SURVEY OF LITERATURE
The survey of literature given here from the available information on the underlying mechanisms of inflammation, degeneration, cell death as the basis of chronic disease processes, has attempted to detail in depth about these phenomena and certain portions of extra elaboration, though in an extent more than required, is only as a part of honest attempt to provide the reader an idea about the intricate mechanisms involved in such disease processes.

INFLAMMATION

Acute Inflammation

Inflammation is the response of living tissue to damage; hence this response in acute inflammation has three main functions:

1. Affected area is occupied by transient material called the acute inflammatory exudates, which contain proteins, fluids and cells from the local blood vessels pouring into the damaged area to mediate local defenses.

2. If an infective causative agent (bacteria) is present in the damaged area, it can be destroyed, eliminated and evacuated by the components of the exudates.

3. Damaged tissue can be broken down and partially liquified, and the debris removed from the site of damage.

4. The cause of inflammation may be a microbial infection, a hypersensitivity reaction to a foreign protein, noxious physical agents (like ionizing radiation, burns, excessive cooling
(frostbite) or corrosive chemicals. Necrotizing inflammation occurs due to inadequate blood supply to a tissue resulting in infarction.

The classical signs of acute inflammation include, Rubor (redness due to blood flow), Calor (rise of local temperature due to increased blood flow and chemical changes of inflammation), Tumor (swelling or oedema due to accumulation of exudates which contains cellular and fluid content) and Dolor (pain). The mediators of inflammation like bradykinin, prostaglandin, serotonin etc. are known to produce registration of painful stimulus which helps in immobilization of the affected part of the body allowing healing processes to proceed undisturbed.

The systemic effects of acute inflammation viz., pyrexia – the secondary to endogenous pyrogens produced by the inflammatory cells (polymorphs and macrophages), constitutional symptoms (malaise, anorexia and nausea) resulting from the catabolic chemical changes in the body, reactive hyperplasia of the reticuloendothelial system due to the multiplication of inflammatory cells. The immune functioning molecules, together with such inflammatory cells bring about the mechanics of 'fight-flight' reaction of the body's immune system.

- The processes of inflammation mentioned above help in dilution of toxins by the fluid exudates, entry of antibodies into the extra vascular space helping in phagocytosis and neutralization of toxins produced by the infecting agent. These help in the delivery of
drugs, nutrients and oxygen to the site of inflammation. Formation of fibrin from fibrinogen in the exudates helps to interfere with the movement of micro-organisms and trapping them by the phenomenon of phagocytosis. The drainage of fluid exudates into the lymphatics allows the transport of the particulate and soluble antigens to the regional lymph nodes, where they further stimulate the immune response (see Janeway et al., 2001; see Ross, 2002)

The markers of chronic inflammation

POLYMORPHS

These polymorphs devour and destroy the noxious inflicting agent by a process called Chemotaxis which is calcium iron dependent. The process of opsonisation (a Greek word meaning 'to prepare the table'), help to render the microorganisms amenable to phagocytosis. This process is activated by immunoglobulins, complement components. Bacterial lipopolysaccharides activate complement via an alternate pathway, generating component C3b, which has opsonising properties. Antibodies binding to bacterial antigen can also activate the complement, generating C3b. In the immune individual, the binding of immunoglobulins to microorganisms by their Fab components leaves the Fc component exposed (Kelso, 1998). Neutrophils have surface receptors for the Fc fragment of immunoglobulins and consequently bind to the micro-organisms prior to ingestion.

The destruction of the microbial agents by polymorphs is partly oxygen dependent and partly oxygen independent. Neutrophil polymorphs
are highly specialised cells, containing noxious microbial agents, some of which are similar to household bleach. The microbial agents may be classified as:

- Those which are oxygen-dependent
- Those which are oxygen-independent.

**Oxygen-dependent mechanisms**

The neutrophils produce hydrogen peroxide, which reacts with myeloperoxidase in the cytoplasmic granules in the presence of halide, such as Cl, to produce a potent microbial agent. Other products of oxygen reduction also contribute to the killing, such as peroxide anions (O$_2^-$), hydroxyl radicals (·OH) and singlet oxygen (1O$_2$).

**Oxygen-independent mechanisms**

These include lysozyme (muramidase), lactoferrin which chelates iron required for bacterial growth, cationic proteins and the low pH inside phagocytic vacuoles.

Release of lysosomal products from the cell damages and local tissues by proteolysis by enzymes such as elastase and collagenase, activates coagulation factor XII and attracts other leucocytes into the area. Some of the compounds released increase vascular permeability, while others are pyrogens, producing systemic fever by acting on the hypothalamic thermo regulatory centre.
Chronic inflammation is an inflammatory response of prolonged duration, which is provoked by persistence of the causative stimulus to inflammation in the tissue. This process inevitably causes tissue damage (degeneration) and is accompanied by simultaneous attempts at healing and repair (regeneration) (Crook et al., 1976). Chronic inflammation can develop as a progression from acute inflammation if the original stimulus persists or after repeated episodes of acute inflammation or de novo if the causative agent produces only a mild acute response.

- Mixed inflammatory cell infiltrate contains macrophages, lymphocytes and plasma cells. Lymphoid cells proliferate at the site of inflammation as well as in the local lymph nodes. In severe cases these cells take a picture of lymphoid follicles with germinal centers (Tsokos, 1995).

- Tissue destruction (necrosis) is caused by both causative agent and by the process of inflammation itself. Attempts at the reconstruction of the damaged tissue occurring simultaneously with the inflammatory process produce regeneration and restructuring of the inflammed organ even after resolution of the inflammation. This process involves removal of the destroyed cells by phagocytosis, angiogenesis and lymphangiogenesis (Stacker et al., 2002). These processes help the fibroblasts to proliferate and produce collagen matrix by and form granulation tissue which at times ends up with dense fibrous scar.
- The above mentioned processes of body fighting mechanisms are mediated and modulated by hormones like β-endorphins, neuropeptides and inflammatory modulatory cells like macrophages, lymphocytes, NK cells, the immunoglobulins and complements, ensuring their own individual roles. Our study investigated the role of growth hormone, testosterone, oestrogen, progesterone, thyroid & parathyroid hormone in modulating the above processes in a favourable way.

**CHEMICAL MEDIATORS OF ACUTE INFLAMMATION**

The spread of acute inflammatory response following injury to a small area of tissue is attended with the release of chemical substances from the affected cells, spreading outwards into the uninjured areas. These chemicals are called endogenous chemical mediators. They cause vasodilation, immigration of polymorphs, chemotaxis and increased vascular permeability (see Galimberti et al., 2004; see Aukrust et al., 2005; see Glabinski et al., 2005).

**Histamine:**

This is one of the prominent chemical mediators in acute inflammation and causes vascular dilation in the immediate, transient phase of increased vascular permeability. It is stored in the mast cells, basophils, eosinophils, leucocytes and platelets. Histamine is released by a process of cell degranulation by these cells. This process is stimulated by complement components C3a and C5a and by lysosomal proteins released from
polymorphs (including cationic proteins which increases vascular permeability and neutral proteases which activate complement) (Hugli et al., 1978)

*Prostaglandins*

These are a group of long chain fatty acids derived from arachidonic acid and are synthesized by many cell types (autocoids). Some prostaglandins potentiate the increase in vascular permeability caused by the other compounds, while the others induce platelet aggregation for which prostaglandin series (PGA1) is inhibitory, while prostaglandin A2 (PGA2) is stimulatory. Anti-inflammatory activity of non-steroidal anti-inflammatory drugs and aspirin is attributed to the inhibition of the enzyme prostaglandin synthetase (Crook et al., 1976).

*Leukotrienes*

These are also synthesized by polymorphs from arachidonic acid and have vasoactive properties. SRS-A (slow reacting substance of anaphylaxis) involved in type 1 hypersensitivity reactions is a mixture of leukotrienes.

*Lymphokines*

Are cytokines synthesized by lymphocytes and have vasoactive and chemotactic properties. They are essential in type IV hypersensitivity reactions.
Serotonin (5-hydroxytryptamine) is present in high concentration in mast cells and platelets and has potent vasoconstrictive action.

The plasma contains four enzymatic cascade systems, the complement system, the kinins, the coagulation factors and the fibrinolytic system, each of which are interrelated and work in union in fighting inflammation.

In the tissue, the dying cells release necrosis enzymes capable of activating complement system. During infection the formation of antigen-antibody complexes activates the complement via the classical pathway, while the endotoxins of gram-negative bacteria activate complement via the alternative pathway. Products of the kinin, coagulation and fibrinolytic system can also activate complement. The important products of complement activation include

- **C5a**: chemotactic for polymorphs, increasing vascular permeability, releases histamine from mast cells.
- **C3a**: fairly similar to C5a but less active
- **C5, 6,7**: chemotactic for poymorphs
- **C5, 6,7,8,9**: having cytolytic activity
- **C4b, 2a, 3b**: helps in opsonisation of bacteria and helps phagocytosis by macrophages.
**Kinin Systems**

The kinins are peptides of 9-11 amino acids, bradykinins being the most important vascular permeability factor are the significant mediators of pain and so happen to be a prime mover in inflammatory processes. The kinins are activated by the coagulation factor XII.

**Coagulation System**

Responsible for the conversion of soluble fibrinogen into fibrin, a major component of the acute inflammatory exudates. Coagulation factor XII (Hagemann factor) once activated by contact with extracellular materials such as basal lamina and various proteolytic enzymes of bacterial origin, can activate coagulation, kinins and the fibrinolytic systems.

**Fibrinolytic Systems**

Plasmin is responsible for the lysis of fibrin into fibrin degradation products. These may have local effects on vascular permeability and which is responsible for the Disseminated Intravascular Coagulation (DIC), encountered in and causative of the multiple organ dysfunction/multiple organ failure occurring in the course of various chronic and acute diseases.
Wound healing

The characteristic features of chronic and acute wounds that fail to heal are excessive leucocytosis and reduced matrix deposition. Oestrogen is a major regulator of wound repair that can reverse age-related impaired wound-healing in human and animals models, characterized by a dampened inflammatory response and increased matrix deposited at the wound site. Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine involved in the hormonal regulation of inflammation. Macrophage migration inhibitory factor is upregulated during healing in a distinct spatial and temporal pattern. The expression of this proinflammatory cytokine is markedly elevated in wounds of oestrogen-deficient mice as compared with intact animals (Swope and Lolis, 1993). Wound healing studies in mice rendered null for the \textit{mif} gene have demonstrated that, in the absence of \textit{mif} gene, the excessive inflammation and delayed-healing phenotype, associated with ageing reduced oestrogen are reversed. Moreover, \textit{in vitro} assays have shown a striking decrease in MIF production by activated murine macrophages, by activation of oestrogen receptors. The role of oestrogen in regulating the local inflammatory response by down regulating macrophage migration inhibiting factor, opens up newer modes of therapeutic interventions in problems of wound healing (Calvin \textit{et al.}, 1998). With the improvement of medical care and progress in epidemiological factors, the problems posed by ageing are on the rise all over the world. Inflammation and the resultant proteolysis of matrix, being a causative factor in age-related delay, it is well established that in the absence of infection the problems posed
by inappropriate inflammatory response, can be tackled by oestrogen which modulates the extent of leucocyte recruitment during the initial stages of injury and tissue destruction (Calvin et al., 1998; Ashcroft et al., 1999).

Recent reports have shown that hormone replacement prevents the development of chronic wounds (both pressure ulcers and venous ulcers) and topical oestrogen has been shown to hasten healing of such slow healing wounds in both elderly women and men. This reflects the marked decrease in local oestrogenic activity secondary to reduced ovarian activity in menopausal women and a decline in the adrenal oestrogenic precursor dehydroepiandrosterone (DHEA) in both men and women. In chronic inflammatory disorders, the influx of leucocytes occurs unabated, leading to enhanced cytokine and chemokine production, which in turn increases the cells in further unchecked leucocyte recruitment and ultimately proteolytic tissue destruction. Oestrogen accelerates the cutaneous wound healing process by enhancing cellular matrix deposition, rapid epithelialization and a dampening of the excessive inflammatory response (Calvin et al., 1998; Ashcroft et al., 1999).

The role of cytokines in inflammation

Cytokines are made by many cell populations, but the predominant producers are helper T cells (Th) and macrophages. The largest group of cytokines that stimulate the proliferation and differentiation of the immune cells belong to the group of interleukins.
Interleukin 1 (IL-1), which activates T cells; IL-2, which stimulates proliferation of antigen-activated T and B cells; IL-4, IL-5 and IL-6, which stimulate proliferation and differentiation of B cells; Interferon gamma (IFNg), which activates macrophages; and IL-3, IL-7, Granulocyte Monocyte Colony Stimulating Factor (GM-CSF), which stimulate hematopoiesis (Tsokos, 1995).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Producing Cell</th>
<th>Target Cell</th>
<th>Function**</th>
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<tbody>
<tr>
<td>GM-CSF</td>
<td>Th cells</td>
<td>progenitor cells</td>
<td>growth and differentiation of monocytes and DC</td>
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<td>IL-1α</td>
<td>monocytes</td>
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<td>co-stimulation</td>
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<td>IL-1β</td>
<td>macrophages</td>
<td>B cells</td>
<td>maturation and proliferation</td>
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<td>B cells</td>
<td>NK cells</td>
<td>activation</td>
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<td></td>
<td>Dendritic Cells (DC)</td>
<td>various</td>
<td>inflammation, acute phase response, fever</td>
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<td>IL-2</td>
<td>Th1 cells</td>
<td>activated T and B cells, NK cells</td>
<td>growth, proliferation and activation</td>
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<tr>
<td>IL-3</td>
<td>Th cells</td>
<td>stem cells</td>
<td>growth and differentiation</td>
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<td></td>
<td>NK cells</td>
<td>mast cells</td>
<td>growth and histamine release</td>
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<td>Th2 cells</td>
<td>activated B cells</td>
<td>proliferation and differentiation IgG₁ and IgE synthesis</td>
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<td></td>
<td></td>
<td>macrophages</td>
<td>MHC Class II</td>
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<td>IL-5</td>
<td>Th2 cells</td>
<td>activated B cells</td>
<td>proliferation and differentiation, IgA synthesis</td>
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<tr>
<td>Cytokine</td>
<td>Producing Cell</td>
<td>Target Cell</td>
<td>Function**</td>
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<td>IL-6</td>
<td>monocytes</td>
<td>activated B cells</td>
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<td></td>
<td>macrophages</td>
<td>plasma cells</td>
<td>antibody secretion</td>
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<td>Th2 cells</td>
<td>stem cells</td>
<td>differentiation</td>
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<td></td>
<td>stromal cells</td>
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<td>IL-7</td>
<td>marrow stroma</td>
<td>stem cells</td>
<td>differentiation into progenitor B and T cells</td>
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<td>thymus stroma</td>
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<td>IL-8</td>
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<td>neutrophils</td>
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<td>IL-10</td>
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<td>B cells</td>
<td>NK cells</td>
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<td>IFN-α</td>
<td>leucocytes</td>
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<td>viral replication</td>
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<td>MHC I expression</td>
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<td>IFN-γ</td>
<td>Th1 cells, Tc cells, NK cells</td>
<td>various</td>
<td>Viral replication</td>
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<td>macrophages</td>
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<td>activated B cells</td>
<td>Ig class switch to IgG₂</td>
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<td>macrophages</td>
<td>pathogen elimination</td>
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<td>macrophages</td>
<td>monocytes, T cells</td>
<td>chemotaxis</td>
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<td>MIP-1β</td>
<td>lymphocytes</td>
<td>monocytes, T cells</td>
<td>chemotaxis</td>
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<td>TGF-β</td>
<td>T cells, monocytes</td>
<td>monocytes, macrophages</td>
<td>chemotaxis</td>
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The other groups of cytokines include interferons and chemokines. Interferons IFNα and IFNβ inhibit virus replication in infected cells, while IFNγ also stimulates antigen-presenting cell MHC expression. Chemokines attract leucocytes to infection sites. Chemokines have conserved cysteine residues in them. This identifies them into four groups:

1. C-C chemokines (RANTES, MCP-1, MIP-1α, and MIP-1β)
2. C-X-C chemokines (IL-8)
3. C chemokines (Lymphotactin)
4. CXXXC chemokines (Fractalkine).

Some cytokines are predominantly inhibitory. For example, IL-10 and IL-13 inhibit inflammatory cytokine production by macrophages.
Helper T cells have two important functions. They stimulate cellular immunity and inflammation, and stimulate B cells to produce antibody. Two functionally distinct subsets of T cells secrete cytokines, which promote these different activities. Th1 cells produce IL-2, IFNγ and TNFβ, which activate Tc and macrophages to stimulate cellular immunity and inflammation. Th1 cells also secrete IL-3 and GM-CSF to stimulate the bone marrow to produce more leucocytes. Th2 cells secrete IL-4, IL-5, IL-6, and IL-10, which stimulate antibody production by B cells (see Shiohara and Koike 2005; see Skurkovich et al., 2005).

T cells are initially activated as Th0 cells, which produce IL-2, IL-4 and IFNγ. The nearby cytokine environment then influences differentiation into Th1 or Th2 cells. IL-4 stimulates Th2 activity and suppresses Th1 activity, while IL-12 promotes Th1 activities. Th1 and Th2 cytokines are antagonistic in activity. Th1 cytokine IFNγ inhibits proliferation of Th2 cells, while IFNγ and IL-2 stimulate B cells to secrete immunoglobulins of the family IgG₂a and inhibit secretion of IgG₁ and IgE. Th2 cytokine IL-10 inhibits Th1 secretion of IFNγ and IL-2; it also suppresses Class II MHC expression and production of molecules that kill the bacteria and production of inflammatory cytokines by macrophages. IL-4 stimulates B cells to secrete IgE and IgG₁. The balance between Th1 and Th2 activity may steer the immune response in the direction of cell-mediated or humoral immunity (see Shiohara and Koike, 2005; see Skurkovich et al., 2005).
Chemokine functions in inflammation

Chemokines are chemo-attractant cytokines that are small, disulfide-linked polypeptides. Most of these are chemo-attractants for leucocytes. Over 40 different chemokines have been identified to date and they can be divided into two major groups depending on the arrangement of the first two-cysteine moieties. Chemokines in the alpha subfamily include interleukin-8, melanoma growth stimulating factor, platelet factor 4 and beta-thromboglobulin. Chemokines in the beta subfamily include monocyte chemo-attractant protein-1, RANTES, macrophage inflammatory protein-1 α, and MIP-1 β. Chemokines can mediate their effects by binding to specific G-protein coupled cell surface receptors, which are expressed on a wide range of cells, including monocytes, T-cells and dendritic cells. The chemokine receptors are constitutively expressed on home cells whereas they are inducible on others (see Galimberti et al., 2004; see Aukrust et al., 2005).

1. Chemokines have many biological activities. They were initially isolated by their ability to stimulate leucocyte immigration and activation.

2. Chemokines have an important and specialized role in the modulations of immune system in response to damaged or infected areas in the process of inflammation.

3. Chemokines have been identified in many types of cells during inflammation in various organs, including the skin, brain, joints, lungs, blood vessels, kidneys and gastrointestinal tract. This
suggests that most, if not all, cells can secrete chemokines, given the appropriate stimulus.

Studies have shown that increased chemokine production may function as part of a protective immune response of the host against human immunodeficiency virus (HIV) (see Belyakov et al., 2004). Studies have also shown that pox viruses have evolved several mechanisms to block chemokine activity, allowing for unabated virus infection and spread. The inflammatory response begins when injured tissue cells release chemical signals (inflammatory mediators or cytokines). These cytokines attract other immune cells, increase the capillary permeability and cause fever. The other immune cells that are attracted to the site of inflammation release their own cytokines. Chemokines play important roles in acute and chronic inflammation and have immunoregulatory functions. The specific effects of chemokines are mediated by binding to seven transmembrane spanning the G-protein coupled receptors on target cells. To date a total of 15 chemokine receptors have been identified. Receptor activation leads to a cascade of cellular activation, including multiple intracellular signalling pathways, which regulate the trafficking of cells. When chemokines activate the immune cells, they respond by ingesting the invaders or by releasing various chemicals that can kill the live pathogens. These actions cause the characteristic redness, soreness and other symptoms of inflammation, which are normally a protective response in order to clear up the infection (see Aukrust et al., 2005; see Glabinski et al., 2005; see Gorska and Alam, 2005).
Scientists have discovered that an overenthusiastic immune response triggered by chemokines can cause or contribute to a variety of diseases. For example, neutrophils create the inflammatory response to rheumatoid arthritis, in which the normal connective tissues of the joints are damaged. Eosinophils have been linked to asthma and monocytes to pneumonia. This dual role of chemokines has caused the investigators to look for ways to block the chemotactic effects of these chemokines, hoping to obtain new and more specific treatments for diseases (see Denbrug and Gauvreau, 2005; see Gorska and Alam, 2005).

Chemokine role in HIV

HIV has been shown to infect a number of cell types, including CD4+ T cells, monocytes and macrophages, as well as bone marrow precursor cells, both in vivo and in vitro. HIV requires fusion cofactors on the CD4+ target cell. Fusin, is a GTP binding protein (G-protein) coupled receptor that serves as a cofactor of the Tcell line tropic HIV-1 isolates. Researchers have shown for long time that by itself CD4+ is not enough for HIV to gain entry into immune system cells. Mouse cells genetically engineered to express human CD4+ on their surfaces could not be infected with HIV. This has resulted in the evolution of another receptor or co-receptor, which is involved in the early stages of HIV infection. A cell membrane bound protein called Cysteine-Cysteine-chemokine Receptor 5 (CC-CKR5) has been found to be the other receptor that HIV uses in addition to CD4+ (Deng et al., 1996).
Macrophage migration inhibitory factor (MIF)

Macrophage migration inhibitory factor (MIF) functions as a pleiotropic protein, participating in various inflammatory and immune responses. MIF was originally discovered as a lymphokine involved in delayed hypersensitivity and various macrophage functions, phagocytosis and spreading tumoricidal activity. MIF has been reevaluated as a proinflammatory cytokine and pituitary derived hormone potentiating endotoxemia. This protein is ubiquitously expressed in various organs like the brain and kidney. MIF is unique in its abundant expression and storage within the cytoplasm, for counteraction against glucocorticoids. MIF has been found to convert D-dopachrome, an enantiomer of naturally occurring L-dopachrome, to 5,6-dihydroxyindole. Anti-MIF antibodies suppress tumor growth and tumor associated angiogenesis. This point adds to the fact that MIF is involved in tumor cell growth. In the present understanding, MIF cannot be clearly categorized as either a cytokine, hormone or enzyme because of its potential functions in various pathophysiological states. Analysis of bronchoalveolar lavage of patients with sarcoidosis, pulmonary fibrosis, associated with systemic sclerosis and idiopathic pulmonary fibrosis showed a predominant MIF cytokine profile (Rottoli et al., 2005). These factors have been shown to activate antigen presenting dendritic cells and induced inflammatory cytokines in the lesions of ulcerative colitis (Loli, 2001).

MIF is expressed in human endometrium and has been implicated in numerous reproductive responses, including ovulation, blastocyst
implantation and embryogenesis. This protein consisting of 115 amino acid residues has emerged as an important regulator of inflammation, influencing both the innate and antigen-specific functions of the immune system.

MIF acts as a counter regulator of the anti inflammatory effects of glucocorticoids, leading to increased monocyte production of IL-6, IL-10 and IL-8. Its spectrum of proinflammatory activities has recently been extended to the direct effects on cytokine expression. MIF can be induced by a variety of inflammatory mediators, including LPS and IFN, leading to unchecked inflammation (Swope and Lolis, 1993). Recent studies have implicated MIF in both acute and chronic inflammatory conditions, including antigen induced arthritis and renal disease.

A previously unrecognized link between oestrogen and MIF has been implicated and is expressed in a number of hormone responsive tissues. LPS can induce MIF production by macrophages and is inhibited by oestrogen in a dose-dependent fashion. Also, in vivo expression of MIF during wound healing is downregulated in the presence of oestrogen. This down regulation is a pathway that may be applicable in many pathophysiological processes involving oestrogen, such as atherosclerosis and osteoporosis. Enough data is available about the correlation of the sexual dimorphism observed in response to sepsis and trauma-haemorrhage. Females are reported to have an improved outcome in these situations due to the action of oestrogen on MIF in modulating the stress reactions to inflammation (Ashcroft et al., 1999; Cutolo et al., 1998; 2002).
MIF has been categorically shown to be the catalyst of atherogenesis, which is a process of ongoing inflammation in the atrial wall and progresses ultimately to the condition of atherosclerosis. The vascular MIF overexpression in blood vessels is seen in patients suffering from atherosclerosis and it induces addition molecules expression in endothelial cells and requirement of macrophages and T cells (Kong et al., 2005). MIF enhances the oxylDL-uptake by macrophages and stimulates smooth muscle cell proliferation and expression of matrix metalloproteinases. Research identifying the genes responsible for this alteration of the cellular mechanics leading to atherogenesis has been going on and promises newer diagnostic tools and development of newer preventive therapies to tackle cardiovascular diseases in very near future.

Evidences for functionally important polymorphism have been identified for MIF and C-reactive protein (CRP). Especially in neuro inflammatory diseases like Alzheimer's disease (AD) have been shown to be attended with increased gene polymorphisms of C-reactive protein (CRP) and MIF. Two functionally important polymorphisms have been identified for MIF and CRP genes the first being a single nucleotide G to C substitution at position 173 of the MIF gene. The presence of C allele creates an activator protein 4 transcription factor binding site that affect MIF expression. In patients with systemic onset juvenile idiopathic arthritis, the MIF-173C allele is associated with significantly higher serum and synovial fluid levels of MIF protein (with poorer clinical out come with higher MIF levels) (Benedetti et al., 2000). This polymorphism consists of G to C substitution and it have been
seen that subjects carrying the CC and GC genotypes have significantly reduced plasma CRP (Zee et al., 2002) MIF has been shown to be a key mediator of several immune and inflammatory conditions associated with septic shock. This cytokine has been detectable in the cerebral cortex of Alzheimer's disease brains (Oyama et al., 2000). MIF is a potent A-beta-binding molecule. CRP binds to phosphocholine in complement damaged cell membranes and co-localizes with the membranes and attack complex in damaged vascular tissues, thus tissue auto-destruction degeneration (Torzewski et al., 1998).

Elevated levels of CRP has been found in the temporal cortex of Alzheimer's disease patients (Wood et al., 1993) Cell adhesion molecules (CAMs) govern the recognition of the stereospecific axons, formation of the guidance cues and promotion of neuronal growth cone.

Compelling evidence implicates the fibroblast growth factor receptor tyrosine kinase as the primary signal transduction molecule in the Cell Adhesion Molecules (CAMs) pathway. These are important constituents of synapses and they appear to play important and diverse roles in NCAM peptide mimetics corresponding to the binding site of NCAM for the fibroblastic growth factor receptor promote synaptogenesis, enhance presynaptic function and facilitate memory consolidation. Dimeric versions of functional binding motifs of N-cadherin behave as N-cadherin agonists, promoting both physically direct neurite growth have also been described. The latter include the myelin inhibitory proteins, Nogo, myelin-associated glycoprotein and oligodendrocyte-myelin glycoprotein. Potentiation of
outgrowth promoting signals, together with antagonism of myelin proteins or their convergent receptor, NgR and its second messenger pathways, may provide new opportunities in the rational design of treatments for acute brain injury and neurodegenerative disorders (Wood et al., 1993).

**Leukemia inhibitory factor (LIF)**

Leukemia inhibitory factor (LIF) is a member of the interleukin-6 family of cytokines, it also includes oncostatin-M, ciliary neurotrophic factor, interleukin-11 and cardiotrophin-1. Members of this family are grouped together based on activation of a common tyrosine kinase receptor, gp130 (Gearing et al., 1987). LIF is expressed during inflammation and has been reported to affect vascular development in culture. In transgenic mice, LIF reduced development of embryonic vasculature in the eye and inhibited retinal vascular development. Inhibition *in vivo* was independent of vascular endothelial cell growth factor (VEGF) expression. In older transgenic mice, the absence of a retinal vasculature resulted in retinal ischemia and elevated VEGF levels. The upregulation of VEGF resulted in the proliferation of pathological vascular membranes in the vitreous and neovascularization penetrating the retina, which in turn resulted in tractional retinal detachment. LIF is a potent inhibitor of retinal vascular development (Aloisi et al., 2000).

LIF expression is clearly linked to inflammatory responses, which suggests that LIF may also have an affect on vascular endothelial cells. In several studies, LIF has been shown to alter vascular growth and differentiation *in vitro* (Aloisi et al., 2000). For example, LIF has been
identified as protein factor secreted by pituitary follicular cells, which acts as an inhibitor of bovine aortic endothelial cell proliferation (Schindler et al., 1995).

However, the effect is dependent on the cell type. LIF does not inhibit the proliferation of capillary endothelial cells of the adrenal cortex (Schindler et al., 1995). Previous studies have suggested that LIF could block arterial angiogenesis but not microvascular angiogenesis. Some other studies have shown that LIF in combination with basic fibroblast growth factor (bFGF) could program an immortalized embryonic cell line to form primitive blood vessels in vitro. Thus it was concluded that the reprogrammed cells could participate in blood vessels development (Ihle et al., 1995).

These studies suggest that LIF can inhibit some angiogenesis and possibly promote vasculogenesis. Interestingly, the inhibitory effects of LIF appear to be partially overcome by increased expression of VEGF, which leads to extensive neovascularization in retinal tissue of the eye. This caused the formation of a contractile vascular membrane and subsequent tractional fractional retinal detachment (Neddermann et al., 1996).

LIF is an anti-angiogenic factor. After the initial involvement of LIF in the inflammatory process the local tissue anoxia seems to be a factor inducing increased expression of VEGF which downregulates to some extent the anti-angiogenic effects of LIF, many studies have come up with opposing evidences for the anti-angiogenic and pro-angiogenic properties of LIF. The switch in vascular phenotype is not due to decreased LIF expression over
time. Instead, observed that the neovascularization occurs after increased VEGF expression in the vascular transgenic retinas. The LIF is anti-angiogenic and that elevated VEGF can partially overcome the effects of LIF.

There are two possible causes of the increased VEGF. It is possible that LIF stimulation of retinal cells directly stimulates expression of VEGF, or more likely that the ischemia in the avascular retina leads to elevated VEGF expression. Even though elevated VEGF could override the inhibition of angiogenesis by LIF, the blood vessels that eventually formed in response to VEGF do not appear to follow the normal temporal or spatial pattern of growth in the nerve fibre layer. LIF known to have properties of inhibiting cell differentiation and in vitro studies have suggested that LIF may promote the differentiation of embryonic stem cells to form angioblast (Ihle et al., 1995).

The brain, being a vulnerable and vital organ, is isolated from the nourishing capillaries by protective shield called the Blood Brain Barrier (BBB). This highly selective barrier consists of a lining of endothelial cells which fit so tightly together and do not allow the passage of substances to cross the barrier. In the course of multiple sclerosis (MS) repetitive disruption of the BBB, as a part of the neurodegenerative inflammation, paves the way to the subsequent infiltration of activated T-lymphocytes and monocytes/macrophages into the central nervous system (CNS). To evade the capillaries and gain access to the CNS these infiltrating lymphocytes and monocytes secrete a variety of (matrix metalloproteins) MMPs, which are capable of degrading the BBB matrix. Among all known MMPs, MMP-9 (a
marker of microglia activation) seems to be more specifically involved in BBB breakdown as increased levels of this protease in MS patients is associated with a leaky BBB as seen in magnetic resonance imaging (Rosenberg et al., 1996).

Moreover MMP-9 overexpression in MS is predominantly localized around blood vessels in the vicinity of acute lesions and in the active borders of chronic lesions.

Besides its contribution to BBB disruption and cellular migration, MMP-9 appears also to be involved in the attack on the protective myelin covering of nerve fibers in MS. Indeed, MMP-9 cleaves human myelin basic protein in vitro and this breakdown leads to remnant epitopes that generate autoimmunity (Opdenakker et al., 2001). This was demonstrated in animal models of MS, by the injection of highly purified MMPs into the brain, which resulted in demyelination and axonal loss.

MMPs may contribute to expand the inflammatory response through the conversion of inactive membrane bound cytokines like TNF-α into an active form that is toxic to myelin (Chandler et al., 1996). Based on these observations, inhibition of MMP-9 activity holds some praise of relief for MS patients. Indeed, when researchers looked at the molecular mechanism underlying the beneficial effects of interferon-beta and steroid treatments in MS, they noticed that both significantly inhibit MMP-9 enzymatic activity and protein expression level in vitro (Makhlouf et al., 2001).
In combination, MMP activation, at least in multiple sclerosis clearly establishes the release of vascular endothelial growth factor (VEGF) in response to inflammatory processes, which acts like an emergency fridge to quickly regenerate the young and immature vasculature in order to repair and restructure the damaged tissue. It has been shown that these new immature blood vessels normally resorb quickly. An additional relationship between VEGF expression and MS was recently described. Using immuno histochemistry and molecular biology, VEGF overexpression was detected in acute and chronic MS plaques. It is suspected that the increased blood vessel permeability induced by VEGF in MS contribute to the leak of fibrinogen from the blood into the damaged nerve. Fibrinogen is a plasma protein that normally helps to form blood clots. In the vicinity of a nerve, it is possible that fibrinogen might be proteolyzed into fibrin by MMPs (Gveric et al., 2001). Fibrin is found in MS lesions where its presence prevents Schwann cells from beginning their repair job.

Neuronal cell damage and regeneration

Schwann cells are involved in regenerating the myelin sheath. Undoing the damage to myelin until very recently, talking about nerve regeneration would have appeared as a heresy. The scientific dogma held that once myelin was lost in the CNS, it was lost forever. Thanks to some unconventional researchers, we now know this to be untrue. The CNS can, to a certain point, regenerate itself. It can remyelinate from cells within the CNS that are still capable of forming new myelin and it can remyelinate from stem cells which are totipotent cells that can differentiate into myelin-making cells (Johansson,
The problem in MS seems to be that myelin loss proceeds to more remyelination. Identifying all the factors at play in the balance of nerve maintenance will undoubtfully one day revolutionize the therapeutic approach to nerve injury.

Meanwhile, studies done with animal models point to signalling factors called “growth” or “trophic” factors as potential mediators of nerve cell repair. CNTF (Ciliary Neurotrophic Factor), leukemia inhibitory factor (LIF), cardiotrophin-1 and oncostatin M, are among the factors shown to induce a strong promyelinating effect (Stankoff et al., 2002).

CNTF is a survival factor for neurons that promote differentiation, maturation and survival of oligodendrocytes. When CNTF-deficient mice were used as an animal model of MS, disease was more severe and recovery was poorer (Linker et al., 2002). Involvement of CNTF in nerve healing is further supported by another animal study showing that astrocytes in the CNS produce increased amounts of CNTF during the remyelination phase (Albrecht et al., 2003). CNTF appears to contribute to remyelination at least partly through stimulation of FGF-2 (fibroblast growth factor) production. Interestingly, glatiramer acetate (one of the current drugs used in MS) was reported to stimulate the production of some neurotrophic factors reinforcing the potential of such signalling factors as tools in MS therapies (Ziemssen et al., 2002).
The role of folliculo-stellate cells (FS) as prime moves in inflammation and degeneration occurring in various tissues

The study of folliculo-stellate (FS) cells of the anterior pituitary dates back to the onset of electron microscopical observation of the pituitary gland. The morphological and electrophysiological characteristics, topographical distribution and contribution to intercellular junctions of these FS cells have been instrumental to the understanding of their putative function. The role of FS cells as a source of newly discovered peptides, growth factors and cytokines like LIF and IL-6 was shown (Vankelecom et al., 1999). It has been known recently that dendritic cells (DC) of the FS cell population are immunocompetent cells belonging to the mononuclear phagocyte system (Ferrera et al., 1989; 1992). Recently more interest has been evinced in the role of FS cell in the cellular transdifferentiation, paracrine control of the pituitary-cytokine system and the control of secretion of lPrl, GH, ACTH and TSH, putting the FS cell access as the chief moderator of stress inflammation and immune cell requirement deciding the fate of the disease processes involved.

The following interesting citations about the extremely different and crucial role of FS cells in relationship with the trans-differentiation of stem cell population of the FS system into macrophages, NK cells, dendritic cells and various haematopoietic and organ specific cells may be noteworthy to understand the involvement of hormones, immune cells and inflammatory processes in neurodegenerative and inflammatory diseases:
1. The postulate that FS cells form the renewal cell system for endocrine cells similar to the adult stem cell lineage (Bjornson et al., 1999).

2. FS cells were found to be a source of newly discovered growth factors and peptides such as basic fibroblast growth factor (bFGF) (Ferrara et al., 1987).

3. The finding that FS cells in the anterior pituitary gland can serve as supportive cell reserve for any type of organ specific cells and can serve as the switch to select the specific cell requirement in situations of different disease processes (Inoue et al., 2002).

4. Differentiation of myocytes from pituitary FS cells and similar endocrine progenitor cells and similar interesting mechanisms of transdifferentiation of pituitary FS cells (Horvath et al., 2002; Childs, 2002; Mogi et al., 2004).

5. Cells producing hormones like prolactin and growth hormone can be differentially made to be transdifferentiated from FS cells by steroids in bovine pituitary cultures (Kineman et al., 1992).

6. Involvement of gonadotrophs in the cyclic transdifferentiation of growth hormone cells (Childs et al., 2002).
7. A recent concept related to stem cells, about being transdifferentiation cells, which can spread across germ cell layers, like neural stem cells giving rise to hematopoietic cells (Bjornson et al., 1999).

8. FS cells acting as chief modulator of paracrine communication in the anterior pituitary, IFN ability to inhibit secretion of ACTH, PRL and GH in pituitary cell cultures (Vankelecom et al., 1997).

9. The established fact that FS cells can produce IL-6 during stress and disease processes (Vankelecom et al., 1999).

10. The relationship between DC cells and other cell type of mononuclear phagocyte system (monocytes and macrophages) and their presence establish in the pituitary FS cells (Hoek et al., 1997).

11. Evidence proving the ability of FS cells to produce several cytokines which are normally produced by peripheral immune cells (Tierney et al., 2005).

12. Proved immunoreactivity to the cytokine MIF in the FS cells-a capacity shared between DC and FS cells (Allaerts et al., 1997a).

13. The effect of thyroid hormone shown in cell culture studies showing the effect of transition of monocytes into DC cells and
the effect of various cytokines like TNF, IL-6 and GMCF in such transdifferentiation (Mooij et al., 1994).

14. The possible relationship of cell lineage between bone marrow cells, dendritic cells and FS cells and the possibility of differentiation into these cell types (Allaerts et al., 1997b).

15. The finding of MIF release from FS cells by endotoxins and dexamethasone (Tierney et al., 2005).

16. The role of nitric oxide synthetase in the regulation of LH secretion by FS cells and the possible biofeedback of raised LH levels affecting the modulation of nitric oxide synthetase and production of NO in inflammatory states. Further elaboration in this direction may open up various explanations regarding the effect of gonadal steroids and gonadotropins in the treatment of inflammatory and degenerative diseases (Ceccatelli et al., 1993).

Arcuate Nucleus frequencies

The resonance mechanisms and reduction in GnRH pulsation rate over time provide an explanation for the elevated gonadotropins at birth. Luteinizing hormone (LH), for example is high at birth, falls between the ages of 2 & 4 years, and then rises again at puberty. The elevation at birth may occur because the frequency of the arcuate nucleus of an infant is very much higher than that of an adult, specifically 1.33 cycles per hour. Resonance of
the ultradian pace maker with the arcuate nucleus will occur, because 1.33 cycles per hour (1.33 hr − 1) is an integral multiple (harmonic) of 0.67 cycle per hour (i.e., almost 2 × 0.67). As the frequency of the arcuate nucleus diminishes between 4 and 8 years of age the resonance is lost and LH levels. But when the frequency of the arcuate nucleus has slowed to 0.67 cycles per hour, resonance again occurs resulting in the gonadotropin surge necessary for secondary sexual development. The LH pulses that occur during sleep in puberty (Boyar et al., 1972) can be explained with the resonance mechanism. During puberty, the arcuate nucleus frequency is very close to the frequency of the ultradian pace maker. When two oscillators are very nearly the same frequency interact and hence the beat phenomenon will occur. The rhythms combine to give a rhythm whose amplitude varies periodically with time. During puberty the beats corresponds with LH pulses during sleep.

During the reproductive years arcuate nucleus intrinsic frequency and GnRH pulsation rate continue to diminish, though at a reduced pace (and that in a logarithmic fashion). There is an LH pulse frequency reduction during the menstrual cycle, from one pulse every one and a half hour (in the follicular phase) to one pulse every 4 hours (in the luteal phase), presumably the result of ovarian steroid feedback.

However, the arcuate nucleus intrinsic frequency, referred to here, is that of the follicular phase of the menstrual cycle. The constant reduction in GnRH pulsation rate during the reproductive years is suggested by the reduction in length of the menstrual cycle from about 31 days at age 16 to about 27 days at age 40.
The continued reduction in arcuate pulse frequency between the ages of 40 & 50 appears to result in menopause. There are at least 3 pieces of evidence to support this idea.

Experimental data confirms that loss of fertility in the ageing rat is definitely a hypothalamic phenomenon. There is known sensitivity of the ovary to gonadotropin pulse frequency. Body weight affects the time of onset of menarche and menopause (Flint, 1976). The increase in the FSH:LH ratio in the years just preceding menopause may or may not indicate a reduction in pulse frequency. The ovulatory irregularities and diminished oestradiol level of perimenopausal women suggest that these could be produced by the reduction in the pulse frequency GnRH. The failure of the ovary and its diminished oestradiol production responsible for the gonadotropin rise at menopause with an absence of ovarian steroid feedback on the pituitary-hypothalamic axis. However, the frequency reduction is necessary to produce menopause is apparently much less than that needed to create a complete loss of resonance and gonadotropin fall comparable to that at age 5 or 6. The frequency diminution from age 45 to age 50, though sufficient to cause GnRH pulse frequency to fall below that needed for normal ovarian function, yet it is not great enough to cause loss of arcuate nucleus resonance. In contrast the much greater rate of fall in frequency from birth to age 5 is sufficient to result in loss of resonance as well as the fall in gonadotropin so prominent in
agonadal girls. Because the elevation of the gonadotropins at birth is attributed to an arcuate nucleus intrinsic frequency (1.33hr-1), which is double that at puberty (0.67hr-1), one might wonder whether the observed gonadotropin pulse frequency at birth would be double that at puberty.

Body weight is known to influence the onset of menarche and menopause. Obese females typically have their menarche earlier than the normal weight females, conversely extremely thin girls have their menarche later than usual. Several studies try to find the relationship of obesity and the time of onset of menopause, without consistent results (Daniell, 1978; Sherman et al., 1981). There are reports about thin women getting their menopause later than obese women. Attempts of explanation of these deviations by postulating that body weight causes a frequency shift in the arcuate nucleus oscillators, have been done by various researchers explaining that extreme thinness causes an upward shift, whereas relative fatness results in the downward shift of the frequency of the arcuate nucleus. This mechanism may predict that very thin women might have menopause later than usual. The arcuate nucleus and the ultradian pace maker weight loss in a young post pubertal women causes a return to the pubertal LH secretory pattern (Kapen, 1981); because the thinness would produce upward frequency shift in the arcuate nucleus oscillator causing slight desynchronization with the ultradian pace maker and restoring ‘LH beats’ during sleep. The above mentioned frequency shift of athletes and thin females causing delay in menarche may be partly due to the stress of exercise, causing increased
catecholamines, which may stimulate in turn the pineal gland to produce more of antigenadotropic melatonin. Exercise is known to be associated with increased levels of melatonin (Carr et al., 1981).

Apoptosis and Hormones

Sex hormones seem to modulate the immune/inflammatory responses by different mechanisms in female and male rheumatoid arthritis patients (Feldmann and Brennan, 1996). The effects of 17β-oestradiol and testosterone were tested on the cultured human monocyctic/macrophage cell line (THP-1) activated with IFN-γ in order to investigate their role in cell proliferation and apoptosis. Activated human THP-1 cells were cultured in the presence of 17β-oestradiol and testosterone (final concentration, 10 nM). The evaluation of markers of cell proliferation included the NF-κB DNA binding assay, the NF-κB inhibition complex, the proliferating cell nuclear antigen expression and the methyl-tetrazolium salt test. Apoptosis was detected by the annexin V-propidium assay and by the cleaved poly-ADP ribose polymerase expression specific methods included flow analysis cytometry scatter analysis, immunocytochemistry and western blot analysis. Cell growth inhibition and increased apoptosis were observed in testosterone-treated THP-1 cells. Increased poly-ADP ribose polymerase cleaved expression and decreased proliferating cell nuclear antigen expression, as well as an increase of IκB-α and a decrease of the IκB-α phosphorylated form (ser 32), were found in testosterone-treated THP-1 cells. However, the NF-κB DNA binding was found increased in 17β-oestradiol treated THP-1 cells (Choen et al., 1983). The treatment with staurosporine (enhancer of apoptosis) induced decreased
NF-κB DNA binding in all conditions, but particularly in testosterone treated THP-1 cells. Treatment of THP-1 by sex hormones was found to influence cell proliferation and apoptosis. Androgens were found to increase the apoptosis and oestrogens showed a protective trend on cell death, both acting as modulators of the NF-κB complex.

Experimental and clinical evidence indicates that immune reactivity is greater in females than in males, which suggests that gonadal steroids may play an important role in the regulation of the immune response (Lockshin, 2001; Straub and Cutolo, 2001; Cutolo et al., 2002; Masi et al., 2005). Indeed, many cells of the immune system have been found to possess functional sex hormone receptors, such as CD8+ T cells, B cells and notably, monocytes/macrophages (Batchelor, 1968; Kanda et al., 1999). Oestradiol (E2) was found to inhibit cellular apoptosis, to increase antibody production by B cells and to exert dose related effects on T-cell functions. Androgens seem to exert effects opposite to those of E2 on immune response. Clinical epidemiology clearly confirms a higher prevalence of autoimmune diseases in female subjects when compared with male subjects (Choen et al., 1983; Cutolo et al., 1998; Joly-Pharaboz et al., 2000).

Oestrogen receptors have been shown to interact with NF-κB factors, via transcription cofactors resulting in mutual or non mutual antagonism, other studies hypothesize that, since oestrogen receptors may repress both constitutive and inducible NF-κB activity, the over expression of NF-κB inducible genes in oestrogen receptors negative cells might contribute to malignant cell growth and chemotherapeutic resistance (Nakshatri et al.,
1997; Shah et al., 2001). On the contrary, further studies report that E2 blocks transcriptional activity of p65 in macrophages. (Ghisletti et al., 2005).

However these opposite observations arise using different cell lines (human and animal) and culture conditions as well as different hormone concentrations (Ma et al., 1993). In addition, multiple mechanisms concerning the interaction between oestrogen receptors (ER) and of NF-κB have been proposed such repression of NF-κB binding by physical association with oestrogen receptors and the regulation of NF-κB expression by oestrogens (Biswas et al., 2004; Seo et al., 2004).

The androgen receptor seems to be closely related to glucocorticoid receptor in terms of both structure and sequence homology. The androgen receptors and glucocorticoid receptor have been shown to interact and repress activator protein 1 via similar mechanisms. It is possible that inflammatory agents that activate NF-κB in vivo may interfered with normal androgen signalling and recent studies indicate that androgen receptor and NF-κB are mutual transcriptional antagonists (Biswas et al., 2004; Seo et al., 2004).

**Adipocytes**

All adipocytes look alike and respond mindlessly to blood borne orders such as insulin, cortisol and glucagon. The ideal adipocytes know their duties and their place, they should mop up excess lipids in the blood and mould themselves into gaps and smooth contours to far rounded breasts, thighs and cheeks, perhaps fill a few internal spaces not required by important tissues,
but otherwise keep quite and make themselves as small as possible until starvation calls them to attention.

Cytokines of the interleukin family are central to the reciprocal relationship between osteoblastogenesis and adipogenesis (Hoek et al., 1997). This may explain the association between the decreased bone formation and the resulting osteopenia and the increased adiposity seen with advancing age.

It has been clarified from experimental and human studies that melanocortin signalling is essential to prevent obesity, as mutations that decrease the melanocortin signal within the brain induces hyperphagia and excess body fat accumulation. Melanocortins are involved in the pathogenesis of disorders at the opposite end of the spectrum of energy homeostasis, the anorexia and weight loss associated with inflammatory and neoplastic diseases processes. The proinflammatory cytokines (interleukin-1, interleukin-6 and tumor necrosis factor α) that are produced in the hypothalamus of rodents during both inflammatory and neoplastic diseases process likely play a role (Kelso, 1998).

The perivascular adipose tissue has been found to be essential for normal response of the tissue to agonists that control vasoconstriction. The perivascular, epicardial and perirenal adipose tissue which are often looked upon contemptuously by the surgeons have been, on the contrary found to be important mediators in the expression of genes and so in the secretion of proteins of the compliment pathway which may directly affect the function of
the critical organ concerned (like the blood vessel, heart, kidney) which the adipocytes cover and protect.

The bone marrow has long known to be a site of intimate relationship between the adipose sites and a variety of other cell types including osteoblasts and hematopoietic cells. The marrow adipocytes which were until recently regarded as mere space fillers, have proved to share the properties with several other cell types and probably contribute to very important functions like myelopoiesis, erythropoiesis and the deposition of bone and its loss in osteoporosis (Seo et al., 2004). Leptin, produced by the adipose sites, has functions in regulation of apetite and body fatness (fat deposits) and also in various steps of haematopoiesis and macrophage function (Zalla et al., 1995).

Studies of in vitro and in vivo nature have shown that the fat tissue around the major lymph nodes (perinodal adipose tissue) participates in immune responses and various interactions with lymphoid cells. The adipose tissue from around mesenteric, omental and popliteal lymph nodes showed lower rates of lipolysis although they released more glycerol in the presence of combinations of noradrenaline, tumor necrosis factor (TNF) and interleukins (IL), than the adipose sites from elsewhere in the same depots. The perinodal adipose tissue can be activated via its lymph node early in local immune response, but fails to respond strongly to the endocrine conditions of fasting.
In both males and females, the hypothalamus and the pituitary gland are closely linked in nervous and endocrine communication by a group of peptide hormones called gonadotropin releasing hormones. The LH and FSH from the pituitary stimulate the production of testosterone by the testis in man, oestrogen by the ovary in woman and small quantities of either are produced by the adrenal cortex (see Marshall, 2005). The oestrogens and androgens are not unique for male or female, what differs is the ratio between the two sexes. A basal level of testosterone in men is needed for spermatogenesis and for prostate seminal vesicle action. However recent works also suggest that high level of oestrogen is also required within the testis to regulate the concentration of sperm in the semen. Both androgens and oestrogens moderate various metabolic processes and affect various modes of behaviour. Ageing brain research has indicated that, oestrogens may counter some of the degenerative conditions of the brain, which occur with old age. This is exemplified by the presence of receptors for the oestrogen in almost all types of cells. In the bone marrow the role of oestrogen in controlling bone metabolism and in the prevention of osteoporosis is well known. The congenital hip dysplasia is more common in girls because during parturition, the mother’s oestrogen rises greatly assisting the articulation or spreading of her pelvis to facilitate the process of childbirth. Some of this oestrogen enters the blood stream of the baby. For a while after birth, the baby’s cartilage is also elastic, so that with this geometry, the hip is likely to pop out of the joint. The problem was more prevalent in cultures that swaddle their babies tightly. If the baby is allowed to keep its legs in a natural position and exercise then the joint soon strengthens. Although testosterone levels in boys are higher at
birth, it seems that boy's cartilage is less plastic under the influence of oestrogen and hence less likely to be damaged. The role that testosterone plays in prenatal development is so important that during that time and even in the late childhood, the concentration of testosterone is much the same in both the sexes (Cutolo et al., 2002).

The set-point hypothesis postulates that, whereas that the levels/set-points of gonadotropins and other tropins may differ among women, the set-points themselves may be modified permanently by “exogenous” events, such as caloric restriction and pregnancy. Over time the net results of these effects depends on the order and the time in a woman’s life when the exogenous events occur. The trophic hormones would thus provide a more proximate link to the risk of cancer than the mediating hormone levels (steroids, IGF-1). From this set-point perspective and the earlier hypothesis that oestrogens can no longer be produced after menopause (when the ovaries have stopped inducing menstrual cycles) and that after menopause, oestrogens can only be derived from adrenal precursors when converted by P450 aromatase in the fatty tissue of women who are over weight. Menopause marks especially a time of increase (compensatory) production of gonadotropins, most likely at the level of hypothalamus. Testosterone and androstenedione are still produced in the ovarian reminence although at lower levels (Carr and Bradshaw, 2001). Apart from the possible relationship of mitochondria to caloric restriction, this molecular pathway may provide at a cellular level, a link to post-menopausal reduction of oestrogens (Elias et al., 2004).
The increase in post-menopausal gonadotropins in non-obese women may also explain the effects of growth and or tropic hormone operating on the breasts after menopause. 20% of post menopausal women (in the range of 54-71 years) told in a questionnaire, that they have to buy a bigger bra (den Tonkelaar et al., 2004). An effect only partially explains by changes in body weight. In famine exposed women there have been a incidence of higher levels of IGF-1, IGFBP-3, gonadotropins and possibly other tropins like thyroid stimulating hormone, melanocyte stimulating hormone, adrenocorticotropic hormone, growth hormone and prolactin, these increases were long lasting and were possible factors for risk of breast cancer in post menopausal women.

Neuronal growth promoting and inhibitory cues

During development of the nervous system, neurons extend axons over considerable distances in a highly stereospecific fashion in order to innervate their targets in an appropriate manner. This involves the recognition by the axonal growth cone of guidance cues that determine the pathway taken by the axons. These guidance cues can act to promote and/or repel growth cone advance. The directed growth of axons is partly governed by cell adhesion molecules (CAMs) on the neuronal growth cone that bind to CAMs on the surface of other axons or non-neuronal cells. In vitro assays have established the importance of the CAMs (NCAM, N-cadherin and L1) in promoting axonal growth over cells.
**Hormones as immunomodulators**

Recent advancements in the field of endocrinology and immunology have unearthed many non-classical roles for hormones, leading to the development of the newer areas of research. The subject of immunoenocrinology is the home for such inventions. Sex steroids, cortisol, thyroid hormones and PRL are immunogenic hormones. Oestrogen is a derivative of testosterone, while both are products of progesterone, which is derived from cholesterol. The steroidogenic pathway encompasses many enzymatic reactions and the intervention of many intermediate products during the complex processes starting from acetyl CoA to oestrogen formation.

Ovary, testis and adrenal are the three major steroidogenic organs in the body. While ovary and testis are involved in the production of sex steroids, adrenal produces corticosteroids predominantly, along with some weak androgens like dehydroepiandrosterone (DHEA). Oestrogens are also formed peripherally in organs like liver, adipose tissue, brain and muscles, from androgens by the action of the enzyme aromatase. The classical mechanism of action of sex steroids like testosterone, progesterone and oestradiol involves the passive diffusion of these ligands into the cell through the lipid bilayer binding to specific intracellular receptors in the cytoplasm/nucleus (Cutolo et al., 1998). These receptors are transcription factors with specific response elements in the promoter regions of their target genes. Ligand binding to these receptors activates them, which in turn binds into specific response element in the gene and induces the transcriptional
activity resulting in the formation of specific mRNAs, which still has to be translated into proteins.

Recent findings suggest that steroid hormones also have cell surface receptors like that of peptides and they transduce signals across the plasma membrane and execute both genomic (delayed) and non-genomic (rapid) effects (Simoncini and Genazzani, 2003). This novel development has helped to resolve many questions about the involvement of steroids in many physiological events, hitherto unheard of. Many mysteries in the immunomodulatory role of steroids would also be unrevealed due to increasing knowledge in these lines.

Perfectly projected and impeccably grant, the endocrine system precisely regulates the most delegate immune processes. The immune and neuroendocrine systems are two essential physiological components of mammalian organisms, important for protection from infection and disease on the one hand and on the other for the regulation of metabolism and other physiological activities. Evidences have been found indicating that there is active and dynamic collaboration of this system in execution of these designated functions. These interactions occur at many stages of embryonic and neonatal development and they are a continual part of normal homeostatic balance which is necessary to preserve health. There is communication between neuroendocrine and immune system via the cytokines, neuro transmitters and peptide hormones which act in both systems through the same receptor molecules. Many investigators have reported the increased thymic weight in experimental animals due to both castration and
The discovery of thymic enlargement in castrated rabbits (Lauriola et al., 1998) has been considered the embryo of hybrid medical discipline i.e., immunoendocrinology. Endocrine influences appear to be a part of bidirectional circuitry. The thymic hormones regulate the release of hormones from the pituitary gland. The neuroendocrine control of thymus appears to be extremely complex, with apparent presence of complete intrathythic biological circuitry involving the production of pituitary hormones as well as the expression of the respective receptor by thymic cells.

It has been observed that oestrogen activates rapid and persistent response that involves the activation of phosphatidyl inositol-3-kinase, without requiring de novo protein synthesis or modification of κB, degradation and mitogen activated protein kinase (MAPK) activation (Pawalk et al., 2005; Watson et al., 2005).

It has also been observed that oestrogen inhibits p65 intracellular transport to the nucleus. This activity is selectively mediated by ERα and ERβ. Oestrogen stimulates the formation of antibodies in circulation. Both cellular and humoral immune response is more powerful in the normal adult women than men of the same age. Such sexual dimorphism is not apparent before puberty. The NK cell and T lymphocyte activities are decreased by the action of oestrogen. The release of thymic hormone is also affected by oestrogen. Cortical reticulo-endothelial cells express a surface antigen gp200-MR6, which plays an important role in thymocyte differentiation. The thymic neuroendocrine polypeptides are the source of cell antigen presented by major histocompatibility complex (MHC), enabling the differentiation of
hematopoietic stem cells. Thymic nurse cells produce thymosin beta-3 and beta-4, which seem to have many neuroendocrine modulatory effects (see Lang, 2004; see Bouman et al., 2005).

Growth hormone enhances the thymocyte (thymic nurse cells) release of these thymosins and hence, modulates the reconstitution of lympho-epithelioid complexes, which in some way affects its regulation of osteoprotegerin (OPG) production. This is complemented by oestrogen, probably through its modulations of thymosin release. The combined modulation of oestrogen and growth hormone on the lympho-reticular complex in the production of CD14 may explain the beneficial effects on immune processes during pregnancy. The intra-thymic T lymphocyte reactions are complex multi-step processes influenced by serially functional and specialized reticulo-endothelial cells, which are modulated by oestrogen and growth hormone (see Bouman et al., 2005). These complex interactions of oestrogen, growth hormone, thymic hormones, reticulo-endothelial system and the immuno modulatory cells, seem to offer promisingly hopeful treatments in future for HIV-1 patients by the scrupulous use of these hormones. The interplay of these mediators of the neuro immuno endocrine axis on the psychological and behaviour aspects of human nature has opened up an entirely new field of endocrinology as psycho neuro immuno endocrinology (PNIE). The effects of oestrogen, growth hormone and probably thymic hormones on mood, behaviour and other psychological factors have been fairly well established by now, explaining the changes in human nature and behaviour in changing phases of human life.
(see Lang, 2004). The changing peculiarities and vulnerabilities that are seen in prepuberal, adolescent, reproductive, menopausal and post menopausal stages of human life are probably explainable by these neuro immuno endocrine orchestrations.

The important cytokines elaborated by TH-1 and TH-2 cells are modulated by oestrogen. Conditions associated with predominance of TH-1 cell activity of the immune axis are rheumatoid arthritis and multiple sclerosis. On the contrary, similar immuno degenerative diseases like SLE, Sjogren syndrome and the various autoimmune diseases of the muscle are characteristically predominated with TH-2 cell immuno potentiation. Thus autoimmune diseases can now be classified according to their TH cell profile. Normal pregnancy happens to be a TH-2 cell predominant immune status. Thus the alteration of cytokine profile of the body immune system can alter the activity and aggressiveness in patients with autoimmune diseases and can also specify the type of treatment that may benefit them, especially the different hormones. Some clinical examples may be cited as follows:

Female patients with rheumatoid arthritis show clinical improvement, while they are pregnant or while they are taking oral contraceptives or any other form of oestrogen (this is because the TH-1 cytokine profile changes to TH-2 profile which is against the basic TH-1 cytokine profile of the disease).

Lupus patients who have TH-2 cytokine profile improve with androgen, which converts TH-2 to TH-1 profile. This is one of the rationales for using DHEA to treat lupus patients (Chang et al., 2004). The variation in
the proneness to infection, especially parasitic infection during pre-menstrual phase can be explained by the shift of cytokine profile from follicular to late luteal phase from TH-2 to TH-1 profile (see Chen and Parker, 2004).

Women with autoimmune diseases tend to have high oestrogen levels, which stimulates apoptosis and other immune functions as explained earlier. Women have a tailor made immune system for maintaining the foetus, which is immunologically a transplanted organ. However, the female body is immuno tolerant to the foetus during the entire period of pregnancy. This is mainly due to the immunoprotective effect of progesterone acting via its cognate receptor. During medical termination of pregnancy the biological protective effect of progesterone is pharmacologically withdrawn by administration of prostaglandins (see Edwards, 2005).

The phenomenon of autoimmunity in endometriosis, in which the disease process is a form of explanting of uterine tissues in other organs and tissues, the uterine tissue continues to respond to changes in menstrual cycle because of the high oestrogen levels. Androgen therapy (Danazol, testosterone, leuprolide) is useful to induce apoptosis in this condition, particularly the process of autolysis of the endometrial cells (see Nakano and Shikone, 1996). Leucocytes within the uterus are the effector cells of inflammatory response and they play an important role in both tissue breakdown and remodelling (see Selak et al., 2001; Cottreau et al., 2003).

In andropause the males have low testosterone titre and a relatively increased, unopposed oestrogenic milieu. This explains the alteration of
immune state and preponderance of immune oriented diseases in aged males. All these common clinical examples highlight the close link between endocrine system and immune manifestations of various chronic inflammatory and degenerative diseases (see Lamberts, 2003).

The proinflammatory cytokines produced by the glial cells, neurons and immune cells involved in inflammatory lesions of the central nervous system impair the permeability of the blood brain barrier (BBB) and promote apoptosis of the neuronal cells and induce myelin damage. Oestrogen can modulate cytokine expression coupled with the gender differences may be helpful in improving such disease processes. There have been reports of elevated serum and tissue levels of IL-2, IL-6, IFNγ and TNF in patients with multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). Similar elevation of IL-6 and TNF has been reported with patients with Parkinson’s disease. In acute neurodegenerative conditions like cerebral stroke and head-injury sequelae, the effect of IL-6 gene G-174c polymorphism has been found to be different in the different sexes (Watanobe, 2002).

Endocrine basis of Maleness and Femaleness

In women, due to the intriguing biochemical peculiarity, oestrogen which was until recently considered the ‘female hormone’ and which has been proved not to be so is produced in female body from the male hormone precursors (androgens). In a way, one can regard this physiological event as being somewhat “problematic” for women, since it is one of the factors
responsible for the phenomenon of follicular atresia or death. From early childhood up to the age of 50, follicular atresia is the main cause for the depletion of the ovarian follicular population that finally culminates with menopause (see Erickson, 2005).

The fact that the oestrogens are produced by the ovaries having androgens as precursors obliges women to first produce male hormones in order to subsequently transform them into female ones. This biochemical peculiarity seems to be an important cause for the atresia (death) of the great majority of the ovarian follicles that begin their growth leading over the years, to complete the ovarian depletion about the age of 50. In the ovarian follicles, the accumulation of androgens produced by the theca cells when they are not adequately turned into oestrogens by the granulosa cells seems to exert an inhibiting effect upon the follicular structures, causing the follicles to become atretic and die (Clement et al., 2005). Now let us see the main stages of the sexual hormones synthesis in the ovaries.

As we can verify, the first important sexual steroid that is formed is progesterone. The androgens (dehydroepiandrosterone, androstenedione and testosterone) arise in an intermediate stage, while the oestrogens (estrone and estradiol) only make their appearance at the final one. The oestrogens are formed directly from the androgens, that is the androgens androstenedione and testosterone are directly turned into the oestrogens estrone and estradiol (Clement et al., 2005).
The relationship between the respective biological potency of the androgens and oestrogens involved in this complex biosynthetic chain and the positions they occupy in it are also very important here. Dehydroepiandrosterone is an androgen that possesses a very low biological potency. Androstenedione, which is the immediate precursor of testosterone, has 20 percent of testosterone’s potency. Testosterone is the most important androgen produced by the testis. Thus, the biosynthesis of the male hormones follows an increasing order of biological potency and activity. Nevertheless, the oestrogens that arise immediately afterwards (estrone and estradiol) are already endowed with an enormous biological potency. Comparing their respective biological activity, we will see that estrone and estradiol are much more potent than their precursors androstenedione and testosterone (see Handelsman, 2005).

It is easy to understand why oestrogens are so potent. Since the ovaries do not succeed in transforming a considerable part of the androstenedione and testosterone into estrone and estradiol, this very high biological potency of the oestrogens becomes a biological need of women. The female nature has to defend herself against the risk of an insufficient transformation of androgens into oestrogens. Regarding this, the women’s “defense mechanism” lies in the capability of their fat tissue has of performing the peripheral transformation of the androgen androstenedione into the oestrogen and estrone. Because of this, a variable part of the estrone produced in women’s bodies does not come from the ovaries but from their fat tissue. Therefore, the fat tissue contained in the female body plays an important role in women’s endocrine physiology
and this is one more reason for advising them to keep their weight within the normal (see Klein and Romijn, 2005; see Handelsman, 2005).

Philosophically, one can say that *at the exclusively hormonal level*, the oestrogens are the main endocrine manifestation of the female principle, and the androgens are the main endocrine manifestation of the male alone. In this way, there are indications that, in women the complete endocrine manifestation of the female principle that characterizes them as women depends paradoxically on a subtle endocrine manifestation of the male principle.

**Oestrogen**

Oestrogen has short-term effects and long-term effects on the cardiovascular system (see Regitz-Zagrosek and Lehmkuehle, 2005; see Mueck and Seeger, 2004; Hisamoto and Bender, 2005). The short-term effects are modulated through oestrogen receptors producing vasodilation and indirectly through the elaboration of nitric oxide (NO). Long-term effects of oestrogen are modulated through genetic signalling, producing the following

- Reduction of vascular tone
- Reduction of vascular injury
- Delay in atherosclerosis
- Enhanced function of vascular endothelium
- Reduction of thrombogenesis
Reduction of vascular smooth muscle proliferation. Nitric oxide release is mainly modulated by oestrogen alpha receptors, whereas the other activities are modulated by both alpha and beta receptors.

Oestrogen replacement changes the consistency equilibrium of the bile. This factor explains why cholelithiasis is more common in females. Chronic oestrogen treatment increases endothelial vasodilator function in medium sized arteries (especially cerebral) (Geary et al., 1998). Endothelial nitric oxide (NO) synthetase (eNOS) is a primary target of the hormone. Oestrogen decreases vascular reactivity through endothelial mechanisms. The endothelium releases multiple factors to regulate smooth muscle tone, including vasodilators like NO, prostacyclin (PGI2) and hyperpolarizing factors (Endothelium Derived Hyperpolarizing Factors – EDHF) (Huang et al., 2001). Oestrogen can increase one or more of these vasodilator pathways, depending on the particular type of blood vessel or the type of vasculature concerned. Local interaction among the endothelial factors may also determine the variability of the oestrogenic influence on the vascular function (Beverelli et al., 1997; Lamping et al., 2000, Osanai et al., 2000) and sex steroids can affect this, this compensation is clearly a protective mechanism for maintaining the endothelium dependent vasodilation. These actions are dependent on the various types of “cross-talk” between these factors and these are determined by the nature and the state of vasculature concerned. The enhancement of PGI2 by oestrogen is by elevating the level of cyclooxygenase (COX-1) and PGI2 synthetase proteins. Koehler et al., (2005)
mentioned categorically that we have known for many years, that oestrogen is more than a female hormone and that in both sexes, oestrogen has function on the skeleton, central nervous system, behaviour pattern in the cardio vascular system and immune system. Diverse actions of oestrogen are now allocated to the two different receptors for the hormone i.e., ERα and ERβ. The ligand binding domains of these are significantly different from each other. Selective ligands can be (and have been) developed to target the oestrogenic pathway i.e., malfunctioning, without interfering with other oestrogen regulated pathways. ERβ is significantly absent in adult pituitary and endometrium. ERβ agonists can be used to target ERβ with no risk of adverse effects from chemical castration and uterine cancer (see Swope and Korach, 2003). Finally to conclude, prostate cancer, autoimmune diseases, colon cancer, malignancy of the immune system and neurodegenerative diseases are some of the areas where ERβ agonist promise a significantly beneficial therapy in the future.

Systemic lupus erethematosis (SLE), is an auto immune disorder characterized by the production of pathogenic auto antibodies, primarily to nuclear antigen. The etiology of SLE is not entirely understood, but it is well appreciated that multiple factors like genetics and environment (i.e., nature and nurture) contribute to the progression of the disease and its pathogenesis. There is also convincing evidence that gender has an important role in the SLE etiopathogenesis. There is a preponderance of incidence of SLE in females (female, male ratio being 9:1), it may be possible that sex- linked gene may contribute to the genetic predisposition of SLE, the other culprits in
the causation of SLE are sex hormones like oestrogen and prolactin. It has been experimentally proved quiet clearly, over the last few years in my study that sex hormone levels can alter the tolerance of auto reactive B cells and thereby exacerbate the disease.

Oestrogen is an immunoregulatory agent i.e., the hormone deprivation increases, while 17β-oestradiol administration blocks the inflammatory response. The transcriptive factor p65/re/A, a member of the nuclear factor Kappa B (NF –κB) family plays a major role in inflammation and drives the expression of proinflammatory mediators. Ghisletti et al. (2005) showed that in macrophages oestrogen blocks the lipopolysaccharide induced DNA binding and the transcriptional activity of p65, by preventing its nuclear translocation. This effective selected activation in macrophages is to prevent p65 activities by inflammatory agents and extends to the other members of the NF-κB family including C-rel and p50.

Elevated C-reactive protein (a marker of inflammation:CRP) occurs due to heightened vascular inflammatory state in vascular condition which is also associated with elevation of IL-6 levels. It is noteworthy that during inflammation, CRP production in the liver is induced by IL-6, thus persistently raised CRP levels are associated with unfavourable outcome in the eventuality of cardiovascular symptoms in post menopausal women, only when simultaneous rise in the levels of IL-6 and oestrogen is known to alter these factors favourably and protectively (see Davison and Davis, 2003).
Naturally occurring sexual dimorphism has been implicated in the risk, progression and recovery from numerous neurodegenerative disorders including multiple sclerosis (MS), stroke, head injury, neurodegenerative diseases (Parkinson’s disease and Alzheimer’s disease and Amyotrophic lateral sclerosis). Accumulating evidence suggest that the observed differences between men and women could result from oestrogens and wide range of effects with mammalian central nervous system with its neuroprotective activity being the most important factor. It is possible that the neuroprotective activity of oestrogen could be partially a result of its anti-inflammatory action. Besides it has been well established that inflammation plays an important role in the etiopathogenesis of brain pathological changes and probably that of all the diseases involving chronic inflammatory process with cell degeneration and cell death of various tissues (Liu et al., 2005; see Czlonkowska et al., 2005).

An important role is played in such neuroinflammatory diseases by a proinflammatory cytokine produced by activated glial cells, neurons and immune cells that invade the inflammatory brain tissue site. Within the central nervous system, cytokines stimulate inflammatory process that may impair blood brain barrier permeability, as well as promote apoptosis of neurons, oligodendrocytes and induce myelin damage. Oestrogen may modulate cytokine expression, coupled with other factor that gender differences of cytokine production are apparent in animal models of Parkinson’s disease and multiple sclerosis. This suggests that an important connection between hormone-cytokine link in incidence and progress of
neurodegenerative diseases (see Czlonkowska et al., 2005). While MS patients and mice subjected to experimental autoimmune encephalomyelitis (EAE) display gender specific alteration of interferon gamma (IFN-γ) and IL-2, varieties of tumor necrosis factor (TNF) and IL-6 were also associated with Parkinson's disease. It has been noted that in cases with more acute neuro degenerative condition such as stroke, the effect of IL-6 gene G-174c polymorphism was different in males and females (see Czlonkowska et al., 2005). These factors stress the importance of oestrogen in the treatment of new hormonal anti-inflammatory modalities for future treatment strategies in thus far desperate degenerative diseases of CNS and generally chronic inflammatory diseases of all tissues including the enigmatic multiple organ dysfunction and multiple organ failure which happens in various diseases as sudden untoward cascade of events, as in our present study.

Myasthenia Gravis (MG) is an autoimmune disease associated with thymic hyperplasia and is much more prevalent in women than men. ER transcription gradually decreased in thymic epithelial cells during culture indicating that the thymic environment influences ER expression. CD4+ helper T cells, expressed high levels of ERs compared to CD8+ cells in myasthenial gravis patients, they found an increased expression of ERα in the thymocytes and both ERs in T cells from peripheral blood mononuclear cells indicating that signals provided by the thymic peripheral and micro environments are clearly distinct. Activation of thymocytes by proinflammatory cytokines induced increased expression of ERs especially in the CD4+ subset suggesting that an excess of proinflammatory cytokines
could explain the increase in ER expression in myasthenia gravis lymphocytes. Dysregulation of ER expression in myasthenia gravis lymphocytes could affect the maintenance of homeostatic condition and might influence the progress of this autonomic disease especially in females (Nancy and Berrih-Aknin, 2005).

Microglia are primary cellular component of the central nervous system and innate immune system. Their response to conserved pathogen motifs is inherent and leads to the release of cytoactive factors that impact surrounding neurons and glial cells. The microglial response is modified by the local tissue environment and by the "global" factors such as gender. Exposure to oestrogen and testosterone in general, downregulate the microglial and peripheral macrophage function promoting an anti-inflammatory phenotype (Liu et al., 2005; Suuronen et al., 2005). Other global factors can however "override" the gender based effects as demonstrated by oestrogen and testosterone. Apolipoprotein E (APOE) genotype and the expression of the specific isoforms of apolipoprotein E differentially regulate the microglial and peripheral macrophage functions (see Colton et al., 2005). It has been showed that the presence of APOE 4 G is a known risk factor for Alzheimer's disease (AD) and other neurodegenerative diseases. This gene promotes a proinflammatory macrophage phenotype in neonatal microglia. However, in adult mice APOE genotype specific effect depends on gender. Peritoneal macrophages from female adult APOE 3 and APOE 4 targeted replacement mice do not demonstrate an APOE genotype specific response, whereas adult males APOE 4 targeted
replacement mice show enhanced macrophage responsiveness (this was significantly more in adult male APOE 3 targeted replacement mice) at least part of the altered macrophage response in APOE 4 male mice may be due to the differences in androgen receptor sensitivity to testosterone (see Colton et al., 2005). These experimental postulates can in a future date conveniently be converted and used as hormonal therapeutic in sex degenerative diseases (as humble beginning like our present study).

Regarding the immunomodulatory actions of oestrogens, several studies have demonstrated the capacity of T cells, B cells and monocytes to respond to oestrogen and ER expression in these cell types. They clarified that ERs are differentially expressed in peripheral blood monocytes subset (PBMC). CD4+ T cells express relatively high levels of ERα mRNA, compared to ERβ; whereas beta cells express high levels of ERβ mRNA, but low levels of ERα. Peripheral blood CD8+ T cells and monocytes express low, but comparable levels of both ERs. This quantitative analysis of ER expression in distinct PBMC subsets may promote a basis for dissecting the mechanisms of immunomodulation by oestrogens and hence future identifications of therapeutic targets for the treatment of inflammatory and immunological disorders (Peeva et al., 2004). Pathogenic autoimmunity required a combination of both inherited and acquired factors. Hormones influence the sexual dimorphism of the immune system and it is possible that they can initiate and or accelerate an autoimmune process contributing to the gender biased expression of autoimmune disorders. Not only natural hormones, but also endocrine disruptors such as environmental oestrogens,
may act in conjunction with other factors to over ride the immunotolerance to self-antigens in lupus erythematosis. Human studies have shown that female sex hormones are implicated in disease pathogenesis. In the B cell compartment, both prolactin and oestrogen are immunomodulators that affect maturation, selection and antibody secretion (see Grimaldi et al., 2005). This impact may be based on their capacity to allow auto reactive B cells to escape the normal mechanisms of tolerance and to accumulate in sufficient numbers to cause the clinically apparent expression of the disease. Both hormones lead to the survival and activation of auto reactive B cells, but they skew B cell maturation towards different directions with prolactin inducing T cell dependent auto reactive follicular B cells and oestrogen eliciting T cell independent auto reactive marginal zone B cells (Gala, 1997; see Colton et al., 2005; Liu et al., 2005). These differential modulations of cytokine milieu by hormone may also affect the development and activation of specific mature B cell subsets. This insight suggests that targeted manipulation of these pathways may represent a promising avenue in the treatment of lupus and other gender based auto immune diseases.

The thymic neuro endocrine polypeptides are the source of cell antigen presented by MHC molecules, enabling the differentiation of haematopoietic stem cells. Thymic nurse cells also produce thymosin β3 and β4 and display a neuro endocrine specific immuno phenotype (IP) Thy-1+, A2B5, TT4, TE4 +, UJ13/A+, UJ127.11+, UJ181.4+ and presence of common leucocyte antigen (CLA+), growth hormone enhances thymocyte release for TNC, as well as the reconstitution of the lympho epithelioid complexes, similar to its role as a
regulator of bone metabolism through regulating OPG production (see Goldstein and Badamchian, 2004). The oestrogen is also involved in the process of thymocyte development, although aromatase mRNA has not been detectable in the thymus. While the increase in number during lactation may be linked to the process of reconstruction of the thymic lymphoid population, the increased activity of lympho reticular interaction on CD14 may be associated with thymic engagement in pregnancy-induced immune processes. The major antigen in the experimental auto immune hypophysitis in rats are growth hormone, thyrotrope and LH. The intra thymic T lymphocytes reactions is a complex multi step process, influenced by serial functionally specialized RE cells and occur under immuno neuroendocrine regulatory control reflecting the dynamic changes occurring in mammalian organism (Goya et al., 2004; see Goldstein and Badamchian, 2004). In HIV − 1 infected adults treated with growth hormone, thymic stress and circulating native CD4+ T cells are altered. This type of treatment would be easier for the diseased as well as the treating physicians, if we are aware of the millennium old wisdom, that the disease is a visit of God.

TH1 cells are inflammatory cytokines and TH2 cells in contrast are anti inflammatory cytokines. Conditions associated with TH1 cells include rheumatoid arthritis, multiple sclerosis; while SLE, Sjogren syndrome and auto immune diseases of muscle are associated with TH 2 cells (all auto immune diseases can thus be classified using their TH cell profile). Interestingly normal pregnancy is associated with TH 2 cells. To summarise, alteration in the cytokine profile may alter disease activity and aggressiveness
in patients with auto immune diseases. For example lupus patients tend to improve when given androgen therapy. In animals treated with androgen, cytokine expression changes, from the interleukins IL - 4, IL - 5 and IL-10 i.e., the classic TH 2 cytokines into TH1 cytokines. Studies of sex steroids and cytokine expression in humans are ongoing. This is one of the rationable for using dehydroepiandrosterone to treat lupus patients. It changes the cytokine profile in these patients from TH2 to TH1 in contrast lupus tends to get worse during pregnancy because pregnancy is a TH2 state (see Matsusaki et al., 2005; see Salem, 2004).

Conversely it was seen that patients with rheumatoid arthritis (RA) often improve while they are pregnant or while they are taking oral contraceptives (OC) or any oestrogen preparations. This is because the TH1 cytokine profile changes to a TH2 profile. Unfortunately this management approach does not work with multiple sclerosis patients. They usually do not improve when the TH1 cytokine profile is changed to TH2 however there are some reports, which states that these patients get better during pregnancy (see Wilder, 1998; see Salem, 2004).

Auto immunity involves much more than cytokines. Our understanding of adhesions, the other molecules known to regulate organ functions, the laying down of matrices, blood vessels and the synthesis of antibodies have also been recently highlighted.

Several areas of research are opening up lately, for example anti phospholipid antibodies may arise from stimulation of the immune system by
the induction of TH2 state. Further studies have also revealed that during the normal menstrual cycle, women's body may shift between a TH2 state to a TH1 state depending on the phase of the menstrual cycle (see Wilder, 1998). This area need to be explored more since here we may find clues as to why and how women are prone to certain types of infections like parasitic infection during pre-menstrual phase.

Oestrogens and androgens play an important role in antigen presentation. Apoptosis is regulated directly by sex steroids (substantiating the theme of our study probing into the possibilities of newer hormonal treatments to change favourably the milieu of the cellular micro environment in the basic inflammatory degenerative processes which lie at the basis of disease mechanisms). A classic example can be sited in auto immunity of endometriosis, which is a process of ex-planting of uterine tissues in other organs and tissues. The uterus continues to respond to changes in menstrual cycle because basally the oestrogen levels are high in endometriosis (see Hastings and Fazleabas, 2003). Another classical example is seen in hormone induced apoptosis resulting in the involution of thymus gland in response to testosterone (see Cutolo et al., 2004).

Women with auto immune diseases tend to have high oestrogen levels, this hormone regulates apoptosis and other immune functions. Women have an immune system i.e., tailored to maintain the fetus (which is immunologically a transplanted organ) for nine months and the female immune system is exquisitely sensitive to any abaration to any shift in hormone levels, any change in diet, indulgence in smoking or other
environmental influences. This sensitivity explains why so many fertility problems occur in women with autoimmune disease (see Cutolo et al., 2004).

In ageing and menopause, women with autoimmune diseases tend to improve and show less clinical disease activity. When women's oestrogen levels drop, auto immunity tends to subside. In men however, the prevalence of auto immunity sequelae increase with ageing because their androgen levels drop and their body oestrogens are unopposed. Further study needs to be done regarding their specific roles in the function and cascade sequentiality of the immune and inflammatory cells to get more conclusive evidences. However, in our small current study we have subjective and collaborative evidences of clinical improvement in inflammatory bone joint conditions when the biochemical hypogonadotropism was corrected with LH dosings along with testosterone dosings, this can be taken as an eye opener even though our study is small.

Recent reports showed the failure of oestrogen usage to alleviate the disease processes in AD (see Baum, 2005). Many authors found that the individuals with AD had elevated levels of LH when compared with controls and both LH and its receptors are seen in increased levels in brain regions, susceptible to degeneration in AD. LH is also known to be mitogenic and could therefore initiate cell cycle abnormalities known to be present in AD afflicted neurons. In cell cultures LH increases amyloidogenic processing of amyloid β protein precursor and in animal models of AD, pharmacological suppression of LH and FSH reduces plaque formation (see Casadesus et al., 2005). Given the evidence of a supporting pathogenic for LH in AD, a trial of
leuprolide acetate which suppresses LH release has been initiated in patients as a part of ongoing trial.

**Testosterone**

Testosterone is a hormone with quite a personality (Freeman *et al.*, 2001). Tainted by a history of abuse by body builders and athletes, testosterone is often pointed as the cause of aggression, bulging pectorals, an insatiable sexual appetite and the almighty hairy chest (Lamb, 1975; Giorgi *et al.*, 1999; see Hartgens and Kuiper, 2004). Its reputation has been somewhat two-faced. Since the 1940’s, the illegal use of testosterone and its relatives, anabolic steroids to increase muscle mass and enhance sports performance has fueled a black market worth millions. On the other hand, its fruits of virility and strength have been well accepted in more mainstream clinical therapies. Being the primary male sex hormone produced by the testis, testosterone tends to be identified stereotypic of male gender (Knorr *et al.*, 1974; see Khosla and Bilezikian, 2003). But despite popular belief testosterone is a male gendered hormone. In 1889 Charles Brown-Sequard, a French physiologist concocted a “rejuvenating therapy for the body and mind”. His bizarre elixir was a liquid extract made from testicles of guinea pigs and dogs (Cusson *et al.*, 2002; Herman, 1982). Brown-Sequard claimed his juicy ‘liquide testiculair’ increase his physical strength, intellectual prowess, relieved constipation and last but not the least, lengthened the arc of his urine. From this early testicular adventure Brown-Sequard has been coined one of the founders of modern endocrinology (Cusson *et al.*, 2002).
For testosterone to have an effect on any particular part of the body there must be testosterone receptors awaiting its call – a case of needing the right key for the right lock. Years of extraordinary investigation culminated in the production of synthetic testosterone in 1935 by Butenandt and Ruzicka who won the Nobel prize in chemistry that year. Being one of the family of hormones called androgens, testosterone gets its first kick into action during embryonic stages of life – a female egg is fertilized with a male sperm each donating a single chromosome to the embryo X-from women and X or Y from men. If the combination of the chromosome is XX then the embryo becomes a female, if XY it becomes a male, until the seventh week of intrauterine life the embryo is undifferentiated regarding the sex (see Bykov, 1986; see Renfree and Short, 1988; see Szarvas, 1990). The differentiation occurs due to the presence of antimullerian hormone arising from the sertoli cells. Testosterone is responsible for the further development and growth of the male genitalia and also is responsible for the regression and degeneration of the female component of the undifferentiated genitalia (see Vignozzi et al., 2005). It promotes the growth of the reproductive tract, increases in the length and diameter of the penis, development of the prostate and scrotum and the sprouting pubic and facial hair, as well as the androgenic or masculanising effects, testosterone also drives anabolic or tissue-building changes. These include thickening of the vocal cords, growth spurts, development of sexual libido and an increasing in strength and bulk of muscles. These powerful effects of testosterone continue well into adult hood (Resko, 1978; Pointis et al., 1980; Berry and Issacs, 1984; see Cunha et al., 1987; Thomson et al., 1997; Sinisi et al., 2003; see Bancorft, 2005). Oestrogen and progesterone are
the so called female sex hormones but it is well known now that women via mainly their ovaries and adrenal glands make there are about 20 times less than the males and out of these testosterone made by the ovaries, oestrogen is made. One quarter of the total circulating testosterone in a female originates from the ovary, conversely males also produce oestrogen which is converted at the tissues to testosterone (see Meaney, 1989; see Reinisch et al., 1991). In menopausal age the circulating testosterone level also decline, while they are experiencing a perceptous drop in oestrogen, Rako says that symptoms of 'deficiency ' or loss of vital energy and feeling of "wellness", a loss of familiar levels of sexual libido, sensitivity of nipples and genitals and thining of pubic hair. Along with these impacts drag along a 'flatness' of mood, dry skin, brittle scalp hair and a loss of muscle tone and strength, and skin turgor. It is understood that testosterone also contributes to the health of a women's vulva, re-grows the vital tissue of the clitoris and plays in curbing osteoporosis by helping to maintain density of bones, testosterone also influences the cognitive functions (see Meaney, 1989; see Reinisch et al., 1991; Sinisi et al., 2003; see Bancroft, 2005). Testosterone receptors are found in the brain, which means the hormone interact and binds with our neurons, relaying to them important messages for action. Deborah Blum, author of sex on the brain says that this indicates, the brain is 'prepared to listen to what testosterone has to say' and that 'most researchers consider that these at least an indirect evidence that this hormone is capable of altering the brain and thus influencing our behaviour'.
Other chemical actions of testosterone are

- Promotion of protein biosynthesis responsible for the highly characteristic anabolic action in shifting to positivity of body metabolism, cell growth and matrix re-building (which is involved in cell regeneration and healing).

- Increases formation of red blood cells and speeds up regeneration.

- It speeds up recovery time after injuries or illnesses stimulating the entire metabolism which results in the burning of body fat.

- Inhibition of the gonad regulating cycles, including the hypothalamo hypophyseal – testicular axis, which regulates the amount of testosterone in the organism. If the testosterone level in the blood is high the testis signals the hypothalamus to release less LHRH (Leuteinizing Hormone Releasing Hormone). In turn the hypophysis releases less gonadotropins such as LH (Leuteinizing Hormone) and FSH (Follicle Stimulating Hormone). Consequently the Leydig cells in the testis reduces the production of testosterone.

Oestrogen and progesterone profoundly affect the brain (see Wang, 2000). The increase in memory loss and mind mental confusion, emerging in middle age are largely due to the declining levels of oestrogen and progesterone. In men the same problems can be caused by the decreasing
levels of testosterone and increasing levels of oestrogen, beginning in the late 30's or early 40's. Insomnia in women is also related to the lack of oestrogen, while in andropause (male menopause) the unopposed levels of oestrogen shifts the balance of personality to a less stable one and a more stable one of gender dimorphism. In effect testosterone replacement offers medical therapy for reversing the effects of ageing, enhancing the immune system, enhancing the sexual potency, hair growth in men and reducing body fat, increasing lean muscle mass, lowering blood pressure, lowering cholesterol, increasing energy and vitality, reversing heart disease, eliminating arthritis pain and treating certain diseases in both genders (see Harman, 2005; see Krause et al., 2005).

Anti ageing therapies in both sexes recently include hormone replacement with testosterone and recombinant growth hormone in males, oestrogen/progesterone with recombinant growth hormone and occasional testosterone in females and this therapy helps in treating injury healing processes and chronic disease processes including, inflammatory and autoimmune disorders (Krause et al., 2005; Harman, 2005). Although categoric positive references are lacking, sporadic statements of researchers finding beneficial effects of the above mentioned schedules are available.

There is substantial evidence that physiological testosterone levels have a beneficial effects on blood vessels and that this effect is actually engineered by its conversion to oestrogen (see Rosano et al., 2005). Testosterone relaxes blood vessels in male animals of several species, as to selective ER modulators like Tamoxifene and Raloxifene. Injury of blood
vessels in male induces the expression of ERβ and disruption of the ERβ gene in mice leads to abnormal vascular function and hypertension. The role of ERs, including membrane receptors in the vasculature extends as well to the more complex processes of the vascular injury response and atherosclerosis (Alzubair et al., 2005; see Hisamoto and Bender, 2005). In the rat carotid injury model, testosterone, oestrogen and selective ER modulators, all can inhibit neo intimal thickening after balloon injury. Using LDL receptor deficient mice, it was shown that castration of male animals increases the extent of atherosclerosis that develops in comparison to intact animals, while aromatase inhibition with anasterazole reverses the protected status of intact male mice and increases the extent of atherosclerosis to the level observed in orchidectomised animals an effect that was abrogated when mice were simultaneously treated with aromatase inhibitor (see Rosano et al., 2005).

Testosterone levels like those of oestrogen, decline with age (see Morely et al., 2005). Most cross sectional studies show an inverse relationship between testosterone levels and the incidence of coronary heart disease in men. Administration of testosterone at physiological doses in men with coronary heart diseases enhances coronary blood flow and endothelial function (see Rosano et al., 2005). It is important to bare in mind that administration of exogeneous oestrogens can alter the expression of components of the very hormone response system targeted. For e.g., oestrogen increases the level of sex hormone binding globulin (SHBG) in blood, which in turn may reduce free testosterone levels and potential tissue bioavailability (see Davis, 2001). Considering the relevance of these factors
it might be more effective to attempt to enhance aromatization of exogeneous testosterone to estradiol instead of trying to increase tissue oestrogen availability with oestrogen supplementation. More work need to be done in this regard to develop a rational approach to hormone replacement therapies (HRT) – tailored selective hormone receptors and/or enzyme – directed therapies for testosterone and oestrogen replacement in the prevention and treatment of vascular disease in men and women.

- Two recent studies (Lew et al., 2003; Kimura et al., 2003) shed important light on the understanding of normal hormonal control of vascular tone in males. In the first study the authors found significant attenuation of flow mediated vasodilation, without significant changes in lipoproteins, homocysteine or C-reactive protein. The second study reveals that aromatase generated oestrogen helps to maintain normal vascular tone.

**Dehydroepiandrosterone (DHEA)**

Dehydroepiandrosterone, has been dubbed the ‘mother of hormones’. DHEA is the most abundant steroid in the human body and is involved in the manufacture of testosterone, oestrogen and corticosterone (Labrie et al., 2005). The decline of DHEA with age parallels that of human growth hormone (HGH) by age 65 and the body makes only 10 to 20% of DHEA and HGH of what it did at age 20. By the age of 75 DHEA levels are only 10-20% of what they were at 20 (see Leowattana, 2001). DHEA is produced by adrenal glands, the production being high even when the fetus is still
developing and it continues to rise up to age 25, when production drops off sharply. As with melatonin and human growth hormone volume, levels of DHEA are closely associated with a number of age related diseases and disabilities (see Watson et al., 1996). Scientists are speculating that ageing men, women can restore their DHEA to youthful levels to regain their youthful health and vigour. According to Dr. Samuel Yen, a reproductive endocrinologist and principal investigator of DHEA study at the Univ. of California at Sandiago, DHEA is a drug that may help people age more gracefully'. Administration of DHEA makes 82% of women and 67% of men score higher test rating their ability to cope with stress, their quality of sleep and their basic well being (see Namiki, 1994). Only 10% of the group not receiving the hormone reported feeling any better. Small amounts of DHEA were found lessen amnetia and enhance long term memory in experimental mice. Even very low levels of DHEA supplementation was found to increase the number of neurons in the brain as well as prevent neuronal loss and/or damage. In animal studies, DHEA has been shown to be useful for fighting obesity, diabetes, cancer, autoimmune disease, heart disease, stress and infectious disease. In other words, it is an allround anti-ageing drug (see Dillon, 2005). It extends life of laboratory animals by as much of 50% experimental mice given the hormone look younger and healthier, maintaining the glossyness and coat colour of their youth. It may have a life extending effect in humans as well, although not as great as was originally reported in a study that now spans nearly 19 years. In 1987 it was reported that 70% drop in mortality from heart disease in men with high DHEA levels. However, the follow-up study found only 20% drop in deaths when
compared with those who had low DHEA levels (see Porsova – Dutoit, 2000; see Wranicz et al., 2004). Higher DHEA levels did not protect women mortality when they are at risk of cardiovascular disease. It was reported that out of a group of men between ages 60 and 80, those with the highest levels of DHEA were younger and leaner, more fit and had higher testosterone levels than those who were in lower DHEA levels. However, no such differences were found in women of the same age group between those with highest and lowest levels of DHEA (Abbasi et al., 1998, Buffington et al., 1998). It is worth pondering on the postulates of the present fairly justified hormone replacement regimes of oestrogen in females and testosterone/DHEA in males needs to lead to the final common pathway of cardiovascular protection. The interim intricacy of biochemical multi directional pathways which is gender bound is only not clear, but all the same this postulate prompted us to the present study of attempting to use similar hormonal modalities to achieve newer modes of treatment of diseases similar to the mentioned above.

Growth hormone deficiency factor in chronic illnesses

The onset of chronic illness begins with inflammation, immune system dysregulation and hyper coagulate state leading to anoxia and dysfunction. At times only one part of the body may be affected while the entire body (different organ systems) including the HPA axis may be involved inproducuing dysfunctional state of altered physiology in various organs (Helmreich et al., 2005). The progress of such illnesses (acute or chronic) tends to achieve a cumulative effect on GI tract, respiratory tract, urinary
tract, liver and endocrine glands mainly thyroid and adrenals and as the disease process advances immune system and lastly the CNS succumb. Components of the immune system implicated in chronic disease are humoral immunity, phagocytic cells, natural killer cells and cytokines (see Turnbull and River, 1995; see Turnbull et al., 1998).

Growth hormone deficiency has been reported in chronic inflammatory diseases especially in stages of immune system dysregulation, adrenal axis dysregulation and hyper coagulation state (Gala, 1997). Such situation of growth deficiency have been seen in relation to chronic fatigue syndrome, fibromyalgia syndrome, rheumatoid arthritis etc. The concept of anoxia caused by the immune system activation of coagulation defect and cytokine excess and vasculitics contributing to the declined and dysregulation of HPA axis leading to the relative deficiency of growth hormone has been reported.

Manifestations of adult growth hormone deficiency are:

- Disturbed lipid pattern.

- Decreased exercise capacity.

- Abnormal body composition with defective sweat secretion and impaired thermoregulation.

- Excess weight and central adiposity.

- Increased tone in sympathetic nervous system.

- Impaired glucose homeostasis.
- Decreased bone mineral content, decreased activity of osteoblast precursor and increased proliferation and differentiation of osteoclasts.

- Impaired fibrinolysis.

- Impaired cardiac functioning.

- Impaired sleep quality.

- Reduction in arterial distensibility.

- Low nitric oxide levels contributing to early atherosclerosis.

- In chronic stages of inflammation & infection associated with release of endotoxins and increased production of cytokines including interleukin 6 (IL-6) by the liver cells.

*Nerve cell regeneration (thus far considered medical impossibility)*.

One of the great unsolved mystery of neuronal growth has been why embryonic or young neuron have the ability to regenerate where as adultself do not, previous studies had suggested that this could not be attributed to the poor environment that the adult central nervous system provides to support the growth and that improving this environment could stimulate adult neuron regeneration. The adult central nervous system expresses very low levels of growth promoting matrix molecules and high levels of myelin associated factors that inhibit the growth of axons (see Hu and Strittmatte, 2004). Also
after injury, there is a pronounced up regulation of inhibitory proteoglycans that are not normally expressed in mature brain (see Mastui and Oohira, 2004). Other studies have suggested the environment unlikely for the only factor that inhibits neurons regeneration, because there are maturation associated changes in the inherent ability of adult neurons to re-grow (see Aguayo et al., 1991). It has also been demonstrated that when embryonic neurons are transplanted into the injured adult CNS, they show significant outgrowth, despite being in this ‘inhibitory environment’ (see Cotman et al., 1984; see Okano, 2002). One cellular factor that could potentially influence neuronal re-growth is a group of proteins known as the integrins, whose receptors mediate axon extension in both embryonic and adult CNS tissues (Condic, 2001). Increased integrin expression has also been shown to mediate adaptation of embryonic neurons to inhibitory environments and has been correlated with superior neurite extension. When neurons were tested for adaptation in conditions similar to those of the adult CNS after injury, adult neurons expressing high levels of either integrin i.e., tested with 3 types of integrin gene, $\alpha_5\beta_1$, $\beta$ galactosidase gene and integrin gene $\alpha_1\beta_1$ had a much greater outgrowth to the adult neuron. This improvement in growth was quiet pronounced representing up to a 2.5-fold increase in the number of neuritis per cell and a 10-fold increase in the neurite length compared with controls. It was also specific for the receptor type that was expressed. Increased expression of integrin $\alpha_1\beta_1$ were associated with increased growth of laminin but not on fibronectin. Integrin $\alpha_1\beta_1$ is the primary laminin receptor in sensory neurons unlike the $\alpha_5\beta_1$ integrin gene which is a major fibronectin receptor, thus explaining the difference. The increase in nerve
fibre growth occurred even in environments that would otherwise prevent regeneration, such as the presence of inhibitory molecules and on weakly growth promoting substrata. And neurons showed improved growth when tested on both high and low levels of extra cellular matrix ligands compared to β-galactosidase expressing neurons. This suggests that in a similar manner to early postnatal neurons, adult neurons with high levels of integrin expression are able to adapt to different substrata by regulating integrin expression. The involvement of stress or injury or disease induced changes in central nervous system cytokines and its alteration of peptide receptors sensitivity and signalling affect the cell degeneration-regeneration-cell death cascade of inflammatory and auto immune disease processes, which in our study has been shown to be favourably modulated by the new hormone treatment modality in post-paralytic and post-stroke patients which is noteworthy although proof reckon which is yet at the bottom of the well.

- Three cytokines viz., IL-1, IL-6 and IL-8 which are associated with chronic ailments associated with inflammation are the mediators for wide spread pain known as polyalgia in chronic ailments, fatigue and impaired sleep pattern. IL-6 is specifically related to fatigue and impaired cognitive functions of the brain (also seen in oestrogen deficiency of menopause in females corrected by oestrogen replacement which reduces the level of IL-6). TNF-α activates hypothalamus, in releasing Corticotropin Releasing Hormone (CRH) and the release of IL-1 which incidentally does not stimulate the pituitary or the adrenal gland.
- Upregulation of N-methyl- D-aspartate (NMDA) receptor at spinal synapse.

- Dysregulation of substance P which is responsible for impaired cognition and sleep dysrythmia.

- Decrease in IGF-1 and decrease in body mass.

Durham et al., (2005) have identified a critical switch that turns on a blood stem cell's prized ability to regenerate itself, while also producing a variety of daughter cells which has a capacity of becoming any type of mature blood and immune system cells. The switch is a protein called Notch that resides on the surface of stem cells. When Notch activity is turned off stem cells quickly lose their potency and begin to change into more mature cells that can no longer produce new bloodforming cells (see Zediak, 2005). Previous results have suggested that activation of Notch, a protein known to be crucial in the development of embryos in virtually all animals from flies to humans could also influence growth of bloodforming cells, but it was not known whether the same was required for the proper development of blood forming stem cells and its exact role in this context was unknown. Mice with engineered gene sequence capable of producing a fluorescent signal when Notch signalling is activated. And this fluorescent protein made it possible to track where and when Notch was active during the blood cell development and to see when the same is turned off, their studies revealed strong signals in the portion of bone marrow where the potent 'haematopoietic' or bloodforming stem cell reside. The levels of Notch signal decrease rapidly as
these stem cells committed to becoming fully mature cells not caring any longer to produce new blood forming cells. When they inhibit Notch activity in stem cells, they rapidly commit to specific lineages before differentiation (see Paziano et al., 2003). This finding together with the data showing Notch function is shut off physiologically as cells commit to specific lineages, suggest that Notch turning off is the mechanism that allows stem cells to become sensitive to differentiation cues (which is crux of blood cell and immune cell proliferation) and thus appears to influence the balance between self renewal and commitment to senescence. The two signal Notch and Wnt are likely to have distinct roles of differing nature in cell cues of self renewal or regeneration. The current status finds out that Notch may have dominant role in maintaining stem cells in undifferentiated state (i.e., senescent state) while Wnt may have a dominant role in proliferation and survival (see Duncan et al., 2005) in which factors seem to be under the currents of the mechanism of healing regeneration and cures of chronic degenerative bone, skin, muscle and neuronal diseases sighted in our small study. The intricate mechanism of alteration of cell signalling by stress and injury induced cellular damage on the provocation of the cascade phenomena of cytokine hormone-cytokine prostacyclin-kallikreins and kinins in modulating autocrine and paracrine signalling for favourable pathways to healing are yet to be individually dissected by further study. Probable role of leukemia inhibiting factor, nitric oxide and pro-opiomelanocortin related cytokines need to be explored in this context.
The importance of Kallikrein-Kininogen-Kinin systems and their diverse pharmacological properties and biochemical characteristics have given modern definitions of the cellular receptors signal transduction mechanisms and the resultant physiological and pathological roles (Kelso, 1998). It is being clearly understood now that tissue kallikreins and their kinin products are important regulators of cardiovascular, renal and skeletal muscle function and participate or interfere in the actions of drugs affecting these tissues (Moreau et al., 2005; see Murakami and Shimamoto, 2005). This phylogenetically ancient system has some responsibility for the regulation of local and perhaps systemic chemo dynamics as well as for the movement of electrolytes, water and cellular metabolites of substrates across the vascular wall or epithelia and into other tissues. These conclusions have resulted from important discovery concerning cellular localization of gene expression for system components and the regulation of such gene expression more complete molecular characterization of components including the kininogen substrates, the formative and destructive proteases and peptidases and the kinin receptors, interrelations among these molecules, other cardiovascular regulatory hormones, the mechanisms and finally new finding illustrating system aberrances in cardiovascular insults or diseases such as diabetes mellitus, cardiac disorders and hypertension.

More information is available about possible regulatory influences on tissue kallikrein, with renal kallikrein being the most studied. The renal kallikrein levels and synthesis rate are increased by diets low in sodium. Diets high in potassium augment urinary kallikrein excretion or renal
kallikrein levels. Chronic high levels of mineralocorticoids can increase renal kallikrein and adrenalectomy reduces it. Renal kallikrein mRNA in female rats is twice as high as that in males (see Ardiles et al., 2003). Various hormones affect the renal mRNA and protein levels including testosterone, thyroxine and insulin. Each significantly increases kallikrein synthesis and the level of enzyme activity. Vasopressin can also increase kallikrein release from rat renal cortical slices, but norepinephrine decreases it (Chapman et al., 1986; Lauar and Bhoola, 1986; Grenfell et al., 1988). The latter effect is accompanied by a reduction in de novo kallikrein synthesis and can be prevented by (β) sup.1adrenergic blockade. Thus there is now ample opportunity to examine alterations in substrate and formative enzyme gene expression or their levels in relation to pharmacologically induced or pathological changes in renal (or other organ) function. This conclusion is reinforced by some recent findings in renal kinin receptors. These receptors were well characterized in binding studies using (125I) (Tyr5)-BK, a partial BK2 agonist and mesangial cells or glomerular membranes. In addition to describing both BK1 and BK2 receptors in rat kidney and evaluating them in Gold Blatt experiments these studies showed that after 28 days of low sodium intake there was a marked reduction in BK2 binding site density concomitant with increased renal and urinary kallikrein. Conversely water deprivation resulted in reduced renal kallikrein and an increase in BK2 receptor density (see Emanueli and Mededdu, 1999; see Zhuo, 2000). Most recently, these clues are being augmented by new technology which is allowing the direct measurement of renal interstitial kinin levels and showing that such levels change markedly in response to various stimuli.
Atrial natriuretic peptides: Tissue kallikrein was capable of forming atriopeptin (atrialnatriuretic peptide) from its precursors, as well as of catabolizing the active peptide in vitro (Briggs et al., 1984). Administration of this powerful natriuretic diuretic agent affects urinary excretion of kallikrein conversely cardiac tissue contains a kallikrein-like enzyme that co-localises in atrionepitin containing granules.

Eicosanoids: Many studies show that kinins are potent stimuli for arachidonic acid release and subsequent eicosanoid synthesis. Vascular prostacyclin synthesis is powerfully stimulated by kinins, but so is vascular or platelet synthesis of the vasoconstrictor thromboxane. Most studies are attempting to interrupt these linkages with for example, cyclooxygenase inhibitors or kinin receptor blockers or to modify relationships with converting enzyme inhibition or lipopolysaccharide induced endotoxic shock disclose the connections between kinins and prostaglandins, especially their vasodilator and natriuretic properties (Wang et al., 1991). These connections may also relate to some actions of vasopressin, catecholamines, endothelins and nitric oxide.

Nitric oxide: NO is another mediator now implicated in kinin-induced vasodilation and hypotension (Fulton et al., 1992). Interesting recent work has disclosed that in the rat and dog renal vasculature the vasodilatation produced by administered bradykinin is significantly but not totally dependent on nitric oxide synthesis and as such could be markedly attenuated by either (N^6)-nitro-L-arginine, inhibitors of nitric oxide synthetase. These studies now bring consideration of abnormality in the endothelial derived relaxing factor (nitric
oxide)-kinin relationship to studies of kinin-induced vascular responses. This relationship is of great interest because there is clear renal vascular hyper-responsiveness to bradykinin, particularly in the spontaneously hypertensive rat in so far, as both nitric oxide-dependent and independent actions are concerned.

Multiple organ dysfunction leading to ultimate inevitable multiple organ failure a cataclysmic cascade of events leading to mortality due to unknown ultimate cause, despite the original disease process being apparently under control and favourably mitigated.

**Multiple organ failure**

**Circulatory failure**

Criteria for diagnosis

- Bradycardia (heart rate <50 bpm)
- Hypotension (mean arterial pressure <50 mmHg)
- Ventricular tachycardia or fibrillation
- Metabolic acidosis (pH <7.2)

**Respiratory failure**

Criteria for diagnosis

- Respiratory rate <5 or >40 breaths per minute
- Hypercapnia (PaCO2 > 6.7 kPa)
- Hypoxaemia
Acute renal failure

Criteria for diagnosis

- Urine output < 400 ml per 24 hours
- Serum creatinine > 150 mmol/l

Haematological failure

Criteria for diagnosis

- Leucopenia (WCC < 1000 cell / mm$^3$)
- Thrombocytopenia (platelet < 20,000 / mm$^3$)
- Evidence of disseminated intravascular coagulation

Hepatic failure

Criteria for diagnosis

- Coagulation defect
- Rising hepatic enzymes

Gastrointestinal failure

Criteria for diagnosis

- Ileus
- Gastroparesis
- Haemorrhage
Neurological failure

Criteria for diagnosis

- Depressed level of consciousness (Glasgow coma score <6)
- Fits

Sepsis has been referred to as a process of malignant intravascular inflammation. Normally a potent, complex, immunologic cascade ensures a prompt protective response to microorganism invasion in humans. A deficient immunologic defense may allow infection to become established, however an excessive or poorly regulated response may harm the host through maladaptive release of indigenously generated inflammatory compounds (Mercier, 1993; Hanasawa and Kodama, 1998).

Lipid A and other bacterial products release cytokines and other immunomodulators that mediate the clinical manifestations of sepsis. ILS, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and other colony stimulating factors (CSFs) are produced rapidly within minutes or hours after interactions of monocytes and macrophages with lipid A. TNF release becomes a selfstimulating process (an autocrine) and release of other inflammatory mediators, including interleukin-1 (IL-1), platelet activating factor, IL-2, IL-6, IL-8, IL-10, TNF and eicosanoids which further increases cytokine levels. This leads to continued activation of polymorphonuclear leucocytes (PMNs), macrophages and lymphocytes, proinflammatory mediators recruit more of these cells (a paracrine process). All of these
processes create a state of destructive immunologic dissonance (see Trappe and Riess, 2005).

Sepsis is described as an autodestructive process that permits extension of the normal pathophysiologic response to infection to involve otherwise normal tissues and results in multiorgan dysfunction syndrome (MODS) (Zimmermann et al., 1989).

*Mechanisms of organ dysfunction and injury*

The precise mechanisms of cell injury and resulting organ dysfunction in sepsis are not understood fully. Multiorgan dysfunction syndrome is associated with widespread endothelial and parenchymal cell injury because of the following proposed mechanisms:

*Hypoxic hypoxia:* The septic circulatory lesion disrupts tissue oxygenation, alters the metabolic regulation of tissue oxygen delivery and contributes to organ dysfunction. Microvascular and endothelial abnormalities contribute to the septic microcirculatory defect in species. The reactive oxygen species, lytic enzymes and vasoactive substances (nitric oxide, endothelial growth factors) lead to microcirculatory injury which is compounded by the inability of the erythrocytes to navigate the septic microcirculation (Arya and Garcia, 1996).

*Direct cytotoxicity:* The endotoxin, TNF-\(\alpha\), and nitric oxide may cause damage to mitochondrial electron transport leading to disordered energy
metabolism. This is called cytopathic or histotoxic anoxia, an inability to utilize oxygen even when it is present (Arya and Garcia, 1996).

**Apoptosis:** Apoptosis (programmed cell death) is the principal mechanism by which dysfunctional cells are eliminated normally. The proinflammatory cytokines may delay apoptosis in activated macrophages and neutrophils, but other tissues such as the gut epithelium may undergo accelerated apoptosis. Therefore, derangement of apoptosis plays a critical role in tissue injury of sepsis (see Papathanassoglou et al., 2000).

**Immunosuppression:** The interaction between proinflammatory and anti-inflammatory mediators may lead to an imbalance. An inflammatory reaction or immunodeficiency may predominate or both may be present.

Chemical painkillers known as endorphins and enkephalins are produced naturally in the body. They are polypeptides, able to bind to the neuro-receptors in the brain to give relief from pain. This effect appears to be responsible for the so called runner’s high, the temporary loss of pain when severe injury occurs and the analgesic effects that acupuncture and chiropractic adjustments of the spine offer.

Four groups of endorphins, α, β, γ and σ have so far been identified. α-endorphin contains 16 amino acids, β-endorphin, another polypeptide (long chains of amino acids) contains 31 amino acids, γ-endorphin contains 17 and σ-endorphin has 27 polypeptides with greater than 50 amino acids in their chain and are called proteins (see Jankovic and Radulovic, 1992).
The enkephalins are pentapeptides, the smallest of the molecules with pain killing or opiate activity. The enkephalins are found in the thalamus of the brain and in parts of the spinal cord that transmit pain impulses. The amino acid sequence of enkephalin is found in the longer amino acid sequence of the endorphin. A chemical called substance P, a polypeptide with 11 amino acids has been found to transmit pain impulses to the brain (Shaw and Ramwell, 1968). Endorphins may act to prevent the release of substance P, which may account for the sedating effects of endogenous endorphins and narcotics given exogeneously such as heroin and morphine. A rise in blood levels of endorphins is measurable after exercise and sexual activity (Harber and Sutton, 1989; see Guszkowska, 2004). Our own opiates may explain how someone severely wounded in battle can continue to fight or have the strength to save someone else. Some scientists feel that endorphin release may be another reason some people pursue dangerous activities such as bungee jumping. So called thrill seekers and adrenaline junkies may not just be addicted to the rush of adrenaline.

Pleasant memories such as our first bike or a great vacation or bad memories, as when a loved one or a pet dies are also linked to the autonomic nervous system and the brain stem. Endorphin research suggest a link between our emotional state of well being and the health of our immune system (see Koob and Bloom, 1983). Endorphins and other neurotransmitters that are flooded into our bloodstream during stressful, as well as good times are often felt “physically” as we get a queasy or nervous feeling in our stomach. That “gut feeling” most people, at one time or another have felt is
our second brain talking to us, according to Jeff Cohen, a Kaiser Permanente neurologist. The placebo effect is also attributed to endorphin and enkephalin release, as the emotional component of receiving a sugar pill. Many types of therapies such as massage or hydrotherapies have also been shown to release endorphins and enkephalins (see Brody, 2000).

Exercise, meditation, relaxation and a good sense of humor may be more helpful than what those in the medical communities once believed. Laughter may help modern therapies and medicine as an adjunctive therapy that cost little or nothing to obtain. Norman cousins reported that, 10 minutes of solid belly laughter gave him two hours of painfree sleep when he was battling a painful degenerative disease. Several studies have shown that pain perception is reduced after exposure to comedy (see Zill mann, 1994). Perhaps this reflects higher levels of endorphins. Further research is needed to learn if the healing power of a comic who can make one breakdown into extreme laughter is as beneficial as taking a pain pill.

For years, it had been suspected that opiates had specific binding sites in the brain. There were several attempts to locate these sites. Technological limitations of the early days made it impossible to distinguish the receptors. The first attempt to isolate endorphins and the receptor sites was done by Vincent Dole in 1970. By the early seventies technology evolved to a point where the discovery was inevitable. The first to shake the scientific community was Solomon Snyder, and his student, Candace Pert, at John Hopkins University in 1973. Using a technique developed at Stanford University by Avram Goldstein, Snyder and Pert located the difficult to find
receptors using Goldstein’s biochemical research. The existence of endorphins could not be proven until receptors on nervous tissue could be proved. With the discovery of the receptor sites, Goldstein asked “why would God have made opiate receptors unless he had also made an endogenous morphine-like substance”. In the mid 60’s, a pituitary hormone named β-lipotropin was discovered (Li, 1964; Lohmar and Li, 1967; Li, 1968). It was noted that one portion of this hormone had analgesic properties. After the receptor sites had been discovered the existence of a morphine-like substance was reported, which was later purified and named Enkephalin meaning “in the head” (Janecka et al., 2004). The peptide sequence was recognised and being the same as the B-lipotropin hormone. Choh Li would later isolate the chemical from his earlier discovered pituitary hormone and named this chemical “Endorphin” which means “the morphine within”.

Today the term opioid is used for all endorphins and morphine-like chemicals, including dynorphin another brain opioid peptide later found by Goldstein. Other psychoactive peptides have been discovered and isolated using the techniques developed in these early laboratories (Surman, 1993). These shared discoveries have advanced the knowledge and led to many other discoveries of modern pain relieving analgesics that are less addictive, have greater potencies and fewer side effects. Other drugs related to the opiates have generated new interest in the function of the brain, making way for a new era in our understanding of the brain and human behaviour as a whole.

A recent clinical use of another opioid has led to new theories concerning autism. One theory states that autistic individuals may have too
much β-endorphin in their central nervous system. This theory goes on to postulate that a synthetic enkephalin called Naltrexone may effectively block opiate receptor sites reducing levels of endorphin uptake at the receptor sites. These high levels of endorphins are thought to be responsible for the trance like state, that many of those afflicted with this little understood disease, autism exhibit. Some of the clinical improvements noted after Naltrexone therapy in autistic patients are increased socialization, eye contact, general happiness, normalized pain sensitivity, and a reduction in self-injury and stereotypic (self-stimulatory) behaviours (Sandman, 1993).

Endogenous opioids have made up the bulk of this discussion and we have even seen how symptoms of autism may be reduced by opiate blockers. How and why scientists became engaged with endorphin research, is answered by the fact that scientists have known the physical effects of drugs like morphine and heroin for many years. Even before we understood their chemical make up and mechanism of action (much is still unknown), the mystery of the botanical origin of all the modern narcotic analgesics perplexed those involved in pharmacology. Enter the opium poppy, papaver somniferum. This flower cultivated in Asia and other countries was known to possess analgesic qualities by the ancient Chinese, who smoked it in its raw form. It is important here that we distinguish the difference between an analgesic and an anesthetic. Analgesics reduce the perception of pain without a loss of consciousness, while anesthetics reduce pain by inducing a loss of consciousness. Opium contains morphine, codeine, noscapine, papaverine and thebaine. Thebaine is a convulsant drug and produces no analgesia, as such it
is not used clinically but is important in the production of other semisynthetic opioids. Opium is a less effective analgesic than morphine, because it is slowly absorbed and has been historically used for its constipating action (paregoric) (Kizilkaya et al., 2005). Morphine itself was discovered in 1809 and has a variety of useful effects, among those of which are analgesia, euphoria and cough suppression. The drawback is its high addictability. The modern chemist works now to create derivatives of morphine that retain the analgesic or other medically useful qualities, while reducing the addictive or constipating effects. The chemical structure of morphine is responsible for its addictive and other undesirable side effects. The ring structure of morphine can be modified chemically and its analgesic effects increased to 100 to 1000 times greater than morphine by acetylation and by hydrogenation at double bonds (Poeaknapo, 2005). If the nitrogen substituent is changed to a bulky alkyl group, the compound produced is a narcotic antagonist. These antagonistic drugs created by modern chemistry are used as antidotes for overdose of heroin and similar drugs that may be abused in our society. Astoundingly, chemically and structurally the drug heroin and the narcotic antagonist used to reverse the opiate effects are virtually identical (see Woolf and Hashmi, 2004). The rapid (almost instantaneous when injected intravenously) onset of action of the antagonist has saved many lives. This quick onset of action responsible for saving the lives of accidental overdose as outrageous as this may seem, has also been known to infuriate hard core addicts, who as they regained consciousness, complain violently demanding to know who ruined their high!