CURRENT TOPIC

The cytokines are coming

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On 31 March 1980 the front cover of *Time* magazine was devoted to 'Interferon—The IF drug for cancer'. Whatever happened to interferon, which seemed to promise so much, and why did it not live up to early expectations? The answer may lie with Dr K Cantell, a pioneer worker with interferon, when he stated that: 'Much second class research was carried out with third class preparations slightly contaminated with interferon'.¹ Such studies were necessarily performed with the tiny amounts of interferon obtained from vast pools of donated blood. The revolution in genetic engineering over the past decade has enabled large quantities of pure interferon and other cytokines to be produced. So 10 years after a false start we are ready to try again: the cytokines are coming.

What are they?

From the evidence of in vitro studies alone, these extremely potent polypeptides seemed to fall into two broad functional groups. Those with a primary regulatory action on mature cells were called cytokines, with the additional distinction that lymphokines were made by lymphocytes (for example, interleukin 2, IL-2) and monokines by monocytes (for example, interleukin 1, IL-1). The others, characterised by their ability to support the proliferation and differentiation of immature precursor cells were labelled growth factors (for example, fibroblast growth factor α) or, more particularly in the haemopoietic system, colony stimulating factors (for example, granulocyte macrophage colony stimulating factor). This distinction has blurred as cytokines have been found to have growth factor activity and vice versa (for example, interleukin 3 was found to be the same molecule as multi-colony stimulating factor). While the terminology is in a state of flux, 'cytokine' remains arguably the most appropriate generic term.

More than 30 peptides are currently recognised as cytokines, having been purified, sequenced, and the coding genes cloned. Together they form a network divided into five broad groups:

- Interferons (IFN- α , β and γ)
Originally recognised by their ability to protect cells from viral infection.
- Interleukins (IL-1 to 9)
Diverse immunoregulatory factors named in historical sequence.

- Haemopoietic colony stimulating factors (erythropoietin, granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), and macrophage colony stimulating factor (M-CSF)
Structurally dissimilar, but functionally related in controlling haemopoiesis.
- Tumour necrosis factors (TNF- α and β)
Structurally related proteins with cytotoxic activity in vitro.
- Assorted growth factors (for example, platelet derived growth factor)
Named according to the cell system in which their activity was discovered.

As our understanding increases, the composition of these basic groups will undoubtedly change. The haemopoietic colony stimulating factors constitute a functionally related group, which is reflected in their orderly nomenclature. Though new members of the group may appear, the names of the existing factors are unlikely to alter. In contrast, interleukins are a heterogeneous group, simplistically numbered as they were discovered, with the consequence that future changes of name are inevitable (for example, IL-6 is the same protein as interferon IFN- β_2).

What do they do?

Cytokines control both specific and non-specific aspects of normal growth, inflammatory repairs, and immune responses throughout the body. The necessary level of integrated control is achieved through a network of cytokines and receptors that can interact rapidly within a locally contained environment.

Each cytokine acts through its own specific receptor situated on the surface of the target cell. A receptor that has been bound by cytokine transduces a signal into the interior of the cell. After further cytoplasmic interactions this signal results in altered gene transcription, protein synthesis, and changes in the metabolic state of the cell. The level of high affinity receptor expression does not remain constant on any given cell, varying with the state of cellular activation. Depending on this state, binding of a cytokine may increase or decrease the subsequent expression of its receptor on the target cell, leading to up or down regulation, respectively, of responsiveness to further cytokine exposure. Additionally, cytokines are able to cross regulate the expression and affinity of each

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other's receptors and thereby ensure a well coordinated response.

Whereas endocrine hormones have their sites of action away from where they are secreted, cytokines tend to act locally as either paracrine (that is, within a small cluster of cells) or autocrine (that is, autoregulating the cell producing the cytokine) factors. The reason for this lies in the requirement for networks like the immune system to produce separate responses to numerous local challenges at any given moment, only resorting to total commitment of resources in the face of a potentially overwhelming attack. To some extent this organisational structure underlies many of the problems encountered with systemic cytokine treatment. Attempting to treat malignancy by flooding a patient's systemic circulation with an immunoregulatory cytokine is rather like defibrillating a malfunctioning computer. One is mechanistically none the wiser—even when successful. On the other hand, some cytokines also seem to have a more generalised action. For example, evidence is accumulating that in addition to its local action in inducing B lymphocyte differentiation, IL-6 may be an important systemic inducer of acute phase proteins.²

Given their pivotal role in the regulation of cell growth, it is not surprising that cytokines and their receptors have oncogenic potential. The viral oncogene carried by the simian sarcoma virus (*v-sis*) induces malignant transformation of fibroblasts in culture. Sequencing shows that the *v-sis* oncogene incorporates the entire code for the β chain of platelet derived growth factor.³ A cell infected with this virus therefore becomes committed to producing unchecked amounts of a potent growth factor. On the other side of the cytokine/receptor interaction, the feline sarcoma virus carries the *v-fms* oncogene, which codes for an aberrant form of the receptor for M-CSF. After infection by the virus the cell manufactures the abnormal receptor, which is then inserted into the plasma membrane. Here it acts as a permanently activated receptor, falsely informing the cell that it is constantly exposed to M-CSF. The end results are unregulated proliferation and in vitro malignant transformation.⁴

Current clinical applications

Trials of cytokines have proliferated with the increased availability of industrially manufactured 'recombinant' proteins. On the basis of their currently understood in vitro actions, cytokines are being used as supportive, cytotoxic, or immunomodulatory factors.

SUPPORTIVE FACTORS

The haemopoietic colony stimulating factors lend themselves to clinical use for several reasons. As a consequence of the availability of good in vitro models, their role in regulating haemopoiesis is comparatively well understood. Their actions are fairly specific and they are relatively non-toxic in vivo. Lastly, their efficacy is reasonably easy to monitor.

Trials of GM-CSF in the treatment of aplastic anaemia have produced some promising early results. Initial improvements in numbers of circulating monocytes and granulocytes have been reported in moderately severe cases, but so far responses have not been sustainable.⁵ This is not altogether surprising as the primary action of GM-CSF is in the later stages of haemopoiesis, whereas the defect in aplastic anaemia is usually at the stem cell level. Perhaps cytokines that do influence pluripotent stem cells will produce better responses in future trials.

Both GM-CSF and G-CSF have been used in the treatment of primary neutropenias with varying success. A recent German trial has shown that while both are clinically beneficial in children with severe congenital neutropenia, G-CSF has more potential for producing sustained neutrophil responses with associated improvements in defence against bacterial infections.⁶

Neutropenia remains the single most important limiting factor in many cancer chemotherapy and transplant regimens. Consequently, the ability of haemopoietic colony stimulating factors to accelerate bone marrow reconstitution is under intensive investigation and plans for the first United Kingdom Children's Cancer Study Group trial of GM-CSF are well advanced. If myelotoxicity can be adequately countered with colony stimulating factors patients will be less vulnerable to opportunistic bacterial infections and may be able to tolerate more intensive chemotherapy. As the results from many centres accumulate, a clear international consensus is emerging that both G-CSF and GM-CSF are beneficial in treating neutropenic adults and are likely to be incorporated into routine chemotherapy regimens.⁵ The potential role of colony stimulating factors in the treatment of thrombocytopenia remains to be explored.

Supportive cytokine treatment is of potential benefit in other groups of compromised patients. Giving GM-CSF to leukopenic AIDS patients has been shown to produce dose dependent increases in circulating white cells.⁷ In paediatric practice treatment with erythropoietin is under investigation as an alternative to blood transfusion in anaemic premature babies⁸ and children with renal failure.⁹ Consideration is also being given to the use of cytokines to enhance neutrophil function during episodes of neonatal infection.¹⁰

CYTOTOXIC FACTORS

IFN- α , IFN- β , and TNF- α each have a direct antiproliferative influence on tumour cell growth in vitro. Clinical trials of IFN- α have shown antitumour activity in patients with chronic leukaemia, lymphoma, carcinoid, glioma, Kaposi's sarcoma, melanoma, and a variety of carcinomas. Response rates vary from 90% in hairy cell leukaemia to 11% in melanoma.¹¹

In contrast, results obtained using TNF- α as a single agent have been disappointing. Its potential for toxicity, as one of the physiological mediators of septic shock, has proved a serious

limiting factor in clinical trials. Tumour regression has been shown in a minority of patients and, consequently, TNF- α may still have a role when used in combination with other cytokines.¹² It remains to be seen whether these anticancer effects are the result of direct cytotoxicity or of as yet unrecognised *in vivo* immunomodulation.

IMMUNOMODULATORY FACTORS

Two cytokines, IL-2 and IFN- γ act through their ability to augment the host's normal immune responses.

During incubation with IL-2, lymphocytes develop greatly increased cytotoxic potential—so called lymphokine activated killing (LAK)—and will kill virtually any cancer cells that they make surface contact with in laboratory assay systems. Initial clinical trials, in which patient's lymphocytes were harvested, activated *in vitro* with IL-2 and then reinfused, were carried out at the United States National Cancer Institute. Promising response rates were obtained in 38% of patients with a variety of metastatic tumours that were resistant to conventional treatment.¹³ Unfortunately the high dose regimens used were severely toxic and many patients required admission to the intensive care unit. The biological responses produced in these trials were so impressive (featured on the cover of *Time* magazine in the mid 80s) that numerous groups throughout the world have designed clinical trials that attempted to dissect out the beneficial from the toxic effects of IL-2/LAK treatment. Interestingly, recent reports have suggested that *in vivo* reinfused LAK cells do not actually go near to the tumours that they destroy.¹⁴ Thus the initial laboratory phenomenon of LAK activity, which requires lymphocyte—tumour cell contact, is probably unrelated to the anti-tumour effects of LAK treatment.

IFN- γ has widespread effects throughout the immune system, including activation of macrophages, T cells, B cells, and natural killer cells and induction of class II major histocompatibility complex molecules.¹⁵ As well as its potential for antitumour immunomodulation, it is an important factor in host defences against microbial attack. Together with the other interferons there are encouraging preliminary reports in various infectious diseases including IFN- α in chronic hepatitis B infection¹⁶ and IFN- γ in leprosy.¹⁷

Cytokine biology is proving to be of interest to other disciplines in which immunoregulation may be therapeutically beneficial. In particular, as a result of the increased appreciation of the inter-relationship of autoimmunity and the cytokine network, there is burgeoning interest among rheumatologists.¹⁸

The future

Numerous candidate proteins are queuing for official admission to the cytokine network. As molecular biological techniques continue to advance the speed with which new factors can be cloned will increase. Each cytokine comes with an individual receptor and, in addition, many seem to have an almost structurally identical but antagonistic 'anti-cytokine'.¹⁹ Consequently, the number of accepted cytokines and associated molecules will grow rapidly during this decade.

As cytokines emerge from the laboratory and move into the clinic, false starts, disappointments, and unexpected problems are to be anticipated. To minimise these set backs it is essential that we direct our efforts to understanding how cytokines function under normal and pathological conditions *in vivo*. As our fundamental understanding of the cytokine network increases, so does the potential to modify it with recombinant cytokines or with drugs—as we have almost certainly been doing for years without realising it.

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