VIEWS FOR FUTURE DEVELOPMENT CYTOKINES IN HEALTH AND DISEASE: IMPLICATIONS FOR CLINICAL MEDICINE

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SUMMARY: The cytokines e.g. IFN-α, IL-2, IFN-γ, G-CSF, GM-CSF and TNF have made tremendous impact in Clinical Medicine (Internal Medicine as well as Laboratory Medicine) either as the potential therapeutic agents or because they can be usefully quantitated in various clinical situations. The therapeutic impacts of cytokines have been felt mainly in the areas of cancer, infectious diseases, blood disorders, rheumatic and autoimmune diseases. However, the occurrence of potentially dangerous complications demands that highly trained and well informed physicians and nursing staffs should be available in centres where cytokines are to be introduced either as therapy or as clinical trial. The medical research and therapy with cytokines have enormous implications in Laboratory Medicine in terms of trained manpower, equipments and costs for commercially available assay kits including efficiency and accuracy of the assay results. The cytokine assay should therefore be based in laboratories where professionally trained immunologists and technical staffs are available, and where the results can be put ethically to further usage as research data, and where improvements in the efficacy and accuracy of the assay results are under routine surveillance. To bring these fruits of advance medical research in 'cytokines' to the developing countries, the authorities concerned should make provisions now for professionally trained manpower (physician and nursing staff, clinical immunologists and medical technologists) and technology (assay kits and equipments) in their national health service planning. This will facilitate the introduction of cytokines in clinical medicine in the third world countries in the very near future.

Key Words: Cytokine, Lymphokine, Growth factor, Interferon, Chemokine, Clinical medicine.

A. WHAT ARE CYTOKINES?

The term 'cytokine' is coined for a group of protein cell regulators, variously called lymphokines, monokines, interleukins and interferons. They are produced by a wide variety of cells in the body, play an important role in the pathophysiology of range of diseases and have therapeutic potential. This heterogenous group of proteins have a number of characteristics in common: (i) cytokines are low molecular weight (<80 KDa) secreted proteins which are often glycosylated; (ii) they are usually produced tran-

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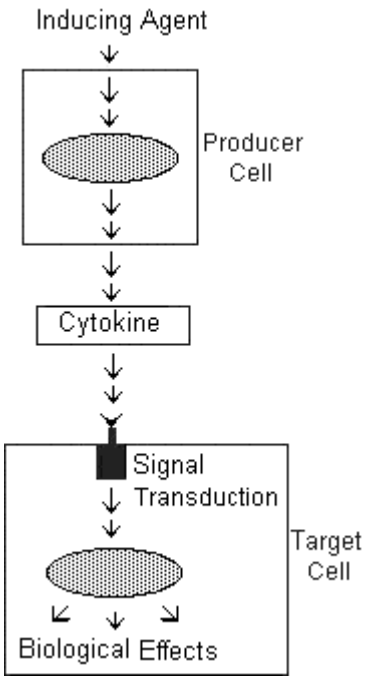
siently and locally, acting in a paracrine or autocrine, rather than endocrine, manner, (iii) they are involved in immunity and inflammation where they regulate the amplitude and duration of response; (iv) they are extremely potent, generally acting at picomolar concentrations; (v) they interact with high affinity cell surface receptors specific for each cytokine or cytokine group; (vi) their cell surface binding ultimately leads to a change in the pattern of cellular RNA and protein synthesis, and to altered cell behaviour; (vii) individual cytokines have multiple overlapping cell regulatory actions; (viii) cytokines interact in a network or a series of cascades by: first, inducing each other; second, transmodulating cytokine cell surface receptors and third, by synergistic, additive or antagonistic, interactions on cell function. Thus, the response of a cell to a given cytokine is dependent on the local concentration of the cytokine, the cell type and other cell regulators to which it is concomitantly exposed (1-3). The fundamental elements of the mode of action of cytokines are shown in Figure 1 and the three types of cytokine-cell interaction are summarized in Figure 2A-B-C. Identification and characterization of the many cytokines now known has resulted in a variety of names and classifications. The major groups of cytokines with examples according to the current nomenclature are stated in Tables-1A and 1B (2-12). However, there is considerable overlap in the biological effects and mechanisms of cytokines currently placed in different categories. It is perhaps important to mention that the name given to a cytokine is often not a very useful guide to its biological functions, e.g. tumor necrosis factor is of interest more for its role in inflammation rather than tumor killing.

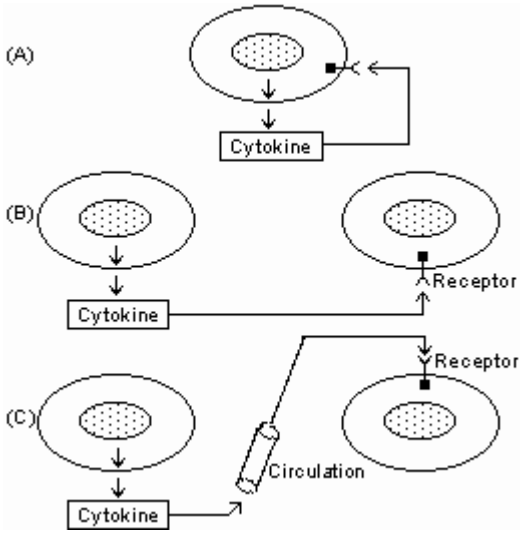
Figure 1: Fundamental elements of the mode of action of cytokines usually secreted as a results of new synthesis by the producer cell in response to inducing stimuli. In most cases signals are sent to the nucleus of the target cells causing changes in gene expression.



B. WHY SO MANY CYTOKINES WITH SO MANY (PLEOTROPIC) ACTIVITIES?

In an alligant review article, Balkwill and Burke tried to explain the interactions of cytokines among various cell types, although it is difficult to understand the complexity of the cytokine network from our current knowledge (1). In an attempt to answer the questions posed, the following reasons may be proposed: (i) the complexity of concurrent messages that any one cell can receive may result in a multiplicity of checks and balances that limit the duration and extent of a cellular response; (ii) another explanation for the complexity of cytokine number and function is that different induction signals result in the production of distinct cytokines with a similar range of actions; (iii) in addition, different subsets of a cell types may produce a distinct spectrum of cytokines. For example, T_H^1 subset of human CD^+_4 T-lymphocytes produces predominantly IL-2 and IFN-γ, but not IL-4 and IL-5 whereas T_H^2 subset of CD^+_4 T-lymphocytes synthesize and secret IL-4 and IL-5 but not IL-2 and IFN-γ.

Figure 2: (A) 'Autocrine' type of cytokine-cell interaction; Autocrine stimulation occurs when a cell possesses receptors to respond to cytokines it produces itself; (B) 'Pracrine' type of cytokine-cell interaction; A localized paracrine stimulation can occur if a different cell type, near the producer cell, has the appropriate receptors; (C) 'Endocrine' type of cytokine-cell interaction; Cytokines may be released in the blood stream or other body fluids and interact with cells elsewhere in the body.



Such selective production may be one of the mechanisms for controlling the potentially pleicotropic effects of cytokines to their appropriate responder cells. Otherwise, given the net balance required to maintain healthy tissues, it is not surprising that abnormal cytokine activitiy may be seen in many diseases (2,3,13-17).

C. WHAT IS THE IMPACT OF CYTOKINES IN INTERNAL MEDICINE?

The therapeutic impact of cytokines has been felt mainly in the fields of cancer, infectious diseases, blood disorders, rheumatic and autoimmune diseases. An account of these impacts is briefly noted below (2,18-20):

(i) Cancer: The majority of successes in clinical trails with cytokines against cancer have been obtained with IFN-α and IL-2. Currently, IFN-α has the approval for the treatment of Hairy cell Leukemia, Kaposi's sarcoma and Basal Cell Carcinoma, and is considered effective in a variety of other tumors. Also, considerable effort is being given to IFN/drug combinations and combined therapy with IFN and surgery, radiation, hyperthermia, monoclonal antibodies, etc. IFN is usually given subcutaneously or intralesionally. Treatment with IFN tends to be long-term (months-years), which makes it very expensive. IFN-γ has not shown consistent therapeutic potential in any malignancies IL-2 is licensed in all European community countries for the treatment of metastatic renal cell carcinoma and licensing is expected for melanoma. Extensive trials with TNF have been disappointing and limited by toxicity, but it may be of value in treating ascites of Ovarian carcinoma.

(ii) Infectious Diseases: The most likely used cytokine has been IFN-α, which now has US-FDA approval for chronic hepatitis B and for hepatitis C. It has also shown promise in other viral conditions, including laryngeal papilloma, warts, common cold (propylactically only) and, though controversial, AIDS. IFN-γ has been recently shown to be of striking benefit in chronic granulomatous disease in cutting down the incidence of serious infections. In the vaccine front there is some evidence that IFN-γ IL-1and IL-2 may have useful adjuvant activity, particularly in immunocompromised patients. A clinical role for inhibitors of cytokines, particularly antibodies to the cytokines (e.g. anti-TNF, IL-10) has also been suggested in life-threatening conditions notably septic shock due to Gram negative infection and cerebral malaria.

(iii) Blood Disorders: Chemotherapy for malignant diseases and intensive therapy used with bone-marrow transplantation often leads to acquired neutropaenia. Both GM-CSF and G-CSF have been shown to accelerate neutrophil recovery in postchemotherapy neu-

tropaenia. Clinical trails in patients with lung cancer have shown that the G-CSF induced recovery of the post-chemotherapy neutropaenia is associated with fewer infections, fewer antibiotics, and a shorter period of stay in the hospital. Similar effects are observed with GM-CSF use after bone-marrow transplantation. G-CSF is now almost the standard therapy for the rare conditions of severe congenital neutropaenias. One of

Table 1A: Some properties of the growth factors and interleukins (However, there is considerable overlap in the biological effects and mechanisms of cytokines action currently placed in different categories).

Cytokines	Molecular Wt. (KDa)	Principal sources
1. Growth Factors (FFs)		
Epidermal growth factors (EGF)	6.0	Many cell types
Platelet - derived growth factor (PDGF)	28 - 35	Platelets, Monocytes, Endothelial cells
Acidic Fibroblast growth factor (Acidic FGF)	16 - 19	Neuroectoderm
Basic Fibroblast growth factor (Basic FGF)	16 - 19	Many cell types
Insulin - like growth factor - I (IGF-I)	7.0	Liver, Neural tissue
Insulin - like growth factor - II (IGF - II)	7.0	Liver
Nerve growth factor (NGF)	130.0	Salivary gland, neural tissue
Neuroleukin	56.0	Muscle, Brain, Kidney
Amphiregulin	9.0	Breast tumor, cells
Hepatocyte growth factor	90.0	Liver, spleen
2. Interleukins (ILs):		
Interleukin - 1 alpha (IL - 1α)	17.5	Monocytes and many other cell type
Interleukin - 1 beta (IL - β)	17.3	Monocytes and many other cell type
Interleukin - 2 (IL - 2)	15.4	T - lymphocytes
Interleukin - 3 (IL - 3)	15 - 25	T - lymphocytes
Interleukin - 4 (IL - 4)	15 - 19	T - lymphocytes
Interleukin - 5 (IL - 5)	45 - 60	T - lymphocytes
Interleukin - 6 (IL - 6)	21.0	T - lymphocytes, Monocytes and many other cell types
Interleukin - 7 (IL - 7)	20 - 28	Bone - marrow stromal cells
Interleukin - 8 (IL - 8)	8.0	Monocytes and macrophages
Interleukin - 9 (IL - 9)	32 - 39	T - lymphocytes
Interleukin - 10 (IL - 10)	18.7	T - lymphocytes, B - lymphocytes macrophages
Interleukin - 11 (IL - 11)	23.0	Bone - marrow stromal cells
Interleukin - 12 (IL - 12)	75.0	T - lymphocytes
Interleukin - 13 (IL - 13)	10.0	T - lymphocytes
Interleukin - 15 (IL - 15)	15.0	Epithelial cells, Fibroblasts, Endothelial cells, Peripheral blood mononuclear cells (except T- lymphocytes)
Interleukin - 16 (IL - 16)	50 - 60	T - lymphocytes

the remarkable features of G-CSF is its safety margin and almost complete absence of side-effects. Although GM-CSF shows similar efficacy to G-CSF in shortening chemotherapy-induced neutropaenia, it has some side effects which increase with modest increases in dose. G-CSF has FDA-US approval for use in post-chemotherapy neutropaenia and GM-CSF has approval for use after bone-marrow transplantation.

(IV) Rheumatic And Autoimmune Diseases: The experimental evidence implicates cytokines in the pathogenesis of rheumatoid arthritis and oesteoarthritis. These are two commonest causes of joint failure as cytokines are involved in the degeneration of connective tissue in cartilage and bone. The following therapeutic approaches with cytokines and antibodies to cytokines are already in the

Table 1B: Some properties of other cytokines such as CSFs, TGFs, TNFs, IFNs and CHKs (However, there is considerable overlap in the biological effects and mechanism of cytokines action currently placed in different categories).

Cytokines	Molecular Wt. (KDa)	Principal sources
1. Colony - Stimulating Factors (CSFs)		
Granulocyte / Macrophage - CSF (GM - CSF)	18 - 22	T - lymphocytes and many other cell types
Granulocyte - CSF (G - CSF)	19.6	Monocytes, fibroblasts, endothelium
Macrophage - CSF (M - CSF)	40 - 45	Monocytes, fibroblasts, endothelium
Erythropoietin (EPO)	34 - 39	Kidney and some other cell types
Leukemia inhibitory factor (LIF)	38.0	T - lymphocytes, carcinoma cells
Stem cell factor (SCF)		Bone marrow stromal cells
2. Transforming Growth Factors (TGFs)		
TGF - alpha (TGF - α)	6.0	Tumor cells, keratinocytes, macrophages
TGF - beta 1	25.0}	Megakaryocytes, macrophages
TGF - beta 2	25.0}	lymphocytes, bone
TGF - beta 3	25.0}	
3. Tumor Necrosis Factors (TNFs)		
TNF - alpha	51.0	Macrophage
TNF - beta	60 - 70	T- lymphocytes
4. Interferons (IFNs)		
IFN - alphas (IFN - αs)	16 - 27	T- lymphocytes, B - lymphocytes, monocytes, Fibroblasts
IFN - beta (IFN - β)	20.0	Fibroblast
IFN - gamma (IFN - γ)	50.0	T- lymphocytes, NK - cells
5. Chemokines (CHKs)		
Monocyte chemotactic protein - 1 (MCP - 1)	8 - 14	Macrophage
Macrophage inflammatory protein - 1 (MIP - 1)	8 - 14	Macrophage
Neutrophil activating protein - 2 (NAP - 2)	8 - 14	Macrophage, T - lymphocytes
RANTES (Released from activated T - cells, expressed and secreted)	8 - 14	T- lymphocytes

experimental stage of evaluation: replacement therapy with cytokine which might be deficient (e.g. IFN-γ, IL-4); neutralization of excessive amounts of cytokines (e.g. TNF, IL-1α, IL-1β, IL-6, GM-CSF, IL-8) by monoclonal antibodies against these cytokines or their receptors; and administration of cytokines which are immunosuppressive or promote tissue healing (e.g. IL-10, TGF-β).

(V) Other Conditions: The renal conditions, IgA nephropathy and mesangial glomerulonephritis, have been shown experimentally to be benefited with anti-IL-6 therapy. There is some suggestions that anti-TNF might alleviate Graft-versus-Host disease. Anti-IL-4 therapy, might be useful in allergy by down-regulating IgE synthesis; while TGF-β and IL-10 might be of value as a general cytokine inhibitor. A strong indication for cytokine therapy would be as replacement of a cytokine deficiency in production. While individual variations in the level of cytokines exist, no complete deficiency has yet been recognized.

Not surprisingly with such biologically potent molecules, toxicity (side effects) following cytokine therapy is common. For example, severe toxic effects of IFN include damage to liver, kidney, bone-marrow and heart. The treatment with IL-2 is complicated by vascular leak syndrome (hypotension, fluid retention and hepatic and renal failure, together with nausea, vomiting and mental alterations-including coma if treatment is continued. The occurrece of such potentially dangerous complications demands that highly trained and experienced physicians and nursing staffs must be available in hospitals where cytokines therapy is expected to be introduced either as therapy or as clinical trial.

D. WHAT IS THE IMPACT OF CYTOKINES IN LABORATORY MEDICINE (DIAGNOSTIC IMMUNOLOGY)?

With the availability of commercial cytokine assay kits, it seems to be useful to know the levels of various cytokines in blood and other body fluids in order to monitor disease activity or monitor treatment or evaluate the need for treatment. Although there is no situation at present where a cytokine assay can be said to be an essential part of management, perhaps the strongest case could be made for TNF levels in shock, since a raised level would be an indication for life-saving measure with anti-TNF. An excellent marker of flaring in inflammatory conditions is IL-6, being raised 24 hours before C-reactive protein. Levels of IL-1, TNF-α, IL-8 and TGF-β in tissues and of IL-2 and TNF receptors in the joint fluids and blood, have also been shown to correlate with disease activity. Levels of TNF, IL-1 and IL-6 can also be predictive of inflammatory bowel disease and graft rejection well before it is clinically apparent. Secondly, research studies of cytokine levels in tissue sections and in patient's cells in *in-vitro* culture are also carried out in collaboration with the Diagnostic Immunology Laboratory. Thirdly, these commercially available cytokine assay kits are considered very expensive to be introduced in the routine diagnostic immunology laboratories. Therefore, these assays should be restricted to laboratories where professionally trained immunologist and technical staffs are available, and where the results can be put ethically to further usage as research data, and where improvements in the efficacy and accuracy of assays are under constant surveillance (2,18,19).

CONCLUSIONS

The scope of medical research in the field of 'cytokines in clinical medicine' has been revolutionized with the availability of recombinant cytokines and some of the corresponding monoclonal antibodies. Firstly, the potential therapeutic impacts of cytokines, e.g., IFN-α, IL-2, IFN-γ, G-CSF, GM-CSF, TNF, antibodies to TNF, have been felt mainly in the areas of cancer, infectious diseases, blood disordes, rheumatic and autoimmune diseases. However, potentially dangerous side effects occur with cytokine therapy having implications in inter-

nal medicine. Secondly, it has enormous implications in laboratory medicine also in terms of trained manpower, equipments and costs for commercially available assay kits including efficiency and accuracy and efficacy of the assay kits. Thus, cytokine therapy or clinical trial should be introduced in centres where highly trained and well informed physicians and nursing staffs are available. The cytokine assays also should be based in laboratories where professionally trained immunologist and technical staffs are present and where the results can be put ethically to further usage as research data, and where improvements in the efficacy and accuracy of the assay results are under constant surveillance. Thirdly, to bring 'cytokines' as therapy or clinical trial in the developing countries, the authorities concern should make provisions for specially trained manpower (physicians and nursing staffs, clinical immunologists and medical technologists) and technology (assay kits and equipments) in their national health service planning now. This will facilitate the introduction of cytokines in clinical medicine in the third world countries in the very near future.

It is probably not an exaggeration to say that we are still only at the beginning of the age of cytokine therapy for serious diseases like cancer, autoimmunity, immunodeficiencies and infectious diseases. The cytokines therapy is the area where we can expect the greatest impact of molecular biology and recombinant DNA in clinical medicine in the near future. One can speculate on many ways in which growth factors, colony stimulating factors and other cytokines and their antagonists, receptors and their antagonists will be developed and engineered in the coming years (2,20-22).

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