunodeficiency diseasesEg:IL-2,IL- α,

5) IL-2 and lymphokine activating killer cells are used in the treatment of cancer.
6) Cytokine therapy has proven to be a novel therapeutic approach in treating patients with advanced malignancies. The purpose of this type of therapy is to manipulate the immune response in such a way as to generate the appropriate immune effector cells to eradicate solid tumors. Cytokine therapy is administrated only after the conventional form of therapies have been performed such as chemotherapy, radiotherapy, and surgery. Various regimens of cytokine administration have been implemented in eradicating solid tumors in patients with melanoma and renal cell cancer.

Lymphokines belongs to class of cytokines that are produced by a type of immune cell known as a lymphocyte. **The lymphokine refers to soluble proteins** produced by lymphoid cells that affect their proliferation, maturation, or function

When the lymphoid cells are exposed to infectious agents, the cells became activated and secreted proteins that acted in an autocrine and/or paracrine fashion. many of the genes for lymphokines have been cloned, sequenced, and characterized. They are protein mediators produced by T cells to direct the immune system response by signalling between its cells. Lymphokines have many roles like attraction of other immune cells, including macrophages and other lymphocytes to an infected site and their subsequent activation to prepare them to mount an immune system. Lymphokines aid the B-cell to produce antibodies. Inhibits T cell growth, activates macrophages, secrete toxins to kill cells, secrete toxins to kill cells and growth inhibiting factors.

In general, lymphokines have effects on hemopoietic cells and cells of the immune system. They have short in vivo half-lives and act locally at very low concentrations. Some are produced constitutively, but most are up-regulated in response to infection, antigen activation. Most lymphokines are produced with a signal peptide that is cleaved at the cell membrane, thereby releasing lymphokine to the exterior.

The lymphokines are divided into three groups, based on their effects on T and B lymphocytes and NK (natural killer) cells.

1) T-cell Lymphokines.
2) B- cell Lymphokines.
3) Natural Killer (NK)- cell Lymphokines

Lymphokines in therapy

1) Treatment of Rickettsia

Rickettsia is a bacterial infection caused by a bacterium (R. rickettsii) that can live only inside the cells of another organism, this is gram-negative bacteria. Rickettsia that infect vascular endothelial cells throughout the body can cause life-threatening and interstitial pneumonia/acute respiratory distress syndrome. There are two types of organisms which causes Rickettsia.

Agent	Protective	Immunopathogenic
Rickettsia conorii	Depletion of CD8 T cells which results in infection	Transfer of CD8 cells to treat disease
Rickettsia Australis	Immune spleen cells are cytotoxic for infected MHC 1 endothelial cells	MH1 cells are treated

2) Treatment of neurobiological disorders

lymphokines are multifunctional immunoregulatory proteins secreted by cells of the immune system. Cytokines can exert neuroprotective effects independent of their immunoregulating properties. However, under certain conditions, cytokines and chemokines promote apoptosis of neuron. The levels of several cytokines (including TNF-α, IL-1, and IL-6) become elevated in a

wide range of CNS disorders, including ischemia, trauma, multiple sclerosis, Alzheimer's disease (AD), and Parkinson's disease (PD). cytokine-driven neuroinflammation and neurotoxicity may modify disease progression in a number of neurodegenerative diseases.

3) Treatment of cancer

Macrophage activation with liposomes containing lymphokines. combinations of these agents results in the eradication of well-established lung and lymph node metastases. successful treatment of metastases by the intravenous injection of liposomes containing immunomodulators have also been reported for several murine fibrosarcoma, melanomas, lung carcinoma, liver metastases, renal cell carcinoma, and primary skin cancers.

INTERFERONS

- Discovered by Issacs and Lindenmann in 1957. They were performing experiment on chicken cell culture and found a substance which interfered virus replication. They named the substance as Interferon.
- Interferons are member of a large group of proteins called cytokines which affect a wide range of target cells and tissue by binding to specific receptors present on the surface of target cells.
- Interferons play an important role in first line of Défense against viral infections. They are part of the non-specific immune system and are induced at an early stage of viral infection before the specific immune system has had time to respond.
- Interferons are produced by cell in response to an appropriate stimulus and are released into the surrounding medium. Then they bind to receptors on target cells and induce transcription of approximately 20-30 genes in target cells which results in antiviral state in the target cell.
- Interferons differ from hormone as these are produced by variety of cell types and not specific endocrine organs.
- Interferons regulate the growth, differentiation and functions of different types of immune cells.

Properties of interferons:

Property	Alpha	Beta	Gamma
Current nomenclature	IFN-α	IFN-β	IFN-γ
Former designation	Leucocyte interferon (Type I interferon)	Fibroblast interferon (Type I interferon)	Immune interferon (Type II interferon)
No. of genes coding	≥15	1 or few	1 or few
No. of subtypes	20	2	3
Principal cell source	Leucocytes	Fibroblasts	Lymphocytes and NK cells
Inducing agent	Viruses, dsDNA	Viruses, dsDNA	Mitogens
Size (MW)	17,000	17,000	17,000
No. of amino acids	143	145	146
Introns in genes	No	No	Yes, 3
Chromosomal location	9	9	12
Stability at pH 2	Stable	Stable	Labile
Glycosylation	No	Yes	Yes

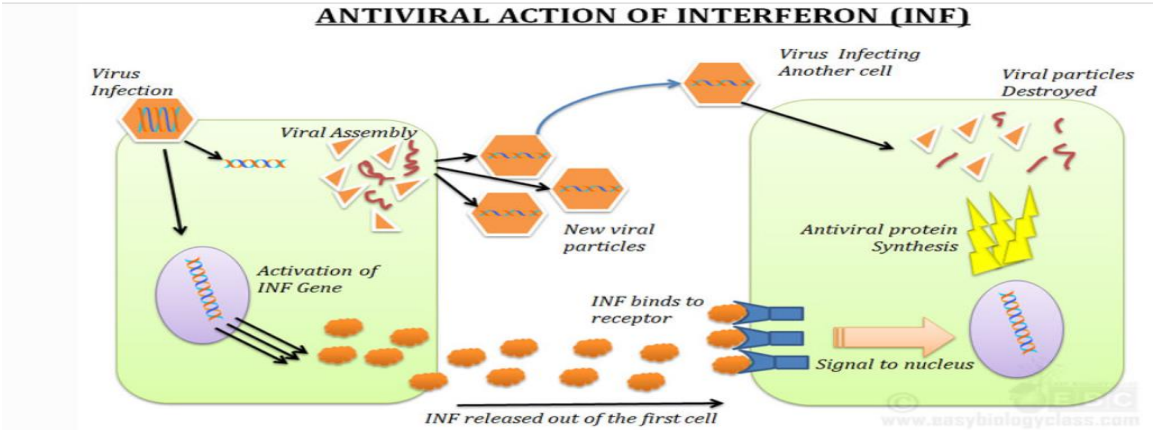
Useful activities of interferons

- Inhibition of virus replication inside target cell
- Protection of cells against intracellular parasites.
- Inhibition division of some normal and transformed cells
- Regulation of cell differentiation
- Induction of cytokines. For example; IL-I , tumour necrosis factor are induced by IFN-Y.

- Activation of NK cells and macrophages
- Increase expression of class I MHC molecules and thus promote recognition by cytotoxic T cells
- IFN-Y increase expression of class II MHC molecules.
- Used in treatment of various virus infection such as Hepatitis B and C.
- IFN-Y is used in treatment of leprosy, leishmaniasis, toxoplasmosis
- Used in inhibition of growth of cancer cells and myeloma cells.
- Used to treat chronic active hepatitis by HBV,HCVby human papilloma virus.
- Used in the treatment of leukaemia and kaposi's sarcoma

Antiviral properties if interferons

The synthesis and release of interferons from a cell is induced by viral particles intact viral particles and even the presence of double stranded viral RNA (dsRNA) in the cell can evoke the production of interferons. Specific interferons are recognized by receptors present on the plasma membrane. Once a cell receives the stimuli, the interferons proteins are synthesized and they are released out of the cell. The secreted interferons molecules then bound to the receptors on the plasma membrane of the cell.



Production of recombinant interferons:

The complementary DNA (cDNA) was synthesized from the mRNA of a specific interferon. This is inserted to a vector (plasmid) which is introduced into E. coli or other cells. The interferon can be isolated from the culture medium. This is the basic mechanism of producing recombinant interferons. The production of interferons is relatively less in bacterial hosts, although E. coli was the first to be used. This is mainly because most interferons are glycoproteins in nature and bacteria do not possess the machinery for glycosylation of proteins.

Production interferons by yeasts:

The yeast Saccharomyces cerevisiae is more suitable for the production of recombinant interferons. This is mainly because the yeast possesses the mechanism to carry out glycosylation of proteins, similar to that occurs in mammalian cells. The DNA sequence coding for specific human interferon can be attached to the yeast alcohol dehydrogenase gene in a plasmid and introduced into 4 yeast cells. The yield of interferons is higher compared to E. coli.

Production of hybrid interferons:

Several attempts have been made to produce hybrid interferons. This is advantageous since different interferons with different antiviral activities can be combined to produce a more efficient interferon. Further, the glycosylation step can be bypassed, and bacteria can be used to produce hybrid interferons. The hybrid interferons are more reactive in performing their function.The creation of hybrid genes from the genes of IFN-α_2 and IFN-α_3 is illustrated in Fig.These genes are digested by restriction endonucleases. The resulting fragments are ligated to generate hybrid genes. The

appropriate hybrid genes can be selected and used for producing hybrid interferons. E. coli can be employed for this purpose.

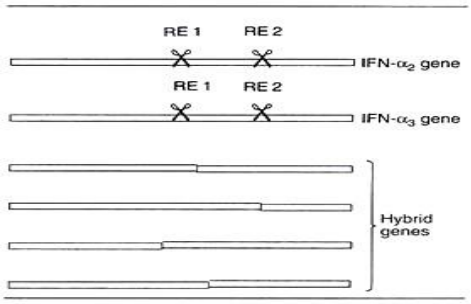


Fig. 15.7 : Creation of hybrid interferon genes (RE-Restriction endonuclease, IFN-Interferon).

Purification of interferons

The alcohol/salt aqueous two-phase system (ATPS) is used in purification of interferons. alcohol/salt ATPS offers other advantages like low toxicity to environment and inexpensive cost.

1.Bacterial strains and plasmid

Recombinant E. coli K12 culture, Rosetta-gami2 (DE3) strain which was chosen for IFN-a2b producing host cell as it combines the advantageous features which will enhance the disulphide bond formation and expression of eukaryotic proteins. IFN-a2b gene was inserted into pET26b plasmid which consists the resistance gene in opposition to kanamycin, this recombinant plasmid was then inserted into E. coli Rosetta-gami2 (DE3) strain with chloramphenicol resistance gene.

2.Fermentation of recombinant E. coli

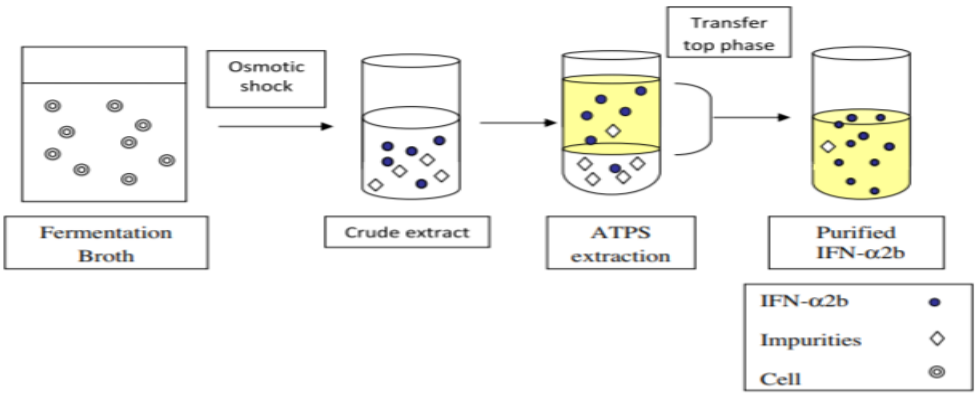
Inoculum was prepared by incubating the recombinant E. coli in 250 mL baffled shake flasks containing 50 mL Terrific Broth (TB) with 34 mg/L chloramphenicol and 30 mg/L kanamycin. The inoculum was incubated at 37 degree Celsius and shaken 250rpmfor 16 hours in an incubator shaker. After the recombinant E. coli culture inoculated with 8% (v/v) inoculum, the culture was then incubated at 37^{o} C, with agitation speed at 250 rpm for 4 h. The IFN-a2b production was then induced by adding 1 mM isopropyl-b-D-thio-galactosidase. Once the IPTG added, the temperature was changed to 30 C with agitation speed at 250 rpm, the cells were then harvested after 8 h and stored at 20 C until further processing.

3. Cell disruption

Osmotic shock was performed to rupture the recombinant E. coli culture cell body, therefore releasing the periplasmic IFN-a2b contained within. The cell pellets were suspended in a buffer solution comprising 20% (w/v) sucrose, 0.03 M Tris–HCl, 5 mM sodium EDTA (pH 8.0). The mixture was rigorously shaken for 5 min to enhance osmotic shock process. Subsequently, the mixture was centrifuged for 10 min at 4000 rpm and 4 C. Next, the cell pellets were re-suspended and shaken rapidly in cold ultra-pure for 10 min. The mixture was centrifuged again for 10 min at 4000 rpm and 4 C. The supernatant from centrifuged mixture was collected.

4. ATPS

The predetermined quantities of alcohol and dissolved salts were prepared in a 15 mL centrifugal tube. The 1 g of crude extract was then added into the mixture to make the ATPS to reach a final total weight of 10 g. The phase system was mixed thoroughly by gentle agitation and then subjected to centrifugation at 4000 rpm for 10 min to speed up the phase separation. After the phase separation, the top and bottom phases were collected, and then the concentrations of total protein and IFN-a2b within the system were analysed. It was always not easy to evaluate the degree of purification by monitoring partition coefficient of IFN-a2b only. Therefore, the PF (top phase) of the ATPS was evaluated as well (Fig.). All the experiments were carried out at room temperature



Purification of interferons

Interleukin (IL), any of a group of naturally occurring proteins that mediate communication between cells. Interleukins regulate cell growth, differentiation, and motility. They are particularly important in stimulating immune responses, such as inflammation. Interleukins are a subset of a larger group of cellular messenger molecules called cytokines, which are modulators of cellular behaviour. Like other cytokines, interleukins are not stored within cells but are instead secreted rapidly, and briefly, in response to a stimulus, such as an infectious agent. Once an interleukin has been produced, it travels to its target cell and binds to it via a receptor molecule on the cell's surface. This interaction triggers a cascade of signals within the target cell that ultimately alter the cell's behaviour. The first interleukins were identified in the 1970s. Initially investigators believed that interleukins were made chiefly by leukocytes (white blood cells) to act primarily on other leukocytes, and for this reason they named them interleukins, meaning "between leukocytes." Because leukocytes are involved in mounting immune responses, interleukins were thought to function only as modulators of immune functions. Although this is an important function of interleukins, it is now known that interleukins also are produced by and interact with a host of cells not involved in immunity and are involved in many other physiological functions. Thus, the role that interleukins play in the body is much greater than was initially understood.

1)Interleukins 1(IL-1): IL-**1** is **produced** predominantly by macrophages and macrophage-like cells but also by endothelial and epithelial cells.

Biological activities of interleukin-1 raises body temperature, spurs the production of interferon, and stimulates growth of disease-fighting cells.IL-1 are classified as IL-α. and IL-β.IL -1 is involved in the central nervous system.IL-1 is involved in the cortico-steriod release,induces fevaer and shivering-useful responses,because eleavted body temeperature reduces bacterial growth.

2) **Interleukins 2(IL-2):** (IL-**2**) a type of cytokine signalling molecule in the immune system. It is a 15.5–16 kDa protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity.

Biological activities of interleukin-2.IL-2is made by a type of T lymphocyte. It increases the growth and activity of other T lymphocytes and B lymphocytes, and affects the development of the immune system.IL-2 IL-2 also controls inflammation by inhibiting T_H17 differentiation. IL-2 prevent diabetes by inducing a repertoire of islet-reactive $CD4^+Foxp3^+$ T_{reg} cells.IL-2 exerts anti-tumour effect through augmentation of its immune system. High dose can produce tumour regression in cancers like metastatic melanomas.IL-2 augments the cytolytic activity of T and NK cells, it is involved in the programming of CD8 memory T cells, which undergoes secondary expansion in viral infections.IL-2 growth factor for B cells and induce class switching and activates macrophages.

3.Interleukins 3(IL-3): (IL-**3**) Interleukin-3 (IL3) is a cytokine that regulates blood-cell production by controlling the production, differentiation and function of granulocytes and macrophages.

Biological activities of (IL-**3**) The protein, which exists in vivo as a monomer, is produced in activated T-cells and mast cells and is activated by the cleavage of an N-terminal signal sequence.IL3 is produced by T-lymphocytes and T-lymphomas only after stimulation with antigens, mitogens, or chemical activators such as phorbol esters. However, IL3 is constitutively expressed in the myelomonocytic leukaemia cell line It is thought that the genetic change of the cell line to constitutive production of IL3 is the key event in development of this leukaemia.IL-3 is also called as colony stimulating factor.

4.**Interleukins 4: IL-4:** Interleukin-4 (IL-4) is a complex glycoprotein produced mostly by mast cells, basophils, a subset of activated T cells, eosinophils and neutrophils

Biological activities IL-4 of Interleukin 4 has many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of B cells into plasma cells. It is a key regulator in humoral and adaptive immunity. IL-4 induces B-cell class switching to IgE, and up-regulates MHC class II production.IL 4 promotes CD 8 cell growth and promotes TH2 cell differention.

5. **Interleukins 6:**IL-6 is responsible for stimulating acute phase protein synthesis, as well as the production of neutrophils in the bone marrow. It supports the growth of B cells and is antagonistic to regulatory T cells.

Biological activities IL-6 plays an essential role in the final differentiation of B-cell into IG-secreting cells, nerve cell differentiation and in hepatocytes.

6. **Interleukins 10:IL-10** is a cytokine with potent anti-inflammatory properties that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. In humans, IL-10 is encoded by the IL10 gene, which is located on chromosome 1 and comprises 5 exons, and is primarily produced by monocytes

Biological activities: Interleukin 10 is an anti-inflammatory cytokine that plays a crucial role in preventing inflammatory and autoimmune pathologies. Elevated levels of IL-10 can hinder host response to microbial pathogenesis and prevent resolution of associated tissue damage and hemodynamic disturbances.

7.**Interleukins 20:**IL-12 is produced primarily by macrophages, dendritic cells, and B lymphocytes and regulates activation. IL-12 appears to play a critical role in Défense against intracellular pathogens, particularly Mycobacterium species, and has been studied for the treatment of nontuberculous mycobacterial infection.

Biological activities IL-12 is involved in the stimulation and maintence of Th1 cellular immune responses, including the normal host defence against various intracellular pathogens, such as HIV.IL-12 plays an important role in pathological Th 1 responses, such as inflammatory bowel disease and multiple sclerosis.

Colony-stimulating factors (CSFs) are secreted glycoproteins that bind to receptor proteins on the surfaces of hemopoietic stem cells, thereby **activating** intracellular signalling pathways that can cause the cells to proliferate and differentiate into a specific kind of blood cell (usually white blood cells).

Types of CSFs

- **GM-CSF or CSF2**, which stimulates the proliferation of granulocytes, macrophages, and also eosinophils, as well as megakaryocytes, the progenitor cells of platelets, at high doses
- **M-CSF or CSF1** which stimulates macrophage colony formation

- **G-CSF or CSF3** which causes granulocyte colony formation but also granulocyte-macrophage colonies to a lesser extent
- **Multi-CSF or IL-3** which stimulates colony formation for a broad spectrum of blood cells.

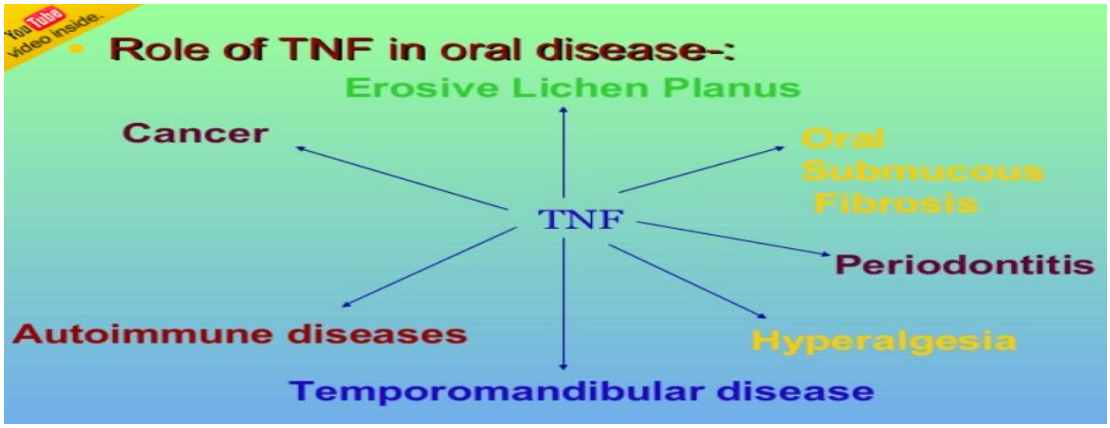
Uses of CSF

- G-CSF and GM-CSF have been used to increase the granulocyte levels in peripheral blood in cancer patients on chemotherapy, with a clear dose-dependent response, and so prevent falling neutrophil counts with fever following chemotherapy. This is associated with a higher risk of infection up to 60%, which not only requires intensive treatment but may delay chemotherapy or make lower doses necessary. This improves patient survival, in turn.
- G-CSF is used in non-Hodgkin's lymphoma and breast carcinoma (early stage). Their use is associated with an almost 50% reduction in neutropenia with fever and death due to infection, while survival improves by 40%.
- A newly approved drug is polyethylene glycol (PEG)-conjugated G-CSF, also called pegylated G-CSF or pegfilgrastim. It is retained for a longer period in the body and can thus drastically reduce the number of injections required to allow normal chemotherapeutic schedules to go on, especially in older and more frail patients. Many major professional oncologic bodies now recommend that these factors be used to prevent infective complications due to neutropenia if a chemotherapy recipient has a 20% or more risk of febrile neutropenia, or other risk factors for such complications. CSFs may prevent the need for bone marrow transplantation in chemotherapy-induced aplastic anemia. The use of GM-CSF or G-CSF can push up peripheral blood stem cell (PBSC) counts, which can then be used to repopulate the blood with neutrophils and platelets, much faster than by bone marrow transplants using bone marrow cells, and comparable to the use of bone marrow grafts with CSF. CSF-stimulated PBSC grafting is now the preferred technology especially since the safety of the CSFs in the normal donors has been proved, because of its relative simplicity, high effectiveness, and range of application.
- CSFs can be used to prevent infection for years in conditions such as chronic neutropenia.
- GM-CSF can also help improve immunity by regulating dendritic cell development. These cells are an essential part of innate immunity as they present captured and processed antigens for antibody and cellular immune responses.
- The use of these CSFs to stimulate local immunity within a tumor and so shrink or remove it is presently being studied.
- A recent area of interest is CSF use to help restore normal bone marrow function in victims of accidental radiation exposure

Tumour necrosis factor

Tumor necrosis factor (TNF) is a multifunctional cytokine that plays important roles in diverse cellular events such as cell survival, proliferation, differentiation, and death. There are two distinct forms of tumour necrosis factor called TNF-α and TNF-β. as a cytokine produced by immune cells having a capacity to suppress tumour cell proliferation and induce tumour regression. Tumor necrosis factor (TNF) (also known as TNF-alpha or cachectin) is a monocyte-derived cytotoxin that has been implicated in tumour regression, septic shock.

The protein is synthesised as a prohormone with an unusually long and atypical signal sequence, which is absent from the mature secreted cytokine. A short hydrophobic stretch of amino acids serves to anchor the prohormone in lipid bilayers. Both the mature protein and a partially-processed form of the hormone are secreted after cleavage of the propeptide. There are a number of different families of TNF, but all these cytokines seem to form homotrimeric (or heterotrimeric in the case of LT-alpha/beta) complexes that are recognised by their specific receptors.



1.) **Role in cancer**: Direct cytotoxic effects on tumour cells by modifying the vasculature so that migration of lymphocyte into tumour is enhanced. Stimulation of immune response by activating T-cells that mediate anti-tumour activity.

2) **Role in Hyperalgesia:** It is a condition of hypersensitivity, other than prostaglandins tumour necrosis factors shows the process of hyperalgesic properties.

3)**TNF is a protein that plays a role in the natural healing process**. When a person sustains an injury or experiences bacterial or viral infections, their body creates inflammation to protect the area and allow it to heal.To create inflammation, TNF proteins begin to circulate in the blood. They arrive at the target area to trigger the inflammation process. In healthy people, the body deactivates any excess TNF in the blood so it does not cause excess inflammation. When this process does not work properly, people can develop an autoimmune condition.