Cytokines: biological function and clinical use

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The last few years have seen the recognition of a multitude of factors involved in cell growth. Molecular techniques have made possible the purification, characterisation and eventual production by recombinant technology of many of these substances. Although the list is growing rapidly, this reflects a clarification rather than an obfuscation of the field.

Cytokines are soluble factors which can modify the behaviour and/or growth pattern of other cells [1]. They resemble hormones in many of their mechanisms of action, but they are typically released and active at a purely microenvironmental level and are rarely detectable in the systemic circulation. The term cytokine is to be preferred to lymphokine or monokine because these terms imply a single lineage source of production, whereas some factors are produced by more than one cell type. Certain cytokines have overlapping effects yet lack homology at a molecular level. Rather than causing redundancy of effect, these factors may be synergistic and thus allow for greater sensitivity of cellular control.

This paper describes four groups of cytokines (Table 1): The *interferons* have a wide variety of biological effects in addition to their antiviral properties, the most profound being an antiproliferative action on many cells. *Colony stimulating factors* promote growth of bone marrow progenitors belonging to a particular lineage. The term *'interleukin'* suggests 'leukocyte interaction' but these molecules are a heterogeneous group in that some are activators of differentiated cell function whereas others have more profound proliferative effects. *Tumour necrosis factor* thought to be mainly involved in tumour lysis has, however, much broader activity.

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Mechanisms of action

Most cytokines must bind to a specific receptor on their target cell to produce an intracellular signal ('second message'), thus effecting a cellular response [2]. It is the ligand-receptor interaction that usually produces the signal, not internalisation of the complex. The affinity of this complex is usually so strong that it requires internalisation via a coated pit system to remove, process and degrade the ligand. The receptor is then returned to the cell surface to be 're-expressed'. This turnover occurs in a matter of minutes. As little as 10 per cent of receptors may be occupied to produce full stimulation of the cell.

Table 1. Cytokine biology

Cytokine	Synonym	Molecular mass	Main cellular source
Interferons (IFN)			
α-IFN	Leukocyte-IFN	20 kd	Monocyte/macrophage
β-IFN	Fibroblast-IFN	26 kd	Fibroblast
γ-IFN	Immune-IFN	17 kd	T lymphocyte
Colony stimulating factors (CSF)			
Granulocyte-macrophage-CSF (GM-CSF)	Pluripoietin, CSF-α	14–35 kd	T lymphocyte
Granulocyte-CSF (G-CSF)	Pluripoietin, CSF-β	19.6 kd	Fibroblast/endothelium
Macrophage-CSF (M-CSF)	CSF-1	70 kd (dimer)	Fibroblast/endothelium
Erythropoietin (Epo)	—	34–39 kd	Renal tissue
Interleukins (IL)			
IL1	Haemopoietin-1	22 kd	Macrophages/fibroblasts/T lymphocytes
IL2	T-cell growth factor	17 kd	T lymphocytes
IL3	Haemopoietin-2, Multi-CSF	20–26 kd	T lymphocytes
IL4	B-cell stimulating factor-1	20 kd	T lymphocytes
IL5	Eosinophil differentiation factor	32–62 kd	T lymphocytes
IL6	B-cell stimulating factor-2	26 kd	T lymphocytes/fibroblasts
Tumour necrosis factor (TNF)			
TNF-α	—	17 kd	Macrophage/monocytes
TNF-β	Lymphotoxin	17 kd	T lymphocytes

A number of second messengers has been described, eg cyclic AMP and protein kinase C [3]. Several factors can activate the same messenger, for example, γ-IFN and IL2 both activate protein kinase C. It has been suggested that each cytokine has a cascade system which involves several messengers and results in giving its own individual signal to the cell. Further, this system may modulate the expression of receptors to other substances, thus altering the response of the cell to these factors.

The end result of the above process is the modulation of gene expression. This may occur at the transcription of mRNA from DNA and/or translation of mRNA into protein. The signal may be stimulatory (α-IFN stimulates 2′5′-oligoadenylate synthetase production) or inhibitory (α-IFN represses c-myc proto-oncogene expression).

Biological properties

The interferons

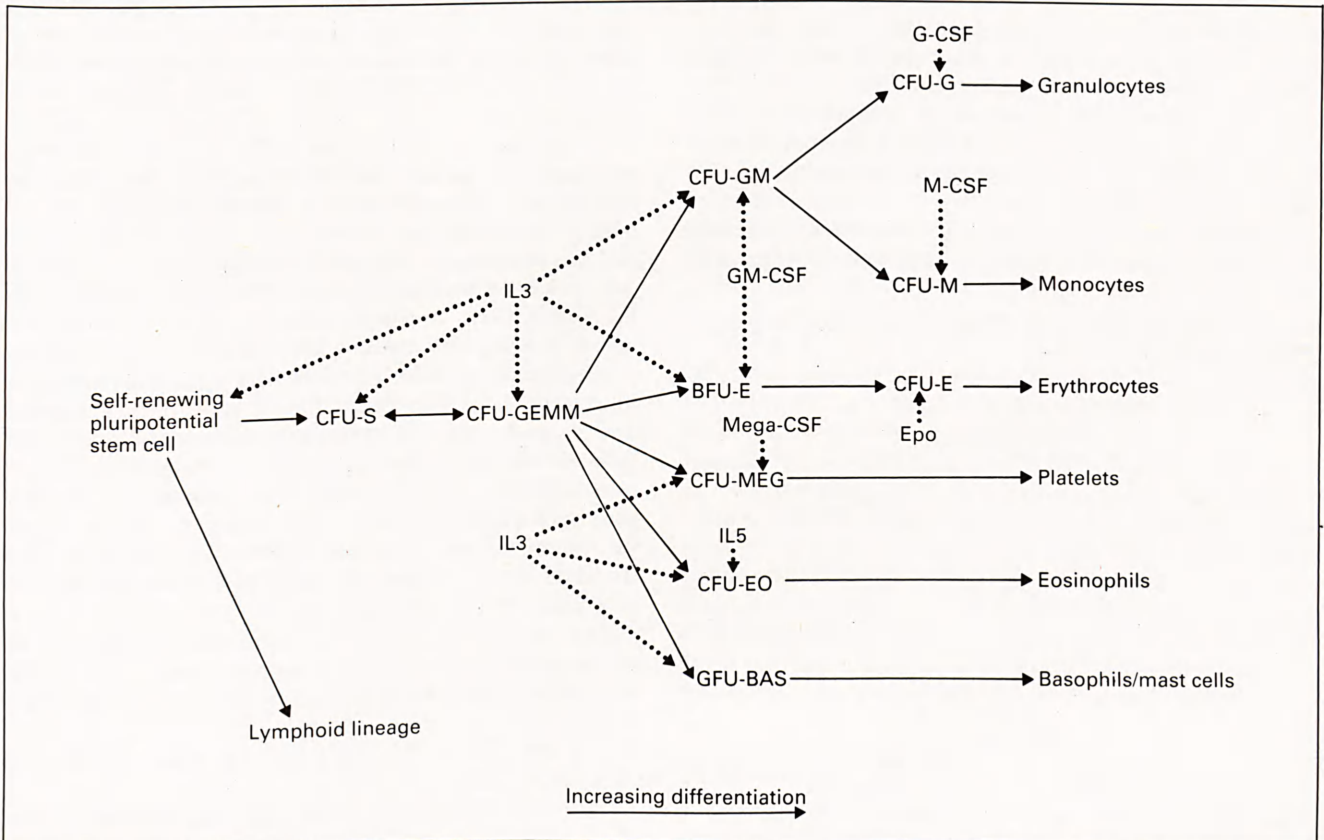
Three types of IFN are recognised: α, β and γ. α-IFN has over 30 subtypes but β-IFN and γ-IFN are unique. All have antiviral activity by definition.

Despite only having 29 per cent homology, α- and β-IFN share the same cellular receptor [4] and are activators of intracellular 2′5′-oligoadenylate synthetase [5]. This enzyme promotes the breakdown of mRNA, hence its potent antiviral effect. The enzyme activity also correlates with advancing cell differentiation [6], but it is not clear how relevant this is to α- and β-IFN's powerful antiproliferative effect. For example, Rosenblum and coworkers demonstrated correlation between the response of chronic granulocytic leukaemia to α-IFN therapy and induction of 2′5′-oligoadenylate synthetase, while non-responders showed no enzyme induction [7].

Both α- and β-IFN are potent stimulators of natural killer (NK) cell and cytotoxic T cell activity [8]. It is unlikely, however, that these actions mediate inhibition of haemopoiesis or their antineoplastic action [9]. It is more likely that α- and β-IFN work by a direct cytotoxic/cytostatic mechanism like other anticancer drugs, non-neoplastic cells being less susceptible to their effects.

γ-IFN has its own specific receptor, bears little resemblance to either α- or β-IFN and does not stimulate 2′5′-oligoadenylate synthetase [10]. γ-IFN is antiproliferative (eg inhibits myelopoiesis) and can promote differentiation

Fig. 1. *The influence of cytokines on haemopoietic development. The direction of normal haemopoietic growth and differentiation is shown in unbroken arrows; the influence of factors in broken arrows.*
Key: *CFU = colony forming unit (or cell); CFU-S = spleen CFU; CFU-GEMM = mixed CFU having potential to develop into granulocyte, erythroid, monocyte or megakaryocyte lineage; BFU-E = burst forming unit for erythroid lineage; CFU-E = erythroid CFU; CFU-GM = granulocyte/monocyte CFU; CFU-G = granulocyte-CFU; CFU-M = monocyte CFU; CFU-Meg = megakaryocyte CFU; CFU-Eo = eosinophil CFU; CFU-BAS = basophil CFU.*



of primitive cell lines (eg HL60), but is a more potent immune modulator than the other IFNs [11]. In particular, γ-IFN stimulates HLA class II expression on monocytes and other cells, which is important for antigen recognition and may be for autoimmunity too. This, coupled with the promotion of macrophage/monocyte killing, has led to γ-IFN's synonym: 'macrophage activating factor' [12].

The colony stimulating factors

Through work on murine marrow, Metcalf established that several factors are involved in stimulation and control of haemopoiesis [13]. Over the last year, the human equivalents of these factors have been purified and cloned. The factors are named according to their main target cell (Fig. 1), but this is inadequate as there is some cross-stimulation between lineages, eg GM-CSF can stimulate BFU-E growth in the presence of erythropoietin. Each CSF bears little homology with the other CSFs, growth factors or oncogene products.

There are two groups of CSFs. Class I factors (GM-CSF and IL3) act on more primitive progenitor cells, being important in self-renewal and proliferation but less lineage-specific. Class II factors (G-CSF, M-CSF, Epo) act on more mature progenitors and influence the survival and function of mature cells [14].

GM-CSF. This acts mainly on the CFU-GM progenitor to produce CFU-G and CFU-M progenitors. In addition to stimulating neutrophil and monocyte production, GM-CSF can activate these mature end cells.

G-CSF. This factor promotes neutrophil production by CFU-G progenitors, but also activates mature neutrophils. G-CSF is unusual in that, unlike other human CSFs, it cross-reacts with mouse G-CSF.

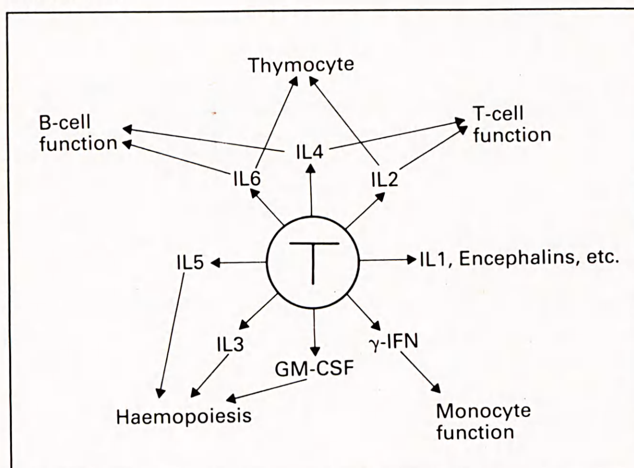
M-CSF. This CSF stimulates monocyte production from CFU-M progenitors but is rather less potent than the other CSFs and may have an absolute requirement for IL1 to achieve optimal effect.

Erythropoietin. Epo is produced by the kidney and was originally purified from the urine of patients with aplastic anaemia. Its main action is the proliferation of CFU-E progenitors to yield normoblasts and erythrocytes.

CSF receptors. Their role may illuminate the leukaemic process itself.

Receptors for the various CSFs are found mainly on cells of their specific lineages. A hierarchical downregulation of receptors has been observed so that IL3 stimulates its own receptor and the other CSF receptors to downregulate; however, GM-CSF downregulates its own receptor plus M-CSF and G-CSF receptors but not the IL3 receptor. If this downregulation results in the stimulation of all these receptors, then it may be mediating a controlling effect on differentiation—proliferation and differentiation in perfect balance [15]. If this equilibrium becomes disturbed then the same hypothesis provides a model for leukaemogenesis.

Fig. 2. *The central role of the T helper lymphocyte in cytokine modulation.*



The interleukins

The helper subset of T lymphocytes exerts an important regulatory influence on many systems (Fig. 2) through its production of interleukins.

IL1. IL1 has the broadest spectrum of activity of all the interleukins. This can be divided into two groups of cellular activity:

(a) *The promotion of differentiated cell functions.* First, IL1 is a potent mediator of inflammation causing fever, cartilage breakdown, acute phase protein release and activation of neutrophils and macrophages [16]. It is also a potent T lymphocyte stimulant.

(b) *The stimulation of cell proliferation.* Although IL1 has a very weak action on haemopoietic progenitor cells, it can prime them for the subsequent proliferative stimulation by other factors [17]. It is thus a powerful synergist with the CSFs.

Two forms of IL1 exist, α- and β-subtypes. Although they have only 23 per cent homology, they share the same cellular receptor and have very similar activities.

IL2. Originally recognised as a T-cell growth factor, IL2 has far broader influence. It is secreted by T-helper cells in response to antigen presentation in the context of HLA class II by 'antigen presenting cells'. Cells exhibiting IL2 receptors become activated: T cells (helper, suppressor or cytotoxic) enter the cell cycle and transform into large blast-like cells; B cell growth is enhanced directly and indirectly by B cell growth factors; NK cells broaden their repertoire of targets by becoming 'lymphokine activated killer' (LAK) cells; monocyte cytocidal activity is stimulated [18]. It has been shown recently that primitive myeloid cells can express IL2 receptors, and that IL2 modulates myelopoiesis indirectly (via T cells) and possibly also directly [19].

IL3. Multi-CSF, or IL3, promotes the proliferation of the most primitive haemopoietic progenitors resulting in erythroid, granulocyte, monocyte, eosinophil and megakaryocyte progenitor production [20]. It has little effect on mature cell function, and does not act synergistically with other CSFs.

IL4. All stages of B-cell development are affected by IL4-activation, from proliferation to regulation of differ-

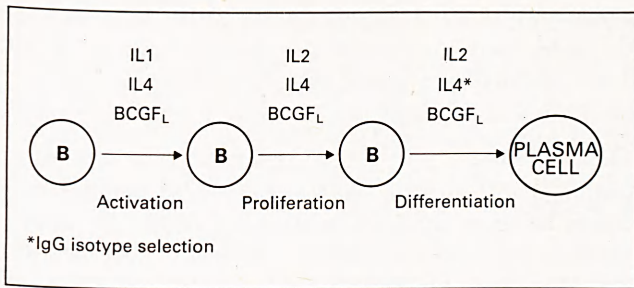


Fig. 3. *The effects of cytokines on B lymphocyte development.* BCGF$_L$ *is a B-cell growth factor of low molecular weight, a molecule distinct from IL4 and IL6.*

entiation [21] (Fig. 3). IL4 is involved in the mediation of the allergic response. It is also said to have an influence by enhancing IgE production and IgE receptor expression. Influence on T cell and myeloid growth is also reported.

IL5. In humans, the influence of IL5 is entirely restricted to the eosinophil lineage: it promotes proliferation of CFU-Eo and differentiation of the resulting eosinophils [22]. It is also a potent stimulator of eosinophil degranulation.

IL6. The most profound effect is upon terminal differentiation of B lymphocytes and consequent antibody production (Fig. 3), hence its former name B-cell differentiation factor [23]. Some confusion has arisen in the nomenclature because some workers described the molecule as IFNβ_2 but it has no antiviral activity and is therefore not an IFN.

IL6 has been implicated in the production of autoantibodies by some unusual tumours such as atrial myxoma.

Tumour necrosis factor

This molecule was originally identified as a macrophage product by injecting mice with endotoxin. It is now termed TNFα [24]. A similar molecule produced by lymphocytes has been recognised—lymphotoxin or TNFβ. Both TNFα and β share the same receptor and function.

The classical effect of TNF on tumour tissue is haemorrhagic necrosis and increased vessel wall permeability. However, some cells may require TNF as a growth factor, eg hairy cells. Extensive *in vitro* analysis has revealed potent effects on inhibition of erythroid and myeloid proliferation, activation of neutrophils and intrinsic antiviral activity. The cachexia of malignancy led to TNF being titled 'cachectin'. Its role in this process is not clear, although TNF levels are higher in patients with progressive than with non-progressive cancer. TNF increases energy production in muscle by promoting 'futile cycles', ie energy that cannot be converted into muscle contraction and so results in loss of muscle bulk [25].

Clinical developments

Cantell pioneered the production of α-IFN from buffy coats, but the early preparations were only 1 per cent pure α-IFN. The next step forward involved stimulating human cell lines with Sendai virus to produce a cocktail of α-IFNs. This was then highly purified by chromatography; the product was called Wellferon. Finally, recombinant technology has made possible the production of all the cytokines mentioned so far. Briefly, mRNA coding for a cytokine is taken and DNA is copied from it (cDNA). This cDNA is then incorporated into a plasmid which is inserted into a bacterium such as *E. coli*. The bacteria multiply rapidly and those cultures producing maximum yield of the cytokine are selected and grown on [26]. The cytokine is highly purified by affinity chromatography.

The interferons

The clinical usefulness of α-IFN has been summed up: 'good in hairy-cell leukaemia, fair in other haematological cancers, poor in solid tumours' [27]. Haematological malignancy has indeed given the best results: 90 per cent of patients with hairy-cell leukaemia (HCL) respond to subcutaneous α-IFN (though only about one quarter actually achieve clearing of the marrow); 80 per cent of patients with chronic granulocytic leukaemia (CGL) respond (10–20 per cent show a fall or disappearance of Philadelphia positive metaphases in the marrow); 60 per cent of patients with low grade non-Hodgkins lymphoma respond. These are the areas where α-IFN may be considered as single therapy. α-IFN will probably best be used as an adjuvant in myeloma, Kaposi's sarcoma and endocrine pancreatic tumours. Other solid tumours, eg melanoma and renal cell carcinoma often show a partial response rate of about 30 per cent with an occasional dramatic cure [28].

The best therapeutic results in terms of antiviral effects have been confined to hepatitis B and non A non B hepatitis, condylomata acuminata (local therapy) and juvenile laryngeal papillomatosis. All these conditions show dramatic responses to α-IFN therapy but nearly always relapse on termination of treatment [28]. α-IFN does not alter the course of AIDS. Although intranasal α-IFN may protect against rhinovirus infection, it is probably best reserved for the immuno compromised host [29].

The early results of γ-IFN trials have suggested possible improvement in lymphoproliferative disorders but little benefit in HCL or solid tumours. γ-IFN may approach α-IFN in its effect upon CGL but is not superior; trials using α- and γ-IFN synergistically will prove interesting [31].

Most diseases show little dose-response above 3 mega units per day for α-IFN and this has become the standard dosage. β-IFN seems to offer no clinical advantage over α-IFN. The side effects of flu-like symptoms improve within days and serious idiosyncratic sequelae are rare [32]. However, because γ-IFN produces more potent and persistent symptoms, lower doses of γ-IFN are being used.

The colony stimulating factors

These molecules seem rather less toxic than the other cytokines when given as therapy.

Improvement in neutropenia and lymphopenia has been observed in AIDS patients infused with recombinant GM-CSF. Whether this will improve morbidity and mortality is under investigation [33].

Marrow suppression following cytotoxic therapy and conditioning for bone marrow transplantation leads to infective morbidity.

Administration of GM-CSF or G-CSF stimulates recovery of myelopoiesis and is reported to shorten the period of dangerous neutropenia in both situations [34]. In about 30 per cent of acute myeloid leukaemias GM-CSF is secreted in an autocrine manner and this therapy would therefore not be suitable for them when they undergo marrow transplantation.

Erythropoietin has improved the anaemia and bleeding disorders or uraemia with consequent reduction in risk of transfusion associated infection and iron overload [35]. This therapy may well improve the quality of life of uraemic patients but, interestingly, erythropoietin has so far found no place in the haematology clinic.

The interleukins

IL2 is the only interleukin used therapeutically. Rosenberg infused IL2 into patients with metastatic cancer and showed improvement in tumour load. When this is combined with an infusion of autologous LAK cells (created *in vitro* by exposure of NK cells to IL2) the effect is amplified. Of 106 patients treated with IL2 + LAKs, 33 responded (8 completely). This treatment is hazardous and often results in patients needing 'intensive care facilities' (four treatment related deaths were described) [36]. Much debate has ensued about quality of life and clinical audit, but it seems that this therapy will only become more widely accepted if potential responders can be identified easily.

A less contentious indication for IL2 may be its use as an adjuvant to vaccination. The response of pigs to Haemophilus pleuropneumoniae vaccination was greatly enhanced by giving systemic IL2 at the same time as the inoculum [37]. Similar results were obtained when vaccinating mice with rabies. (Toxicity was less of a problem in these studies because a lower dosage of IL2 was used.) The implications of this early work are wide ranging for man, and for animal husbandry.

Following allogeneic bone marrow transplantation, the graft versus leukaemia effect may be mediated by NK/LAK cells. IL2 stimulation enhances this effect *in vitro* [38]. Clinical work will therefore hope to define a role for IL2 in the post-transplantation period.

Tumour necrosis factor

Despite its name, TNF has not had much therapeutic success in clinical oncology [39]. Fears of severe reactions to TNF therapy have not really been borne out, although fever and fatigue do occur. *In vitro* work suggests that combining TNF with other therapy, such as γ-IFN, may yield a better antineoplastic effect. TNF may mediate the cerebral consequences of Plasmodium falciparum (in a mouse model). This raises the possibility of treating it with monoclonal antibodies against TNF. Such therapy has been used to prevent the development of 'septic shock' in baboons injected with endotoxin [40].

Conclusion

Optimal tissue survival relies on cell growth and differentiation being in equilibrium with cell death. By understanding the cytokine mediated control of this balance, one can begin to unravel the mysteries of abnormal growth. The next step is intelligent manipulation of cytokines in therapy. This will probably be most successful when more than one agent is used, maybe as an adjunct to chemotherapy. At last immunotherapy has gained a place in the fight against human cancer.

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