Acta virol. **37**: 276–289, 1993

CYTOKINES: THE FOURTH HOMEOSTATIC SYSTEM

L. BORECKÝ

Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Slovak Republic

Received February 1, 1993

Summary. – The cytokines represent a network of small polypeptides produced by a variety of immune, blood and somatic cells. They can be considered the fourth homeostatic system serving the integrity of the organism. The cytokines cooperate with, substitute or, regulate the activity of cells and products of hormonal, neural and immune systems and function as physiological and/or emergency regulators. The attempts to exploit them in the therapy of viral and/or neoplastic diseases brought, so far, only partial success. However, they remain the goal of a new, more physiological therapy.

Key words: cytokines; interferon; interleukin; tumor necrosis factor; haemopoietic factors; toxicity; therapy

Introduction

"Parts of a system can be understood only in its context or not at all."
K. Lorenz

From the middle of sixties, a great variety of cellular products with regulatory activities has been reported. Most of them were found *in vitro* during studies of the immune response (interleukin, IL), search for antiviral substances (interferon, IFN) and/or, cell-growth inhibitory factors (tumor necrosis factor, TNF). The activity found in functional tests usually determined their designation (for instance interferon for interference with virus replication etc). However, employing the methods of molecular biology, several of new factors were found to be related or indistinguishable. So the "lymphocyte activating factor" (LAF) and the "endogenous pyrogen" are now considered identical with interleukin-1 (IL-1) (Berlin and Wood, 1964). As result of numerous studies, many of "new" regulatory factors were grouped under the name "lymphokines" since lymphocytes seemed their main producers. Later, when the regulatory activity and/or source of lymphokines were extended to stromal cells, the designation "cytokines" (Cy) was coined.

The number of various Cys operating in animal organism was estimated of about 200. But only a relatively small part of them (about 30) were sufficiently characterized as yet (Kawade, 1990). Along with the hormonal, neural and

immune systems, the Cys can be imagined as a network of agents forming the fourth homeostatic system of the animal organism. However, the borderline between the four homeostatic systems proved not sharp. Several of Cys were initially classified as hormones by functional criteria (thymus-hormones, immune hormones), but the cross-neutralization between ACTH or, gamma-endorphin and anti-IFN sera suggested a structural relationship between them (Blalock and Smith, 1980). Also, a relationship to neurotransmitters is emerging from finding somnogenic, pyrogenic and hyperalgesic agents among Cys (Krueger and Karnovsky, 1987; Ferreira *et al.*, 1988).

Cys differ from immune products mainly by non-specific effects on cells, shared specificity of some cellular receptors (IFN and thymosin alpha), quick release from the producer cell and, pleiotropic activity in the Cy-treated cell (Logan, 1992).

Cys are coded by genes which can be found in various phyla. From classical hormones (endocrine gland products) they differ by ubiquitous presence of cells that produce them and/or respond to them and the unclear role of classical intracellular signal transducers (second messengers) such as cAMP-cGMP in their regulatory activity.

The Cy-system can be viewed also as a "sensor-system" which signalizes the presence of agents that were not recognized by the nervous or immune system in the organism. The system is composed of families of factors that share such properties as polypeptide character, molecular weight below 80 M_r, production by cells of various provenience, receptor-mediated pleiotropic effect, and, participation on both physiological and pathological processes (Balkwill, 1989) (Table 1). The list on Table 1 is provisional and a better characterization of included agents in the future may require its additional modifications.

The release of one Cy from cell is usually accompanied by release of various other Cys and/or not yet identified cell products. This leads to a cascade phenomenon which may be followed by cooperative or antagonistic interactions (Belosevic *et al.*, 1988). The release of Cys requires an appropriate stimulus

Table 1. The cytokine system

1. Interferons (IFN)	3 types, 20 subtypes (?)
2. Interleukins (IL)	11 types (?)
3. Haemopoietic factors (CSF)	5 types (?)
4. Thymus hormones	3 types (?)
5. Somatic growth factors	5 types (?)
6. Other growth factors (complement (?), chalones, tuftsin, transfer factor etc)	

(?) present state

which may be biological (antigenic), chemical (drug) or physical (heat, exercise) etc. The production of Cys is autoregulated by the amount of the released Cy and is followed by a phenomenon called tolerance or hyporeactivity (Stewart, 1979). Cys are not stored in producer cells but may function as inducers of their own production. The amounts produced by cells are minutious but the activity of Cys per cell is high: 10^{-12} to 10^{-15} mol per mg of tissue. The activity can be potentiated or mitigated by other Cys functioning as enhancers or antagonists. In consequence, the regulatory activity of Cys may oscillate between stimulation and inhibition of cell-activity.

The mechanism of Cy induction in the cell is far from clear. In addition to the mediating role of cellular receptors interacting with the inducer, several reports support the view that arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid) released by phospholipase A_2 from membrane lipids in answer to stimulation of the cell and products of its catabolism (such as 5- and 15-hydroxyeicosatetraenoic acid) during the lipoxygenase pathway, may play important role in mediating the helper signal for Cy (IFN gamma) production (Johnson *et al.*, 1986). In contradistinction, the products of cyclooxygenase (PG-synthetase) pathway (such as prostaglandins, leukotrienes etc) may exert an antagonizing effect on Cy (IFN gamma) induction. This is supported by finding that the inhibitors of cyclooxygenase pathway (aspirin, indomethacin etc) enhanced the production of IL-2 and IFN (Hsia *et al.*, 1989). Such inhibitors proved effective also in inhibiting some cellular effects of Cys (IFN) such as membrane rigidity, prolongation of the cell-cycle etc (Sreevalsan *et al.*, 1979; Rhodes and Stokes, 1982; Chandrabose and Quatrecasas, 1982). However, others could not confirm the abrogating effect of cyclooxygenase inhibitors on the anti-viral, or, anti-proliferative action of IFN (Tovey *et al.*, 1982). In general, it remains presently unclear whether Cys exert the variety of activities in cells as a result of concurrent effect of different, or, only a few (or one) "specialized" pathways. In the former case, the protein synthesis might be the common denominator. However, some studies failed to find a correlation between suppressed protein synthesis and inhibitory activity (De Maeyer and De Maeyer-Guignard, 1988).

More light was shed on the antiviral (AV) and anti-proliferative (AP) activities of IFNs. Two Cy-iduced mechanisms seem to play a central role in these activities and both are dsRNA dependent. One is the 2'-5'oligoadenylate synthetase pathway which leads to production of 2'-5'adenylated oligomers via activation of cellular ribonuclease L. In non-stimulated cells, the 2'-5'oligoadenylate synthetase probably regulates the break-down of cellular RNA, while in stimulated infected cells the enzyme can degrade both the cellular and viral nucleic acids. In non-infected cells, the dsRNA needed for enzyme activity may be supplied by sequences present in some cellular products, while in virus-infected cells the amount of available dsRNA increases through formation of replicative intermediate viral dsRNA (Silverman, 1987). This system might be responsible also for the AP effect in non-infected IFN-treated cells as a consequence of enhanced cellular RNA break-down. Less is known about the

regulation of cellular oncogene transcripts (ras, myc) which are inhibited by IFN and may be involved in 2'-5'adenylate synthetase activity as well (Kimchi *et al.*, 1988).

The second dsRNA dependent regulatory system that may participate on the AV and AP activities of Cys (IFN) is the protein kinase P1 system. The mechanism of its regulatory activity is more complex and less transparent. It phosphorylates three cellular substrates: (1) an endogenous protein P1 with an M_r 68; (2) the alpha subunit of the eukaryotic protein initiation factor (eIF2A) with M_r 35; and (3) histones. Phosphorylation of eIF2A inhibits the cellular protein synthesis by forming and accumulating an inactive complex eIF2B from eIF2A and the GTP recycling factor. After viral infection, a viral inhibitor or protein kinase P1 interrupts the normal regulation of protein synthesis (Silverman, 1987).

In addition to these 2 systems, further proteins factors are synthesized and/or activated in Cy (IFN) treated cells suggesting further intervening mechanisms. They may participate on the modulation of Cy receptors, their interaction with various growth factors etc (Table 2).

The role of cytokines in the organism

The Cys have both physiological and emergency functions (Bocci, 1987; Borecký, 1992). The physiological function can best be demonstrated on the haemopoietic system which is regulated by haemopoietic Cys (CSFs), and provides for continuous replacement of mature haemopoietic cells (with a relative short life-span) with cells recruited from a common pluripotent stem cell-pool (normally restricted to the bone marrow). Ordinarily, about 4×10^8 white cells and 10^{10} red cells are replaced each hour. However, in disease states such as trauma, infection, cytotoxic therapy etc, the production of CFSs may be dramatically increased turning the physiological function of CSFs into an emergency activity. Such natural "autotherapy" by Cys motivates the idea of therapeutic exploitation of CSFs and other Cys.

Table 2. Proteins produced in response to cytokine (IFN) activation by cell

2-5-oligoadenylate synthetase
Protein kinase
HLA-complex I and II
Indoleamide dioxygenase
Guanylate-binding proteins
M_x-protein
Metallothionein II
Tumor associated antigens (CEA)

The idea is based on the following premises: (1) Cys are "normal" cell products with physiological regulatory role in the organism (Moore, 1991; Sachs, 1991). (2) Their main task is regulation of growth and differentiation of various cell systems and, normalization of those altered by transformation, infection, physical and other injury (Backer, 1984). (3) Cys may act on the cells directly or by secondary, indirect mechanisms which may follow Cys release or action. (4) In their homeostatic function, they represent a "first line" barrier devoid of immunological constrains and, appearing, in contradistinction to antibodies, in minutes after stimulation (Schattner *et al.*, 1983) (5) Since several immunomodulators of bacterial origine and their synthetic analogs (MDP, BCG, LPS etc) proved potent inducers of Cys, their immunomodulating activity may result from the activity of released Cys (Galleli and Chedid, 1986). (6) As normal cell products with physiological destination, the Cys were expected to be non-toxic for human organism.

Unfortunately, this last premise proved non-valid. In contrast with numerous experimental tests in laboratory animals, the trials in man were often interrupted because of toxic side effects. They can be explained by the following considerations: (1) The Cys are predominantly short-distance reactors acting in minutious amounts on cells in their immediate vicinity (0.1 to 1/μ) (Bocci, 1978). (2) When appearing in circulation, they usually signalize an overproduction (in disease), or, overdosing (in therapeutic trial). (3) The "toxicity" of Cys may be of various gradation reflecting thus inherent properties of the molecule and/or sensitivity of the contacting tissue. (4) The appearance of Cys in inproper place and amounts may contribute to pathology of disease and/or signalize its exacerbation (in AIDS, SLE etc). An overspill of Cys into circulation may be the consequence of unsettled dose-activity problems. (See later.)

Such interpretation of Cy-toxicity in man found support in results of animal tests by Gresser *et al.* (1982) and by Scott *et al.* (1983) in man. Gresser *et al.* found that the "wasting disease" of newborn mice infected with the lymphocytic choriomeningitis virus (LCMV) can be mitigated by administration of anti-IFN alpha serum and, on the other side, some of cardinal symptoms of disease, such as liver necrosis, glomerulonephritis etc could be reproduced by treating non-infected mice with IFN. In man, influenza-like symptoms similar to "true" flu (coughing, sneezing etc) were observed after repeate intranasal administration of IFN to non-infected volunteers serving in IFN therapeutic trials as controls in G. Britain by Scott *et al.*

In acute infectious diseases such as bacterial meningitis, viral Argentinian haemorrhagic fever etc, the appearance of Cys (IFN, TNF etc) in the blood was followed by aggravation of the basic disease (Lewis *et al.*, 1983). In chronic diseases such as SLE, AIDS and cancer, the appearance and level of IFN in the blood correlated with the exacerbation of the basic disease (Eyster *et al.*, 1983 etc) (Table 3).

However, some controversions about the nature of such IFNs were claimed concurrently. In SLE and some other chronic diseases, the detected IFN was

Table 3. Clinical symptoms caused or activated by IFN and other cytokines

In humans	Lymphopaenia in viral diseases	
	Exacerbation of clinical symptoms:	
	Argentine haemorrhagic fever	IFN
	AIDS	
	Psoriasis	
	Meningococ. meningitis - TNF	
	Influenza-like symptoms after application	
In mice	Wasting disease (as after LCMV)	
	Accerelation of SLE-like disease	

characterized as "atypical" since it was acid- and thermolabile (in contradistinction to classical IFN alpha), and, its appearance in the blood was accompanied by impaired production of IFN gamma when the patient's cells were tested *in vitro*. Such reports raised the speculation that the "pathological" IFNs were causing "inclusions" in the leukocytes obtained from patients producing them etc. However, such speculations were not approved as yet and some results of studies dealing with "acid- and thermolabile" IFNs alpha suggested that they might be a subtype of IFN alpha family (Borecký *et al.*, 1989; Kontsek *et al.*, 1991). However, even if so, it remained unclair whether such IFNs participated on the exacerbation of the basic disease, or the registered exacerbation resulted from the activity of (secondary) Cys released in answer to stimulation by acid- and thermolabile IFN found in patient's organism.

Despite of reports of unfavorable side effects accompanying the administration of Cys to man, the hope that Cys may represent a fundamentally new approach to treating various diseases persists. Cys may substantially increase the present therapeutical arsenal of medicine. The aptitude of Cys to fulfil such expectations follows from considerations that: (1) Cys have a "normalizing" role on cellular level; (2) their preventive and curative efficacy in model experiments was high; (3) they have broad applicability including infectious, immune and neoplastic disorders.

In the same time, the available experience makes clear that any advance in successful exploiting of this type of potential remedies requires a new application technology that must result from a better understanding of the regulatory activity of Cys (Wersall *et al.*, 1990; Johnson *et al.*, 1990; Sachs, 1991; Moore, 1991 etc).

Some therapeutically prospective cytokines and their potential toxicity for man

1. "Toxic" cytokines (Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF))

Some Cys may exert an inherent toxicity in man which can be associated with peculiarities of their structure and/or mode of action (Vilček, 1984). To such Cys belong IL-1 and TNF. They are also called "proinflammatory Cys".

IL-1 was known since 1972 under the name "lymphocyte activating factor" (LAF) or "endogenous pyrogen" (EP) (Berlin and Wood, 1964). IL-1 is produced by a great variety of cells (T- and B-lymphocytes, neutrophils, endothelial, glial, certilage cells, fibroblasts and their tumorigenic variants) after injury, infection or antigenic stimulation. IL-1 appears in two molecular forms: IL-1 alpha is a neutral peptide and IL-1 beta is an acidic protein. There is a 26 % amino-acid homology between the two molecular forms, but they are antigenically distinct. After release from cells, they acquire a similar molecular weight (17.5 M_r). The cytoplasmic (precursor) form is 271 amino-acid long which is processed to the mature form before release which lasts a few minutes. IL-1 can be induced by exogenous inducers as well as other Cys or IL-1 itself. Concurrently with IL-1, other ILs, TNFs and/or acute phase proteins (APP) may be released from the stimulated cell.

In addition to pyrogenic, inflammatory and hyperalgesic activity of IL-1 which may be mediated by products of enhanced phospholipase A_2 and cyclooxygenase pathways, IL-1 has also sleep-inducing and anorectic activities as well as hormon-releasing potency for neutrotransmitters and/or somatic peptide hormones such as insulin. While the inflammatory activities of IL-1 can be suppressed by inhibitors of cyclooxygenase pathway (aspirin, non-steroidal antiinflammatory drugs), the somnogenic and anorectic activities apparently run a different mechanism (liver?) (Smith, 1990).

In some diseases it might be desirable to suppress the inflammatory activity of IL-1 resulting in shock, exacerbation of autoimmune symptoms etc. This can be done by: (1) treatment of patient with antagonistic Cys (IFN) or hormons (corticosteroids); (2) preventing the IL-1 production by suppression of lipooxygenase (leukotriene) pathway; (3) a diet containing polyunsaturated eikosapentaenoic acid (EPA) in fish oil concentrates. A similar benefical effect of EPA could be observed also in patients with burns, shock and polyarthritis (Speck and Lauterburg, 1991). (4) Also a treatment with cAMP-enhancing methylxanthin, or, a direct IL-1 antagonist found in feverish sera and urine may be recommended as remedy in the future (Dinarello, 1990).

TNF also appears in two molecular forms: TNF-alpha (cachectin) and TNF-beta (lymphotoxin). While TNF-alpha was discovered in 1975 by Carswell *et al.*, TNF-beta was reported by Granger and Williams in 1968. However, the activities of both TNFs are probably, identical with that of the "Coley-toxin" reported by Coley in 1906 as responsible for spontaneous regression of tumors in some patients after an intercurrent bacterial infection.

The two molecular species of TNF share a 30 % homology in aminoacids and both are genetically regulated from the 6th chromosome. While TNF-beta has a M_r 17 and is unglycosylated, TNF-alpha is 25 M_r and is glycosylated. As in the case of IL-1, the cytoplasmic form of TNF is larger (M_r 26) than the secreted (released) form. However, as yet, no correlation was found between the number of cell receptors present on cell-surface and the susceptibility toward TNF action (Tsujimoto and Vilček, 1986). The released form of TNF is a trimer.

It is noteworthy that in the 3'untranslated region of TNF-gene (and in GM-CSF and IL-1 gene as well) a stretch of T-A exclusive construct was found consisting of octameric pattern "TTATTTAT" (in mRNA the corresponding UUAUUUAU) which is uncommon in animal genes and/or products. The octamer is of interest because it might be involved in regulation of instability of Cy mRNA in general (Beutler, 1990).

TNF synergizes with IL-1 and IFNs but not all TNF activities are inflammatory or destructive for tumor cells. Small quantities of TNF may have a stimulatory effect on cell-growth which may, or may not result from the direct effect of TNF (Griffin and Lowenberg, 1986 etc).

2. Cytokines with dose dependent cytotoxicity (Interleukin-2 (IL-2), Interferons (IFNs), Interleukin-6 (IL-6))

The toxicity of several Cys for man may be the consequence of therapeutic overdosing. To such Cys belong the interleukins (ILs) and interferons (IFNs). IL-2 is a member of the interleukin family comprising about 9 members. They were formerly called "immune hormones". The ILs share a similar genomic structure and a molecular size of 15 to 20 M_r. (By similar criteria also the CSFs can be included in this group.) As the majority of other Cys, IL-2 is normally not present in the blood or urine. It acts on the cell via receptors that consist of two peptide chains, 55 and 75 M_r, respectively. However, the further steps in its biochemical pathway remain unclear. Neither cAMP nor the phosphoinositol catabolic products seem to be operative in IL-2 activity (Smith, 1990). While the resting T- and/or B-cells are devoid of IL-2 receptors, NK-cells express constitutively the heavy chain (M_r 75) of receptor. (The monocytes were controversial in this respect.) As can be expected for small proteins in general, the half life of IL-2 administered to animals is short, about 25 minutes. It is eliminated from the organism predominantly by the kidney.

After antigenic stimulation, IL-2 initiates the proliferation of stimulated T- and B-lymphocytes and determines, in this way, the development and duration of the immune response. In addition, IL-2 stimulates the T-cell cytotoxicity, generation of IgM molecules abd the secondary immune response.

IL-2 was so far successfully used (alone or with LAK cells) for treatment of diseases with a depressed specific T-cell immunity (leprosy, immunodeficiencies of various provenience) (Rosenberg *et al.*, 1987). However, IL-2 can be used also for an antigen-specific immunosuppression when the enhanced activity of immune mechanisms treatens the patient. This may occur after organ transplantation, in autoimmune diseases etc. In such cases, also IL-2 analogs, or, anti-IL-2 antibodies could be instrumental.

In dose dependency, the gravity of IL-2 toxicity in patients did not differ from the gravity of symptoms observed during application of IFNs and/or some other Cys. This raises the problem of adequate therapeutic doses of Cys in man. Some therapeutic trials, especially in veterinary field, suggested that large doses i. e. exceeding 3×10^6 units (per about 70 kg) were not more effective than

substantially lower doses. This may be important because the large and continuously applied doses of IL-2 are feared for the "capillary leakage syndrom" (pulmonary and brain oedema). However, the pathogenesis of this complicating syndrome is not clear and it cannot be excluded that secondary Cys induced by IL-2 (such as TNF) might be responsible for the registered cases (Lotze *et al.*, 1986; Talmadge *et al.*, 1987).

Also IL-6, functionally a haemopoietic factor which mediates many final effects of CSFs in the process of differentiation, may become cytotoxic when overproduced, as observed in Castelman's disease - a lymphoid hyperplasia (Cicco *et al.*, 1991).

The interferons (IFN alpha, beta and gamma) are produced by a family of genes located on the 9th (IFN alpha and beta) and 12th (IFN gamma) chromosome. They have a molecular size of 17 to 23 M_r. While 23 IFN alpha genes were identified, up to now, only one beta and one IFN gamma genes were found. Recently, the structural and functional differences among the members of the IFN alpha gene family led to the proposition to consider one of IFN alpha class-II genes (IFN omega) as a new, fourth type of IFNs (Adolf, 1990). The "classical" three types of IFNs (alpha, beta, gamma) differ antigenically. However, the IFNs alpha and beta share common cellular receptors and, recently, an antigenic link between them could be established (Kontsek *et al.*, 1988; 1990). While the members of IFN alpha class-I are glycosylated, the class-II members are devoid of glycosylation. However, no difference in biological activity among members of these two classes could be observed so far.

Supposedly, the IFN alpha genes contain two regulatory sites: the "negative" one regulates the constitutive production of IFN molecules, while the "positive" site is normally suppressed by an imaginary repressor. When induced, the repressor is removed and the production of IFN mRNA is going on. During the phenomenon known as superinduction of IFN beta by cycloheximide and/or actinomycin D, the imaginary repressor is probably blocked allowing an increased production of IFN beta.

The cellular receptor for IFN alpha and beta is coded on the chromosome 21 and chromosome 6 (IFN gamma). While the direct inhibitory effect of IFNs on virus multiplication in infected cells is evident from *in vitro* experiments, *in vivo* participation of immune mechanisms in the antiviral effect seems plausible.

Several reports indicate that IFNs (and other Cys as well) may appear in the blood of patients during various acute and chronic diseases. Neither their origine nor their inducers are known. In 1975, Skurkovich and Eremkina expressed the view that IFN (alpha?) found in the blood of patients with autoimmune and allergic diseases might represent a "defective" IFN species participating on the pathogenesis of such diseases which can be effectively treated with anti-IFN sera (Skurkovich *et al.*, 1987). Such IFNs were considered "atypical" because, in contradistinction to classical IFN alpha/beta, they were acid- and thermolabile and, only partially neutralized by anti-IFN alpha sera. They may show also a lowered species specificity (Preble *et al.*, 1982). Skurkovich hypothetized that

such IFNs exerted a negative immunoregulatory affect in the organism leading to disturbances in Cy and Ig production as well as in hypothalamic-pituitary-adrenal (HPA) axis.

Atypical IFNs were found in several autoimmune (systemic lupus erythematosus, psoriasis, Behcet's disease etc) and chronic diseases (cancer, Down's syndrome, AIDS etc). Several interpretations of acid-and thermolability of IFN alpha were published meantime (Yee *et al.*, 1989; Capobianchi *et al.*, 1991). However, other studies pointed out that the "atypical" IFNs can be also found in "normal" leukocyte IFN preparations obtained during the processing of human leukocyte extracts (Chadha *et al.*, 1985) or, in the blood of patients with acute viral diseases (Green and Spruance, 1984) and, finally, as an artifact resulting from testing mixtures of various IFNs which appear in the blood of patients with autoimmune diseases (Minagawa *et al.*, 1989). Recently, however, we arrived at conclusion suggesting the possible identity of the "atypical" IFN alpha with the IFN omega (Adolf, 1990; Kontsek *et al.*, 1990). This preliminary conclusion resulted from finding a cross-neutralization when the acid- and thermolabile IFN (from leukocytes) was tested with anti-omega serum and vice versa. (The IFN omega and the monoclonal anti-IFN omega antibodies were a gift of Bender and Co., Wien, Austria.)

IFN alpha/beta proved effective in treating viral diseases and malignancies both in man and animals. The most impressive results were seen in hairy-cell leukemia (over 80 %) and in chronic myelogenous leukemia (over 70 %). In the low grade lymphoma an about 65 % efficacy was registered (Quesada *et al.*, 1984; Talpaz *et al.*, 1987). With regard to toxicity in man, it is noteworthy that in hairy-cell leukemia both "standard" (about 3×10^6 units per m^2) and "low" doses of IFN alpha (Welferon) (0.2×10^6 per m^2) proved effective (Smalley *et al.*, 1987). According to reports presented at the IInd International Symposium on Combination Therapies (Catania, Italy, 1992), the toxic side effects of chemotherapeutic agents were reduced and the efficacy of therapy enhanced by combining IFNs, IL-2, CSFs and/or thymic hormones with the chemotherapeutic agents.

3. The "harmless" cytokines (colony stimulating factors (CSFs))

Only minimal cytotoxicity was reported in human trials using haemopoietic factors (CSFs) described by Bradley and Metcalf (1966). Presently, this group of cell regulators consists of three myeloid and one erythroid cell growth factor. They are: the Granulocyte CSF (G-CSF), the Granulocyte-Macrophage CSF (GM-CSF), the Macrophage CSF (M-CSF) and, Erythropoietin (EPO). EPO is a classical, long known cell regulator (1850) considered as a tissue hormon produced in the kidney. Among CSFs, is long known also the Multi-CSF which was recently included into interleukin group as IL-3 (Glaspy, 1989).

The basic task of Cys of this group is the continuous replenishment of the myeloid and erythroid cell pool in the organism. They are also necessary for normal functioning of mature blood cells. These functions can be considered as physiological. A division of labor seems to exist among CSFs because, while G-,

GM- and M-CSFs function mainly as inducers of proliferation of stem-cells, interleukins IL-1 to IL-9 function as differentiating agents and, maturation factors in the later phases of proliferation of stem cells.

The haemopoietic factors (CSFs) are regulated by specific genes located on chromosome 5 (GM- and M-CSF) and chromosome 17 (G-CSF). They have an M_r between 14 and 30 and their glycosylation shows extreme heterogeneity. However, the glycosylation (as by other Cys) does not seem to play a significant role in the biologic activity of CSFs since the recombinant preparations (devoid of CH) had a comparable (or higher) activity in colony stimulating tests.

All CSFs act on cells through cellular receptors which have a M_r between 50 and 130 in mice. Data on human receptors for CSFs are incomplete. The CSF receptors, in addition to myeloid stem cells, can be found also on placental trophoblasts (M-CSF) but not on erythrocytes or megakaryocytes. CSFs, in addition to their haemopoietic activity, stimulate various cells to produce Cys like TNF, IFN alpha/beta as well as protooncogenes (c-fos, c-myc), arachidonic acid, prostaglandins, plasminogene activator etc. They alter the chemotactic activity of mature granulocytes. The lineage-specific activity of CSFs is directed to proliferation of various myeloid cell-subclasses and/or erythrocytes (EPO). M-CSF stimulates the development of macrophages also *in vitro*. It is released in two molecular forms: as a 70 to 90 M_r glycoprotein and, a 40 to 50 M_r glycoprotein consisting of two 25 M_r subunits forming a dimer. G-CSF stimulates predominantly the growth of granulocyte colonies. It is a 18 to 20 M_r peptide used as recombinant preparation in the therapy. EPO with a M_r 18.3 has a native 34 to 39 M_r form. It acts on stem cells to differentiate them into pronormoblasts and, during following 72 hr, into normoblasts containing the bulk of the synthesized homoglobin.

In view of the continuous demand of CSFs in regulation of homeostasis and, their continuous presence in the serum and tissues (which seems to be regulated by hormons of the HPA axis) the constitutive production of CSFs was advocated. However, it was not confirmed as yet. Nonetheless, their homeostatic significance and therapeutic prospectiveness is stressed by prompt and impressive rise of CSFs during blood-loss, cytotoxic therapy and/or pregnancy. In addition, it is remarkable that the therapeutic application of CSFs to man was, so far, not accompanied by any significant adverse effects (Balkwill, 1988).

In chronic diseases like cancer, AIDS, or haemopoietic disorders of unknown and iatrogenic provenience, the primary disturbances of Cy-system may play a pathogenetic role. Both overproduction and suppresion of production of CSFs as well as inappropriate receptor expression on cells may lead to diseases. In acute myelogenous leukemia 6 of 11 patients that had activated CSF genes were found to secrete GM-CSF (Young and Griffin, 1986). Sher *et al.* (1985) have found inappropriate M-CSF receptor expression via the activated oncogene v-fms. In myeloma multiplex, the hyperactivity of IL-6 gene seemed to be responsible for the disease since treatment with anti-IL-6 antibodies led to a reduction of plasma cell proliferation in patients (Klein *et al.*, 1990).

Conclusions

Systems can be viewed as integrated and mutually communicating structures of components. In this sense, the Cy network in the animal organism can be regarded as a sophisticated security system which guaranties proper functioning of basic mechanisms of cellular growth and differentiation through specialized peptides produced by a variety of cells in response to physiological and pathological stimuli (Roberts *et al.*, 1989). The efficacy of the Cy-system is based on the abundance of its specialized components, dose dependent stimulatory activity and, capacity to synergize, or, antagonize other regulatory molecules. The quick release of Cys from the producing cell and the high per mol activity suggest that the Cy network, along with hormonal, neural and immune systems, functions as the fourth homeostatic system of the animal organism. As an example of homeostatic regulation may serve the regulation of the growth activity of various lines of stem cells in the bone marrow by several different CSFs, the final differentiation of the growing pool of stimulated cells to maturity by IL-6, and modification of functions and viability of mature cells by several further Cys. Such system proved a reliable safeguard against the unexpected collaps of various organs that may follow a sudden regulatory failure if the Cys were solitary and in number restricted agents. Due to pleiotypic activity, the Cys can exert various additional functions (such as anti-infectious, anti-cancerous and/or immunoregulatory) by mechanisms of substitution, synergism and/or antagonism. There is a wide range of both infectious (viral, bacterial, parasitic) and non-infectious diseases (cancer, autoimmune disorders etc) that may require Cy substitution and/or enhanced cooperation.

Despite of the fact that various Cys are presently available in purified recombinant form, their exploitation in human and animal therapy still meets difficulties. In the sixties, when several Cys were first used for treatment of diseases, neither their purity nor the available amounts were sufficient for controlled trials. Later, the unexpected "toxicity" hindered their evaluation in man. It is note-worthy that the therapeutic trials as yet did not arrive at a definition of recommended and/or safe doses for treating various diseases. Some results of therapeutic trials suggested that satisfying therapeutic results could be obtained with doses lower than used formerly (Blalock *et al.*, 1980). The present situation in exploiting Cys for the therapy was recently characterized by Tomlinson (1986) as a period making the necessity to find a new technology for successful application of Cys to human diseases *decisive*. The new technology should (a) allow facilitated access of the used Cys to the diseased tissue, (b) reach an effective concentration in it, and (c) by convenient timing, avoid the inactivation of Cy by potential antagonists present in tissues (Bocci, 1978). As Moore (1991) reminds us, Cys in the organism resemble presently an orchestra with known instruments but unknown way for producing the symphony played.

References

Adolf, G. R. (1990): *Virology* 175, 410–417.
Balkwill, F. R. (1989): *Cytokines in Cancer Therapy*. Oxford Univ. Press, Oxford.
Becker, S. (1984): *Cell. Immunol.* **84**, 145–153.
Belosevic, M., Davis, C. E., Meltzer, M. S., and Nacy, C. A. (1988): *J. Immunol.* **141**, 890–896.
Berlin, R. D., and Wood, W. B. Jr. (1964): *J. exp. Med.* **119**, 715–726.
Beutler, B. (1990): In E. Borden (Ed.): *Regulatory Cytokines*, Amgen-Roche, Basel, pp. 59–71.
Blalock, J. E., Georgiades, J. A., Langford, M. P., and Johnson, H. M. (1980): *Cell Immunol.* **49**, 390–394.
Blalock, J. E., and Smith, E. M. (1985): *Immunol. Today* **6**, 115–120.
Bocci, V. (1978): In S. Baron, F. Dianzani, G. J. Stanton and W. R. Fleischmann (Eds): *The Interferon System. A Current Review to 1978*, Univ. Texas Press, Austin, pp. 436–442.
Bocci, V. (1987): *Pharmac. Ther.* **34**, 1–49.
Borecký, L., Kontsek, P., Novák, M., and Lackovič, V. (1989): *Antivir. Res.* **12**, 195–204.
Borecký, L. (1992): *World J. Microbiol. Biotechnol.* **8** (Suppl. 1), 54–57.
Bradley, T. R., and Mecalf, D. (1966): *Austr. J. exp. Biol. med. Sci.* **4**, 287–300.
Capobianchi, M. R., Mattana, P., Gentile, M., Conciatori, G. C., Ankel, M., and Dianzani, F. (1991): *J. Biol. Regulat. Homeost. Agents* **5**, 147–153.
Carswell, E. A., Old, L. J., Kassel, R. J., Green, S., Fiore, N., and Williamson, B. (1975): *Proc. natn. Acad. Sci. U. S. A.* **72**, 3666–3670.
Chadha, K. C. (1985): In F. Dianzani and G. B. Rossi (Eds): *The Interferon System*, Raven Press, New York, pp. 24, 35–41.
Chandrabose, K., and Quatrecasas, P. (1982): *Biochem. Biophys. Res. Comm.* **98**, 661–668.
Cicco, N. A., Lübbert, M., Oster, W., Lindemann, A., and Mertelsman, R. (1991): *Hematol./Oncol. Clin. N. Amer.* **5**, 1053–1066.
Coley, W. B. (1906): *Amer. J. med. Sci.* **131**, 375–430.
De Maeyer, E., and De Maeyer-Guignard, J. (1988): *Interferons and other Regulatory Cytokines*, J. Wiley and Sons, New York.
Dinarello, C. A. (1990): In E. C. Borden (Ed.): *Regulatory Cytokines*, Amgen, Roche, pp. 41–57.
Eyster, M. E., Goedert, J. J., Poon, M. C., and Preble, O. T. (1983): *New Engl. J. Med.* **309**, 583–586.
Ferreira, S. H., Lorenzetti, B. B., Bristow, A. F., and Poole, S. (1988): *Nature* **334**, 698–700.
Galelli, A., and Chedid, L. (1986): *J. Immunol.* **137**, 3211.
Glaspy, J. A. (1989): In E. C. Borden (Ed.): *Regulatory Cytokines*, Mediscript, London, pp. 31–40.
Granger, G. A., and Williams, T. W. (1968): *Nature* **218**, 1253–1254.
Green, J. A., and Spruance, S. L. (1984): *New Engl. J. Med.* **922**
Gresser, I. (1982): In I. Gresser (Ed.): *Interferon 4*, Academic Press, New York, pp. 95–127.
Griffin, J. D., and Lowenberg, B. (1986): *J. Amer. Soc. Hematol.* **68**, 1185–1195.
Groopman, J. I., Ronald, M. D., Deleo, M. J., and Golde, D. W. (1987): *New Engl. J. Med.* **317**, 393–397.
Hsia, J., Sarin, N., Oliver, J. H., and Goldstein, A. L. (1989): *Immunopharmacol.* **17**, 167–174.
Johnson, H. M., Rusell, J. K., and Torres, B. A. (1987): *J. Immunol.* **137**, 3053–3056.
Johnson, V. A., Barlow, M. A., Merrill, D. P., Chou, T.-C., and Hirsch, M. S. (1990): *J. inf. Dis.* **161**, 1059–1067.
Kawade, J. (1990): *J. Interferon Res.* **10**, 101–107.
Kimchi, A., Shure, H., and Revel, M. (1979): *Nature* **282**, 849–851.
Klein, B., Wijdenes, J., and Jourdan, M. (1990): *Blood* **76**, 357*a*.
Kontsek, P., Borecký, L., Kontseková, E., and Novák, M. (1988): *J. Interferon Res.* **8** (Suppl. 1), 555.
Kontsek, P., Borecký, L., Kontseková, E., Novák, M., and Krchňák, V. (1990): *J. Interferon Res.* **10**, 119–128.
Kontsek, P., Borecký, L., and Novák, M. (1991): *Virology* **181**, 416–418.
Krueger, J. M., and Karnovsky, M. L. (1987): *Ann. N. Y. Acad. Sci.* **343**, 510–516.
Levis, S. C., Snavedra, M. C., Ceccoli, C., Feuillade, M. R., Euria, D. A., Maiztegui, J. I., and Falcoff, R. (1985): *J. Interferon Res.* **5**, 383–389.

Logan, A. (1992): *Lancet* **340**, 420–421.
Lorenz, K. (1963): Das Sogenannnthe Böse, Borotha-Schoeler Vlg., Wien.
Lotze, M. T. (1986): *Cancer* **58**, 2764–2772.
Minagawa, T., Asano, M., Nakane, A., Numato, A., Kohanawa, M., Kaneko, F., Hiraga, J., Kachimori, K., Negishi, M., Yamazaki, S., and Sato, Y. (1989): In J. Kawade and S. Kobayashi (Eds): *The Biology of the Interferon System*, Local Org. Comm., Osaka, pp. 363–368.
Moore, M. A. (1991): *Cancer* **67** (Suppl. 10), 2718–2726.
Preble, O., Black, R. J., Friedman, R. M., and Klippel, J. H. (1982): *Science* **216**, 429–431.
Quesada, J. R., Reuben, J., Manning, J. T., Hirsh, E. M., and Gutterman, J. U. (1984): *New Engl. J. Med.* **310**, 15–18.
Rhodes, J., and Stokes, P. (1982): *Immunology* **45**, 531–536.
Roberts, R. M., Kronenberg, L. A., Malathy, P. W., and Imakawa, K. (1989): In J. Kawade and S. Kobayashi (Eds): *The Biology of the Interferon System*, Local Org. Comm., Osaka, pp. 315–321.
Rosenberg, S. A., Avis, F. P., Chang, A. E., Lee, R. E., Leitman, S., Linehan, W. M., Lotze, M. T., Muul, L. M., Robertson, C. N., Rubin, J. T., Seipp, C. A., Simpson, C. G., and White, D. E. (1987): *New Engl. J. Med.* **316**, 889–897.
Sachs, L. (1991): *Cancer* **67**, 2684–2694.
Schattner, A., Meshorer, A., and Wallach, D. (1983): *Cell Immunol.* **79**, 11–25.
Scott, G., Cartwright, T., Le Du, G., and Dicker, D. (1978): *J. biol. Stand.* **6**, 73–82.
Sherr, C. J., Rettenmier, C. W., Sacca, R., Ronel, M. F., Look, A. T., and Stanley, E. R. (1985): *Cell* **41**, 665–676.
Silverman, R. H. (1987): In B. N. Dhawan, Maheshware and N. B. Sengh (Eds): *Interferons in Biomedical Research*, Central Drug Res. Inst., Lucknow, pp. 24–33.
Skurkovich, S., and Eremkina, E. J. (1975): *Ann. Allergy* **35**, 356–359.
Skurkovich, S., Skurkovich, B., and Bellanti, J. A. (1987): *Clin. Immunol. Immunopathol.* **43**, 362–373.
Smalley, R. V., Glaspy, J. A., Connore, J. H., Venner, P. M., Bottomley, R. L., Vutte, R. L., and Whisnant, J. K. (1987): *J. Interferon Res.* **7**, 679–681.
Smith, K. A. (1990): In E. C. Borden (Ed.): *Regulatory Cytokines*, Amgen, Roche, pp. 9–29.
Speck, R. F., and Lauterburg, G. H. (1991): *Hepatology* **13**, 557–561.
Sreevalsan, T., Taylor-Papadimitriou, J., and Rozengurt, E. (1979): *Biochem. Biophys. Res. Comm.* **87**, 679–684.
Stewart II, W. E. (1979): In *The Interferon System*, Springer Verlag, Wien.
Talmadge, J. E., Phillips, H., Schnidler, J., Trebble, H., and Pennington, R. (1987): *Cancer Res.* **47**, 5725–5732.
Talpaz, M., Kantarjian, H. M., Mc Credie, H. M., Keating, M. J., Trupillo, J., and Gutterman, J. (1987): *Blood* **69**, 1280–1288.
Tomplinson, E. (1987): *Documenta Ciba/Geigy* **5**, 8–13.
Tovey, M. G., Gresser, I., Rochette-Egly, C., Begon-Lours-Guymarho, J., Bandu, M.-T., and Maury, C. (1982): *J. gen. Virol.* **63**, 505–508.
Tsujimoto, M., and Vilček, J. (1986): *J. biol. Chem.* **261**, 5384–5388.
Vadhan-Raj, S., Brozmayer, H. E., Gutterman, J. V., Henney, C., Hittelman, W. N., Keating, M., Lemaistre, A., Mc Credie, K., and Trujillo, J. M. (1987): *New Engl. J. Med.* **317**, 1545–1552.
Vilček, J. (1984): *Prog. Med. Virol.* **30**, 62–77.
Wersall, P., Masucci, G., Nielsen, J., Kierulff, N. I., Elsen, H., Pihlstedt, P., Wigzell, H., and Mellstedt, H. (1990): *Cancer* **3**, 147–153.
Yee, A. M. F., Buyon, J. P., and Yip, Y. K. (1989): *J. exp. Med.* **169**, 987–993.
Young, D. C., and Griffin, J. D. (1986): *Blood* **68**, 1187–1191.