REVIEW ARTICLE

Peptide regulatory factors. Intercellular signalling molecules regulating tissue (re) modelling

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Definition

Peptide regulatory factors (PRFs)[1] refer to a group of factors that regulate cell growth and differentiation and are therefore closely involved in the control of morphogenesis and tissue modelling in the embryo as well as inflammation, tissue repair and regeneration in neonates and adults. PRFs bind to specific receptors present on the surface membrane of target cells. Unlike classical endocrine hormones, PRFs are produced locally from diverse sources, acting mainly as paracrine or autocrine regulators of cell function. They thus act as intercellular signalling molecules influencing the functions of a variety of target cells and their actions are typified by intricate interactive networks that serve to amplify the responses triggered by the initiating event.

Nomenclature

Different disciplines have used a variety of names to describe these chemical mediators released from cells capable of modifying biological functions. Immunologists referred to immunoregulatory molecules as lymphokines or monokines until the term 'interleukins' was adopted. Virologists have used the term 'interferons' to identify peptides that interfere with viral replication. In haematology, substances defined by virtue of their ability to support haematopoietic colony formation *in vitro* were named 'colony stimulating factors'. In recent years, the more generic terms 'cytokines' or 'peptide regulatory factors[1] have been used to define soluble proteins, non-immunoglobulin in nature, that are released by living cells and act non-enzymatically to regulate cell functions.

Fracture Repair – A Model of Tissue Remodelling

The role of PRFs as regulators of tissue remodelling is exemplified by the model of fracture repair. Healing of a fracture can be divided into 3 phases:

(a) The inflammatory phase
(b) The repair phase
(c) The regenerative phase

(A) The Inflammatory Phase

Soon after a fracture is sustained, an extensive blood clot forms in the subperiosteal and soft tissue as well as in the marrow cavity. There is often extreme muscle and bone necrosis. After 2– 5 days, neo-vascularisation occurs and organisation of the clot by collagen begins. The purpose of this phase is to eliminate the pathogenic insult and remove injured tissue components.

Inflammation involves the recruitment of leucocytes into sites of injury or infection followed by release of vasoactive mediators that increase vascular permeability such as leukotrienes, histamine and serotonin. Micro-organisms are destroyed and specific immune responses initiated. The major cellular components of inflammatory reactions that are attracted to the site of injury are haematopoietic cells

represented by platelets, mast cells, monocyte phagocytic cells, lymphocytes and polymorphonuclear leucocytes and their functions are controlled by **colony stimulating factors, interleukins** and **interferons.** These factors have multiple functions. For example, colony stimulating factors activate mature neutrophils to enhance their function while committed progenitor cells are stimulated to proliferate before differentiating to form more functional haematopoietic cells, replacing those that have completed their tasks (Fig. 1). The requirement for cell replacement is based on the finite life span of functional, differentiated cells in normal renewing cell populations. This is explained by the following model of tissue maintenance.

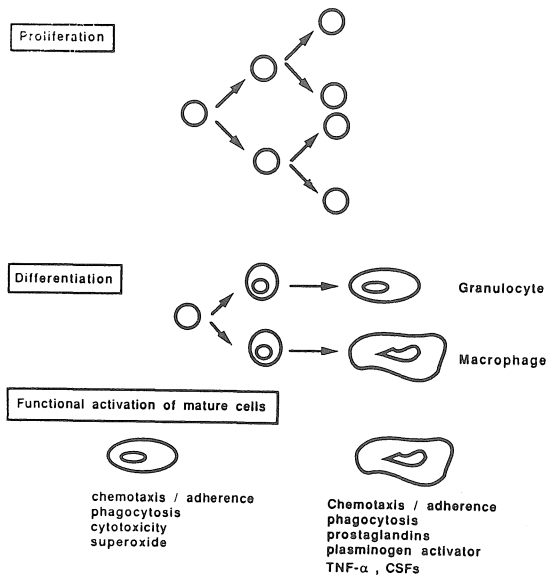

Fig. 1:
Multiple functions of colony stimulating factors (CSFs). CSFs are involved in the functional activation of mature cells. At the same time, progenitor cells are stimulated to proliferate and differentiate to form more functional cells, replacing those that have completed their tasks

Stem cell model of tissue maintenance

In this model, the properties of a cell renewal system is represented as a hierarchy of cells with a spectrum of proliferative potentials (Fig. 2). Stem cells occupy the top of the hierarchy and are distinguished by their capacity of self-renewal to form a minority, subpopulation of cells in any tissue. Alternatively they can generate cells committed to differentiate. Committed cells undergo a number of cell divisions (clonal expansion) on the way to producing functionally differentiated end cells. However, as cells differentiate, they also lose proliferative potential so that terminally differentiated cells are unable to divide.

It will become apparent that PRFs are capable of influencing events at all points along this pathway of differentiation.

Colony stimulating factors

Colony stimulating factors (CSFs) that regulate granulocyte/macrophage proliferation and differentiation are derived from such diverse sources as stromal cells, endothelial cells, fibroblasts, macrophages and lymphocytes in response to stimuli such as endotoxins released by fibroblasts and endothelial cells or foreign antigens coming into contact with T lymphocytes. These cells can produce one or more CSFs.

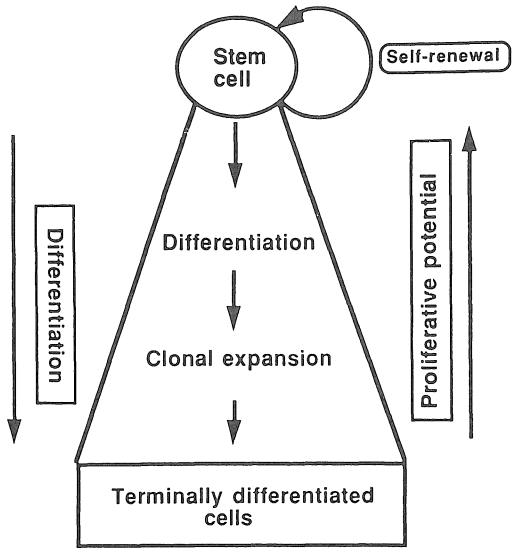

Fig. 2:
Stem cell model of tissue maintenance. Stem cells can renew themselves or generate cells that are committed to differentiate. As cells move down the hierarchy, they become progressively more differentiated but losing their proliferative potential at the same time. The resultant end cell population is fully differentiated but non-dividing.

The four major CSFs responsible for regulating granulocyte/macrophage formation are granulocyte CSF (**G-CSF**), granulocyte-macrophage CSF (**GM-CSF**), macrophage CSF (**M-CSF**) and multi-potential CSF (**Multi-CSF** or **Interleukin 3**). There is little structural homology among the CSFs and each binds to its specific receptor. Some selectivity in the actions of CSFs occur eg G-CSF selectively stimulates granulocytes while M-CSF is specific for macrophages. Some biological activities overlap. For example, GM-CSF and multi-CSF stimulte eosinophils and megakaryocytes as well while mast cell proliferation is also regulated by multi-CSF. Other CSFs involved in the regulation of granulocyte/ macrophage formation are stem cell factor (**SCF**), **IL 1** and **IL 6** which act on cells earlier in the lineage than committed progenitor cells (Fig. 3). Leukemia inhibitory factor (**LIF**) is a potent stimulator of megakaryocytic growth.

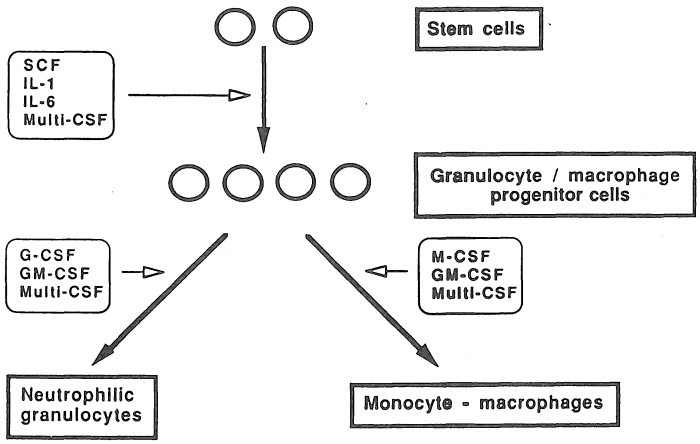

Fig. 3 : The influence of peptide regulatory factors on the differentiation of cells of the granulocyte-macrophage series. SCF, stem cell factor, IL, interleukins; multi-CSF, multipotential CSF; G-CSF, granulocyte CSF; GM-CSF, granulocyte-macrophage CSF; M-CSF, macrophage CSF.

CSFs have multiple biological functions. They enhance the functional activity of mature granulocytes and macrophages resulting in an increase in their phagocytic capacity or, in macrophages, to produce a variety of PRFs eg interferons, tumour necrosis factor alpha (TNF-α), plasminogen activator or other CSFs. They also stimulate the proliferation of committed progenitor cells of the lineage and regulate their differentiation into mature cells (Fig. 1). Individual cells tend to express receptors for more than 1 type of CSF. This allows several CSFs to stimulate proliferation simultaneously, often leading to super-additive responses.

CSF producing cells are widely dispersed throughout the body and in locations likely to make early contact with products of invading micro-organisms or of cells damaged by such organisms. CSF production is highly responsive to the relevant stimuli, activating existing cells within hours. Should infections persist, CSFs can stimulate the formation of additional mature cells and act to sustain this elevated production until the infection is resolved. Following withdrawal of the stimulus, the decline in CSF synthesis is equally rapid. These properties enable CSFs to respond rapidly to meet fluctuating demands.

Interleukins and gamma-interferon

The immune response is mediated by macrophages, T and B lymphocytes (Fig. 4). Antigens are first processed by macrophages before they are displayed on the cell surface in physical association with a Class II histocompatibility (DR) molecule. Contact with antigen also stimulates macrophages to secrete interleukin I. The antigen receptor on the surface of resting T lymphocyes recognise the processed antigens and, on binding the antigens, become activated. Activated T cells synthesise a variety of peptides, some of which are secreted as interleukins and others become integral components of the cell membrane such as receptors for interleukin 2.

Interleukins function to amplify the response to antigen in a non-specific fashion. Interleukin 1, released by macrophages, augments the synthesis of interleukins 4, 5, 6 by activated T cells. A different subset of activated T cells, not sensitive to interleukin 1, synthesise interleukin 2 and gamma-interferon. **Gamma-interferon** enhances DR expression on the macrophage, thereby increasing the T-cell response to the antigen. When IL 2 binds to specific receptors on activated cells, it stimulates the proliferation of the activated clone eg. the clonal expansion of cytotoxic T cells and at the same time, enhances the cytolytic activity of that population of natural killer cells.

Interleukins 4 and 6 provide the critical signals for the growth and maturation of antigen-primed B cells. When an antigen activates a resting B cell by binding to the immunoglobulin on the surface, it is converted into a large proliferating B cell. To progress further, it requires the stimulus of IL 4. Once proliferation has begun, IL 6 drives the cells to become specialised immunoglobulin-secreting plasma cells.

Activated T cells are also a source of GM-CSF and multipotential CSF (IL 3), thus ensuring the adequate supply of functional neutrophils to help combat infection.

Clinical applications

The availability of recombinant forms of CSFs has benefited many patients whose survival would otherwise have been threatened by neutropenia. Recombinant G-CSF and GM-CSF have been used successfully to stimulate marrow regeneration and shorten the period of neutropenia following transplantation and chemotherapy; in AIDS and in myelodysplastic syndromes. Encouraged by these results, their use have been broadened to include the stimulation of leukaemic cell proliferation to enhance cytotoxic sensitivity in acute leukaemia patients undergoing chemotherapy. They are also used to stimulate efflux of marrow stem cells into the circulation to allow their harvest for autologous

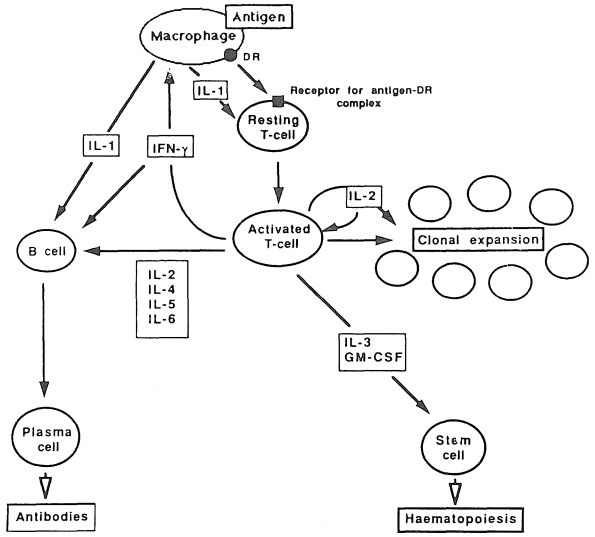

Fig. 4
Schematic representation of the immune response showing the intricate interactive networks mediated by interleukins, gamma-interferon and CSFs which serve to amplify the immune responses triggered by presence of foreign antigen.

marrow transplantation. This shortens the time to recovery compared to conventional bone marrow transplantation. Stem cell factor and leukaemia inhibitory factor will soon be entering clinical trials. **Erythropoietin** has been successfully used in the treatment of the anaemia of chronic renal failure.

Clinical diseases

PRFs are involved in the pathogenesis of many diseases in which an inflammatory response is the predominant tissue abnormality. For example, interleukins, gamma-interferon and TNF-α have been implicated in the acute inflammation and joint destruction of rheumatoid arthritis. TNF-α has also been implicated in many of the metabolic derangements and tissue injury often seen in cachexia and fatal septic shock syndrome. TNF-α is synthesised by activated macrophages, lymphocytes and natural killer cells. Its deleterious effects are largely caused by the induction of mediators such as interleukins, prostaglandins and leukotrienes. Ultimately, it may be possible to devise new treatment for such diseases by manipulating PRFs and their activities *in vivo*.

(B) The Repair Phase

The reparative phase overlaps with the inflammatory response. Following the initial influx of haematogenous inflammatory cells into the wound site, additional cells capable of synthesising extracellular matrix migrate into the injured area. This second wave is composed of mesenchymal stem cells of connective tissues, histiocytes, fibroblasts, endothelial cells, macrophages, platelets and cells that initiate formation of the callus. A key PRF involved in the reparative phase is platelet derived growth factor.

Platelet derived growth factor

Platelets initiate clotting and are involved in the inflammatory response through the release of vasoactive mediators. They also play an important role in the reparative phase through the actions of platelet derived growth factor (PDGF). Although PDGF was first isolated from platelets, it is also synthesised and secreted by many other kinds of cells such as activated vascular endothelial cells, macrophages and fibroblasts. It is principally a mitogen for connective-tissue forming cells, vascular smooth muscle and capillary endothelial cells. PDGF consists of 2 peptide chains denoted A and B. The B chain has a striking homology with the protein product encoded by the v-sis oncogene of Simian

sarcoma virus. This provides a crucial link between a naturally occurring mitogen (PDGF) and a protein capable of inducing tumours *in vitro*.

PDGF receptor and transmembrane signal transduction

How do PRFs instruct cells to carry out the necessary functions? Many peptides do not readily penetrate cell membrances because they are hydrophilic molecules. They impart their information by first binding to the extraordinary portion of a ligand-specific receptor present on cell surface membrane. The activated receptor generates a signal that is transmitted across the membrane to the intracellular portion of the receptor.

There are several classes of receptors. PDRF belongs to a class of receptors whose intracellular domain contains the enzyme tyrosine kinase. Other receptors belonging to this class include the receptors for epidermal growth factor, insulin and insulin-like growth factor. Tyrosine kinase catalyses the reaction that phosphorylates selected tyrosine residues within the molecule. This is the trigger that initiates an array of cellular responses which include stimulation of Na+/H+ exchange, calcium influx, activation of phospholipase C-γand stimulation of C-γleads to the generation of phosphotidylinositol metabolites such as 1,4,5 trisphosphate (IP3) which cause the release of calcium from intracellular components (eg mitochondria), and the generation of diacylglycerol (DG), the natural activator of protein kinase C (PKC). It is assumed that the phosphorylation of cellular substrates, together with alterations in the ionic content of the cell, provide an internal stimulus for cell growth. However, a lot more work needs to be done to define the sequence of intracellular events that link initial transmembrane signalling processes which is completed within seconds to minutes, to the long term (hours to days) patterns of gene regulation that are needed for cell proliferation and differentiation. Other PRFs stimulate the formation of cyclic AMP as a second messenger while many PRFs do not appear to act via any of the transmembrane signalling mechanisms that have so far been identified.

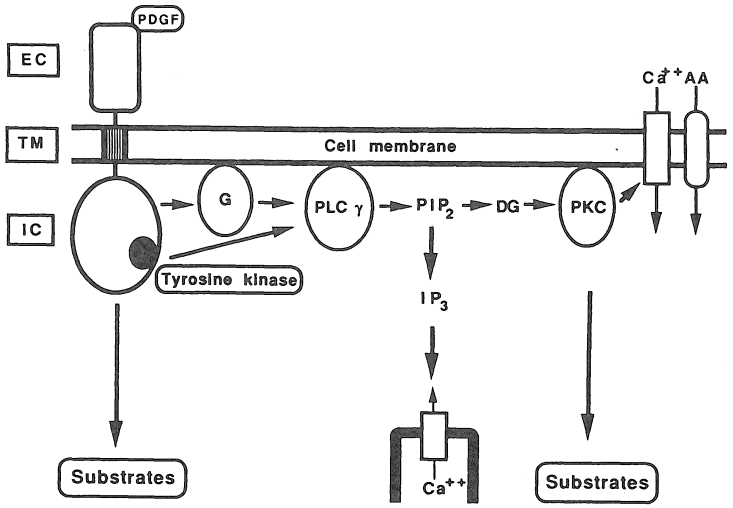

Fig. 5 : Signal transduction occurring as a result of platelet derived growth factor (PDGF) binding to the extracellular (EC) portion of the PDGF receptor. The signal is transmitted across the transmembrane (TM) portion of the receptor to reach the intracellular domain (IC) which contains tyrosine kinase. Stimulation of tyrosine kinase activity results in the phosphorylation of phospholipase C-γ (PLCγ) PIP2 = phosphatidylinositol 4,5-bisphosphonate; DG = diacylglycerol; IP3 = inositol 1,4,5-trisphosphate; Ca++ = calcium ions; AA = amino acids; G = G protein.

(C) The Regenerative Phase

The regenerative phase begins with the laying down of cartilage at the fracture site. The cartilage undergoes calcification to form a callus. This is eventually resorped and replaced with bone by a process identical to endochondral ossification.

Bone formation

Two factors, bone morphogenetic protein (BMP) and transforming growth factor beta (TGF ß) have recently attracted a great deal of interest because of their ability to stimulate endochondral bone formation *in vivo* – raising the distinct possibility that they may be used therapeutically to hasten fracture repair.

Urist pioneered the work showing that bone can be formed *in vivo* by bone derived protein extracts named bone morphogenetic proteins.[2] A family of BMPs (BMP 1 to BMP 7) has now been isolated. The sequence of events leading to bone formation starts with the initial recruitment and proliferation of mesenchymal cells which differentiate into chondroblasts and chondrocytes. Cartilage is formed by the first week. From days 10 to 14, vascularization occurs followed by the appearance of osteoblasts and osteoclasts and then resorption of the calcified cartilage. By day 21, bone formation is complete with a functional bone marrow. Analysis of the protein structure of BMPs revealed that BMP 2 to BMPT belong to the TGF ß superfamily.

TGF ß is the prototype of a family of polypeptides that play important regulatory roles in growth and development. Members of the family include Inhibins, Activins, Mullerian Inhibitory Substance (MIS), the Drosophila decapentaplegic (dpp) gene product and the Xenopus Vg1 gene product. TGF ß is synthesised by many cell types but is highly enriched in bone where it is synthesised and secreted by osteoblasts. TGF ß regulates several processes relevant to bone formation, namely, osteoblast proliferation, differentiation, extracellular matrix synthesis as well as inhibition of matrix degradation. Local injections of TGF ß into the subperiosteal region of femurs results in chondrogenesis which is followed by endochondral ossification when the cartilage is replaced by bone.[3]

How do BMPs and TGF ß work together in order to coordinate cartilage and bone formation? Recent evidence suggests that the coordinated expression of several members of the TGF ß superfamily is required to control the progression of specific cell types through their differentiation pathways.[4] This model proposes that the expression of BMP promotes the condensation and differentiation of mesenchyme into activity proliferating chondroblasts and chondrocytes which results in the expression of a different set of TGF ß-like gene products in the more differentiated cells. The newly induced growth factors may then regulate the proliferation and differentiation of the cells in which they are produced in an autocrine manner, as well as act on additional cell types in the chondrocyte differentiation pathway in a paracrine manner to ensure a coordinated progression through this lineage. It is assumed that a similar sequence of events occur in osteoblast differentiation.

Bone resorption

Multinucleated osteoclasts are formed by the fusion of mononuclear precursors. Current evidence suggets that the osteoclast is derived from a pluripotent haemopoietic stem cell. Osteoclast progenitors proliferate before differentiating into mononuclear pre-osteoclasts. It is likely that osteoclast precursors, monocytes and macrophages share a common origin, diverging later as they differentiate along their respective lineages. The proliferation and differentiation of osteoclast progenitors is dependent upon CSFs and osteoblasts. The most potent is M-CSF, secreted by osteoblastic stromal cells, followed by GM-CSF, IL 3 and G-CSF. M-CSF also plays an important role in the diferentiation of osteoclast progenitors. Mature osteoblasts also play a major role in modulating the function of osteoclasts. Local PRFs such as TNF-α, TNF-ß, IL 1 and systemic factors such as parathyroid hormone and 1,25 (OH)2

vitamin D_3 regulate osteoclastic function indirectly by first binding to specific receptors present on osteoblasts. This results in the release of 'coupling factor(s)' that in turn, modulate osteoclastic activity.

Finally
The fresh marrow spaces excavated by osteoclasts are populated by haematopoietic cells under the influence of stem cell factor, colony-stimulating factors and erythropoietin.

And so the circle is complete.

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