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Meeting Report: First Meeting of the "Task Force Cytokines" of the EORTC Research Branch

H. Zwierzina

THE RESEARCH BRANCH (Chairman: M. Rajewsky) of the EORTC was founded to narrow the gap between basic scientists and clinicians. A very promising field for cooperation between these groups is represented by cytokines, because these factors have already been introduced into *in vivo* clinical trials while, on the other hand, much scientific work remains to be done toward understanding the mechanisms of action underlying this promising new biological therapy.

Cytokines are produced by a large number of cells and serve to transmit signals mainly within the haemopoietic and the immune systems either by themselves or by inducing the production and release of other cytokines. These factors enable clinicians to carry out completely new therapy concepts by allowing them to intervene in and modulate physiological processes. The term "cytokine" includes more or less well known factors such as interferons, tumour necrosis factors (TNFs), haemopoietic growth factors and many others such as transforming growth factors (TFG-α, TGF-β), platelet-derived growth factor (PDGF) or epidermal growth factor (EGF). For historical reasons, all of these maintain their names, although these often do not correspond to their actions. Since the 6th International Congress on Immunology in 1986, every newly discovered human cytokine has been called "interleukin" (IL) once the aminoacid sequence has been defined. The number of interleukins is steadily increasing and, at the moment, 11 of these factors are cloned.

Therapy studies conducted to date with cytokines such as growth factors or IL-2 have shown that only limited conclusions can be drawn from *in vitro* and animal experiments since the intact human immune system appears to be required. Thus, new research concepts must be developed and close cooperation between clinicians and basic scientists is essential to understand the complex mechanisms of the cytokine network within the human body and the multiple aspects of a potential *in vivo* application of these factors. For this reason, the Task Force Cytokines of the EORTC Research Branch was founded and a first meeting was held at the Institute of Cell Biology (Cancer Research) in Essen, 23–24 November 1990. This meeting brought together distinguished scientists and clinicians not only from the EORTC but also from other European groups involved in cancer research, who presented their contributions in the field of cytokines and discussed future strategies for cooperation.

INTERFERONS

O. Kloke (Essen) reported on *in vitro* and *in vivo* investigations with interferon-α (IFN-α) and interferon-γ (IFN-γ) in chronic myeloid leukaemia (CML).

IFN-α is a very efficient drug in the stable phase of CML. When Philadelphia chromosome (Ph) positive CML patients are treated with IFN-α in combination with IFN-γ, neither in

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the number of complete and partial remissions nor in the prolongation of remission time is a significant advantage achieved as compared with IFN-α alone. The combination seems, however, to have a more pronounced influence on the genomic alterations specific to CML.

In order to identify phenotypic features associated with responsiveness to the growth-inhibiting action of IFN-α, the IFN-sensitive myeloid cell line U937, the IFN-resistant subline U937-R and the IFN-resistant cell lines HL-60 and K562 were studied. All these cell lines express comparable numbers of high-affinity IFN-α receptors and are IFN-reactive with regard to the induction of intracellular 2′,5′-OAS (oligoadenylate synthetase) activity. Based on the known defective activation of distinct transcriptional factors in IFN-resistant cells, IFN-α-induced activation of these DNA-binding proteins was examined. Cell lines were also assayed for expression of a series of cell surface antigens associated with leucocyte differentiation. IFN-sensitive U937 cells were found to express the myelomonocytic differentiation marker CD11b while the antigen was absent on the cell lines HL-60, K562 and on most of the U937-R cells. In addition, IFN-mediated induction of DNA-binding activity was undetectable in all IFN-resistant lines studied. The significance of these findings in monitoring response to IFN treatment in patients with myeloid leukaemias is under investigation.

HAEMOPOIETIC GROWTH FACTORS

One subgroup of cytokines is represented by the haemopoetic growth factors (HGFs). Binding of HGFs to specific receptors triggers a cascade of intracellular events resulting in cell proliferation and differentiation (R. Kaczmarski, London). Knowledge of ligand-receptor signal pathways is essential in understanding the pathophysiology of malignant disease and devising new strategies for therapy.

Receptors for IL-2a, IL-2β, IL-3, IL-4, IL-5, IL-6, IL-7, erythropoietin, granulocyte colony stimulating factor (G-CSF) and granulocyte–macrophage colony stimulating factor (GM-CSF) have recently been isolated and cloned. It has become apparent that they have structural similarity and make up a new receptor superfamily.

The finding of sequence homology of these receptors suggests that they are evolutionarily related. These receptors are transmembrane proteins, 257–856 aminoacids long. The extracellular ligand-binding domain contains four conserved cysteine residues and a Trp-Ser-X-Trp-Ser motif; these features define the members of this superfamily.

There is no sequence homology of the intracytoplasmatic domains of these receptors, although many are rich in proline and serine residues. This may be important in mechanisms of signal transduction, which are poorly understood at present. No receptor in this superfamily functions as a receptor tyrosine kinase or has intrinsic protein tyrosine kinase activity. However tyrosine and ser/thr kinases have been shown to be involved in signal transduction, acting as second messengers.

Detailed study of individual receptors holds clues to the regulation of receptor expression, ligand-receptor interactions and mechanisms involved in signal transduction, which may apply to other members of this group. Such knowledge might explain the pleiotropic effects one cytokine may have on different cell types and the overlap in function of many of the cytokines.

Autocrine secretion of IL-3, G-CSF and GM-CSF may play a role in the pathogenesis of leukaemias. Growth factor receptors have been demonstrated on many leukaemic cells and an increasing number of non-haemopoietic cells (e.g. in small cell lung cancer). As growth factor therapy is becoming part of cancer treatment, a knowledge of growth factor receptor distribution and expression by malignant cells may guide us to the appropriate choice of growth factors, avoiding those that may cause proliferation of the malignant clone.

Where proliferation of the malignant clone is dependent on autocrine or paracrine growth factor secretion, antireceptor therapies may be used to block the response. Similarly, soluble receptors, incapable of signal transduction, might prevent the action of a cytokine *in vivo*.

Further understanding of the cytokine-receptor-signal pathway will increase our understanding of the pathogenesis of cancer and manipulation of this axis has prospects for new cancer therapies.

The haemopoietic system is a very complex system with early and late acting HGFs which influence haemopoiesis at very distinct localisations. Due to their *in vitro* effects on the haemopoietic precursor cells, the HGFs are also termed colony stimulating factors (CSFs). Aside from erythropoietin there exist at least four different CSFs: GM-CSF, G-CSF, macrophage CSF (M-CSF), also known as CSF-1, and the so-called "multi-CSF", interleukin-3 (IL-3).

Recently these factors have been introduced into large clinical trials as single agents but little is known about their possible future application in combination (D. Krumwieh, Marburg). As some of these recombinant factors are very species-restricted, preclinical *in vivo* investigations must be performed in healthy cymologous monkeys to obtain results applicable to humans. The growth factor receptors in the monkeys also recognise human material but the dose must be somewhat higher. In bone marrow culture, the human and the primate cells behave rather identically. When combining these factors, the idea is to not administer all the factors together as they may counteract, but to apply them sequentially and prime the haemopoietic cells with one broader acting growth factor before applying a very restricted one.

When monkeys are treated with recombinant human granulocyte/macrophage colony-stimulating factor (rhGM-CSF) sequentially after application of IL-3, only a tenth of the dose of GM-CSF is required to obtain the same effects as with GM-CSF alone. When the time of IL-3 application is shortened to 3 days, the effect on platelets is not reduced significantly but the synergistic effect on neutrophilis is abolished.

With IL-3 as an early-acting growth factor in combination with erythropoietin as a late-acting factor, a synergistic effect on red blood cells but no effect on leucocytes can be expected. Indeed, regarding red blood cells, IL-3 is a precursor factor for erythropoietin, and a reduction of the erythropoietin dose is possible to influence erythropoiesis with IL-3 pretreatment.

Erythropoietin used in combination with GM-CSF gives only additive effects as both factors have very distinct non-overlapping activities on red blood cells or myelopoiesis, respectively.

In conclusion, it seems reasonable to combine GM-CSF with IL-3 *in vivo* in a synergistic way to generate neutrophils and eosinophils and to stimulate megakaryopoiesis in a synergistic manner. As shown by the generation of reticulocytes, a synergistic effect also occurs when combining IL-3 and erythropoietins. Whether this combination leads to an increase of red blood cells *in vivo* remains to be determined.

W. P. Steward (Glasgow) reported on the experience of the EORTC Soft Tissue and Bone Sarcoma Group in the treatment of metastatic soft tissue sarcomas with high-dose chemotherapy

and GM-CSF. As these tumours are not too frequent, therapeutic questions can only be solved in large cooperative trials.

Once soft tissue sarcoma becomes progressive and metastatic, only two drugs are available with significant activity: doxorubicin and ifosfamide. Used as a single agent, these drugs maximally show an approximately 25% response rate. In a randomised prospective trial done by the South Western Oncology Group comparing two different doses of doxorubicin, a good relationship between dose and response was apparent.

On that background, the Soft Tissue and Bone Sarcoma Group performed a randomised trial, comparing doxorubicin as a single agent (75 mg/m^2) vs. doxorubicin (50 mg/m^2) plus ifosfamide vs. CYVADIC (cyclophosphamide/vincristine/doxorubicin/dacabarzine). In more than 700 patients who had been randomised in 2½ years, the response rate was shown to be almost identical. The higher dose of doxorubicin (75 mg/m^2), however, was only applied in the single-agent arm. In the combination treatment with doxorubicin (50 mg/m^2) and ifosfamide there was profound neutropenia lasting more than 7 days; the white blood cell count did not recover within 3 weeks and most of the patients had their third course of chemotherapy delayed or the dose reduced.

As in soft tissue sarcoma a clear dose-response relationship for doxorubicin is apparent, a haemopoietic growth factor seems to be required to escalate the dose of doxorubin to 75 mg/m^2 in combination with ifosfamide and thus perhaps achieve more and better responses.

On that basis, a trial was designed for patients with advanced inoperative disease. Patients received six courses of ifosfamide in combination with the so-called optimal dose of doxorubicin (75 mg/m^2) 24 h before the commencement of GM-CSF (Behring) application at a dose of 250 μg/m^2 per day given subcutaneously for 2 weeks.

In about 110 patients it was demonstrated that with the addition of a growth factor, the chemotherapy regime was still myelosuppressive but well applicable. There was no trend to cumulative myelosuppression regarding neutropenia while the nadir of platelet count fell successively during the courses of chemotherapy. None of the courses had to be delayed, however, and there was a lower infection rate as compared to previous studies.

In conclusion, the regime showed an acceptable tolerability and, compared to previous trials, an approximately 50% higher response rate. These results provide evidence that in metastatic soft tissue sarcoma an increased dose of doxorubicin in combination with ifosfamide leads to a better response rate.

Preclinical and clinical investigations of cytokines in myelodysplastic syndrome (MDS) were discussed by H. Zwierzina (Innsbruck). MDS represents a clonal disorder of the haemopoietic stem cell characterised by dyshematopoiesis and by a variety of phenotypic manifestations. The malignant clone has the tendency to gradually replace normal haemopoiesis and lead to overt leukaemia. Thus, these syndromes are an unstable state with, on the one hand abnormal maturation of blood cells and dyshaematopoiesis and, on the other, the risk of developing acute leukaemia. As acute leukaemia, however, develops in only about 30% of patients, therapy is usually dictated more by treatment of cytopenia than by cytotoxic drugs. The incidence of MDS is at least that of acute leukaemia but there are no reports of cure.

Besides the blast cell count in the bone marrow, prognosis in MDS is closely correlated with the acquisition of lethal infections. Their development is encouraged as neutrophils are not only reduced in number but also show functional abnormalities which make neutropenia even more severe.

The EORTC Leukemia Group recently studied the effect of GM-CSF derived from Chinese hamster ovary cells (Sandoz/Schering Plough) given subcutaneously in MDS patients. Patients with a relatively low risk of developing overt leukaemia (<10% blasts in the bone marrow) were treated with GM-CSF every 12 h for 8 weeks at two different dosages (75 μg vs. 37.5 μg per injection). The most common finding in the peripheral blood was a significant increase of white blood cells (WBC) with eosinophilia often reaching 40% or more in the differential blood count. Monocytes generally only slightly increased while there was mainly a significant increase in lymphocytes accompanied by an increase of soluble IL-2 receptor. In the bone marrow, blast cell count usually remained stable, suggesting that the malignant clone was not stimulated. Based on these data the EORTC Leukemia Group is currently studying the application of IL-3 in patients with low-risk MDS.

High-risk patients (≥10% bone marrow blasts) received three cycles of low-dose cytarabine (10 mg/m^2) every 12 h for 2 weeks plus GM-CSF 150 μg every 12 h either in the second or third week. The overall response rate was 46% without significant differences between the two arms of the protocol. Good responses occurred mostly in the patients who received three or more cycles of low dose cytarabine plus GM-CSF. Toxicities were mainly caused by low dose cytarabine, while fever with GM-CSF was generally mild. As a major aim of this palliative therapy was the maintenance of life quality, the patients were taught to self-administer both drugs and were treated as outpatients.

The question as to whether survival can be prolonged by the addition of GM-CSF to low dose cytarabine cannot be answered by this study. Thus a randomised trial has now been launched which compares low dose cytarabine with low dose cytarabine plus GM-CSF versus low dose cytarabine plus IL-3 in patients with high-risk MDS.

Some studies in MDS demonstrated abnormalities of neutrophil function, which aggravate the consequences of neutropenia even more. Growth factors such as GM-CSF may induce stimulation of the leukaemic pool in patients with a high blast cell count, and IL-3 does not stimulate neutrophil function as neutrophils express no receptors for this cytokine.

IL-8, originally called "neutrophil activating factor", is a very potent inducer of neutrophil function. In bone marrow culture IL-8 only mildly stimulates growth of myeloid progenitor cells via GM-CSF induction. Neutrophil function measured in different assays such as elastase release, *Escherichia coli* killing and by chemoluminescence, is stimulated by IL-8 not only in healthy individuals but also in a large number of patients suffering from MDS. Thus IL-8 is able to repair the functional abnormalities of neutrophils in patients with MDS without essentially stimulating myelopoiesis and potentially triggering the malignant clone to overt leukaemia.

Cytokines influence the adherence of CR3-bearing mononuclear cells to the endothelium by triggering the expression of complement regulatory factors (W. Schwäble, Mainz). When cytokine-stimulated endothelium becomes adhesive for leucocytes, the process requires synthesis of membrane proteins such as intercellular adhesion molecule 1 (ICAM-1). For the rapid induction of neutrophil-endothelial adhesion, the deposition of iC3b, a cleavage product of the third component of complement, is required on the membranes of the vascular endothelium. On monocytes, granulocytes and large granular lymphocytes CR3,

a iC3b receptor, was identified as the responsible molecule for this complement-mediated cell–cell binding.

The complement regulatory proteins factor H and properdin (factor P) are involved in the conversion of C3 to iC3b. Properdin strongly supports the conversion of C3 to C3b, while factor H mediates the conversion of C3b to iC3b. Interestingly, monocytes and monocytic cell lines like MonoMac 6 express an abundant amount of properdin mRNA. IFN-γ, interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) influence the expression of properdin mRNA in MonoMac 6 cells. The amount of properdin secreted by these cells was determined with a properdin-binding ELISA. While IFN-γ showed only little or no effect on the secretion of properdin, IL-1 increases properdin levels up to 1.5-fold and TNF-α approximately 3-fold as compared to the amount of properdin detected in supernatant of untreated cells. The level of properdin measured in the binding ELISA was shown to follow the enhancement of properdin mRNA expression in northern blot experiments.

The monocytic cell line HUVEC expresses mRNA for human factor H. In primary cultures of HUVEC cells, IFN-γ increases the abundance of factor H mRNA up to 6-fold, while IL-1 showed counterregulatory and TNF-α no effect on the expression of factor H mRNA. Thus, the endothelium can assemble all factors required to convert C3 to iC3b, which was shown to be an important *in vivo* mediator in inflammatory cell recruitment.

M. Aapro (Geneva) summarised the experience of the EORTC Clonogenic Assay Screening Study Group (CASSG) with the application of recombinant cytokines.

The clonogenic assay is an interesting tool for investigating of direct effects of various agents on tumor cell lines. Although cytokine action has to be seen in connection with the complete organism, it is important to know the possible effects on *in vitro* tumour growth. For example, an inhibited growth of CML cells has been demonstrated under the influence of IFN-α. Also, combinations of IFN-α and cytotoxic agents have been tested and in some of the malignant cells a synergic action has been found.

There are several reports that GM-CSF would stimulate the growth of tumour cells via receptors at the surface of these cells. Small cell lung cancer cells, for instance, often express GM-CSF receptors, and clinicians might be afraid of using the factor. Based on these reports the CASSG group studied the effects of human recombinant GM-CSF in continuous human cell lines. A broad spectrum of doses was applied to various malignant cell lines (mainly breast, ovarian and colon cancer) in three different types of assays.

The conclusion from this collaborative study is that moderate increases of tumor cell line growth may occur under GM-CSF. The *in vivo* relevance of this finding, which must be compared with the dose of GM-CSF is absolutely unknown.

INTERLEUKINS

As a highly potent pyrogen and mediator of the acute-phase response, IL-1 is responsible for many reactions connected with the onset of an infection and is an important regulator of immunity and inflammation (W. Fibbe, Leiden). Furthermore, IL-1 possesses a broad range of action within the haemopoietic system and is involved in manifold physiological processes of the body. IL-1 is produced in a wide variety of cell types: keratinocytes, fibroblasts, endothelial cells and cells of the smooth muscle, of the renal mesangium and of the microglia and certain B and T cell lines.

When added to human bone marrow culture in a semisolid assay without any other stimulus, IL-1 induces colony formation to an extent similar to GM-CSF. For this reason, it was originally termed haemopoietin 1. After depletion of the bone marrow culture of mononucleated cells, IL-1 is no longer able to stimulate colony formation, suggesting that the effect on haemopoiesis is indirectly mediated by growth factors produced by accessory cells. Indeed, colony formation is shown to be dependent on the number of accessory cells added.

IL-1 acts synergistically with other growth factors on haemopoietic progenitor cells. In primates, the factor accelerates the recuperation of haemopoiesis after chemotherapy when it is administered for less than 7 days. Longer administration produces the opposite effect, probably being dependent on an induction of TNF-α production.

IL-1 on the one hand leads to an activation and proliferation of earlier haemopoietic precursor cells and to the upregulation of their receptors for CSFs, and on the other induces the production of CSFs through various accessory cells. In costimulation with IL-3, and also with M-CSF, it stimulates the proliferation of multipotent stem cells. There is also an effect on relatively mature progenitor cells which is mediated by the production of G-CSF. All these activities of IL-1 appear to be indirect.

IL-1 can be used as a priming agent to mobilise stem cells from the bone marrow to the peripheral blood and therefore might be of benefit in an autologous transplant model. After injection of IL-1, a significant dose-dependent increase of the number of progenitor cells occurs, and neutrophilia usually develops with peak levels 4–8 h after injection. When a transplantation with peripheral blood cells is done after the donor is treated with IL-1, the survival curve shows that pretreatment leads to a much better survival rate due to acceleration of haemopoietic reconstitution. Thus IL-1 induces an increase in neutrophils and CFU-GM in a dose-dependent manner and this effect might be used to improve long-term survival after autologous bone marrow transplantation. Furthermore, IL-1 induces an increase of the bone marrow repopulating cells, which might provide a means for its clinical use.

IL-6 was originally identified as B-cell stimulating factor 2 (BSF-2) present in culture supernatants of mitogen-stimulated or antigen-stimulated peripheral mononuclear cells and inducing Ig production in Epstein–Barr virus-transformed B-cell lines or in mitogen-stimulated normal B-cells. Among a number of other physiological functions, it interacts with various haemopoietic growth factors to enhance the growth of multipotential progenitor cells and is involved in megakaryocyte development in bone marrow cultures. It is, however, also a potent growth factor for myeloma cells, suggesting a possible role in the generation of multiple myeloma.

In this disease, malignant plasma cells are often disseminated throughout the skeleton at a very early stage of the disease, while they are not found in the circulation (F. Caligaris-Cappio, Turin). A possible explanation for this phenomenon is that plasma cell precursors circulate in the peripheral blood in disguise and having once reached the bone marrow, find an optimal microenvironment in which to settle. In this process, cytokines appear to play a major role.

When bone marrow stromal cells of myeloma patients are cultured, fibroblasts and some macrophages can be detected after approximately 1 week. Furthermore, there are rare giant cells with the morphology and the cytochemical staining of osteoclasts. The finding that in multiple myeloma bone marrow

stromal cells are also represented by osteoclasts is of major interest, because the interaction of proliferating malignant cells and osteoclasts is of great relevance in the light of the generation of osteolytic lesions.

In various myeloma patients studied, stromal cells produce large amounts of IL-6 measured in the culture supernatants with ELISA. Another cytokine produced by these cells is IL-1β, while no TNF-α, IL-2, IL-3 or IL-4 can be detected.

Peripheral blood mononucleated cells which have been depleted of residual lymphoid cells can be seeded over the feeder layer of the stromal cells of the same patient and co-cultured for 3 weeks. In this autologous system, malignant monoclonal B-lymphocytes and plasma cells develop and a striking increase can be found in the number of osteoclasts. In contrast, no osteoclasts or osteoclast-like cells can be detected. In co-culture of stromal cells and mononucleated cells the amount of IL-6 and IL-1β is significantly increased and IL-3 also becomes measurable in the culture supernatant.

Thus, in the pathophysiology of the disease which is characterised by the development of osteolysis, stromal cells are necessary for the generation of osteoclasts, and cytokines like IL-6, IL-1β and IL-3, which are able to activate osteoclasts, become measurable in the supernatants.

The growth of normal and malignant B-lymphocytes is strictly regulated by cytokines in an autocrine and a paracrine manner. There are more and more indications that these cytokines may also be involved in the course of neoplastic B-cell disorders (A. Biondi, Monza).

The proliferation of some lymphoid cell lines, which constitutively express the IL-6 gene, fulfills the criteria of autocrine growth as these cells only grow when they have a certain density. When adding a polyclonal antibody against IL-6 to these cell lines, growth decreases significantly while it is induced by addition of exogeneous IL-6.

IL-6 gene expression can also be detected in lymphoid diseases which represent more mature stages of lymphoid development. No IL-6 expression can be found in the B-cell type of acute lymphoblastic leukaemia (ALL), which is considered to be derived from an early stage of B-cell development. In contrast, approximately 50% of B-cell chronic lymphocytic leukaemia (B-CLL) cases express the message for IL-6 constitutively, as measured by northern blot analysis. This constitutive expression of IL-6 transcripts is associated with production of a biologically active protein. Besides IL-6, IL-1β and TNF-α have also been reported to be produced by B-CLL cells.

The biological significance of IL-6 expression by B-CLL patients remains to be elucidated. IL-6 is a B-cell growth factor and differentiation factor, and expression of growth factor genes may play a role in autocrine or paracrine growth regulation of malignant cells, as demonstrated for plasmocytoma. However, as B-CLL cells have little proliferative activity, it remains to be determined whether IL-6 gene expression is indeed important in the regulation of B-CLL growth or its clinical manifestations.

IL-7 is a product of bone marrow stroma cells which controls the growth and differentiation of B-cell precursors without affecting cells of the myeloid lineage (T. Rollink, Basel). This led to the hypothesis that IL-7, produced in bone marrow, is responsible for differentiation of the pluripotent stem cell in the direction of lymphopoiesis and not of myeloid differentiation. While mature B-cells seem to be unaffected by IL-7, highly purified T-cells cultured with ConA plus IL-7 have significantly increased levels of IL-2 receptor expression, IL-2 production and proliferation. Thus, IL-7 also exerts costimulatory activity on purified T cells.

Most of the studies of factors involved in B-cell growth and differentiation were performed in normal B-cells, since it is imperative that we know the physiological effects of cytokines in order to possibly direct these cells to attack neoplastic cells in future. Much information can be gained from the *in vitro* establishment of pre-B-cell lines having the capacity to differentiate into antigen-reactive mature B-cells.

In germ line configuration, immunoglobulin genes are not able to produce immunoglobulins. During pre-B-cell development the different stages are defined by a stepwise rearrangement of the immunoglobulin locus, which finally leads to mature B-cells able to produce immunoglobulin. For their maturation, B-cells require strong stroma cell contact. The proliferation of pre-B-cells furthermore demands the presence of IL-7, which is a very potent growth factor for these cells and cannot be replaced by any other interleukin. However, IL-7 is not able to fully substitute for stroma cells, which means that other factors are also necessary for the growth of pre-B-cells.

IL-8, which was recently cloned (I. Lindley, Vienna), is a 72-aminoacid polypeptide originally isolated from endotoxin-stimulated monocytes, also called neutrophil activating factor 1 (NAP-1). Closely related factors concerning the activity of NAP-1 are NAP-2, melanoma growth stimulating activity and platelet factor 4. IL-1 and TNF induce the production of IL-8.

Mediated by a selective surface receptor, IL-8 induces exocytosis and respiratory burst in neutrophils and leads to improved chemotaxis. Furthermore, it triggers histamine release from IL-3-primed basophils and induces dose related effects on adherence of neutrophils as well as monocytes and melanoma cells. When given to experimental animals by systemic injection, it seems to induce granulocytosis. Intradermal injection of rIL-8 into rats causes not only dose-dependent accumulation of neutrophils but also of lymphocytes. This strong chemotactic effect on lymphocytes, however, is only seen in rats in which lymphocytes account for more than 90% of peripheral blood cells. When IL-8 is injected subcutaneously into human skin, only neutrophils move to the injection site.

Improved neutrophil function can be determined by the *E. coli* killing assay. If in mice IL-8 is injected into the peritoneum together with *E. coli*, a protection vs. *E. coli* growth is effected. This protection cannot be seen, however, when *E. coli* and IL-8 are given intravenously or subcutaneously.

High serum levels demonstrated with ELISA are found in septic shock patients and to a lesser extent in patients suffering from rheumatoid arthritis. In rheumatoid arthritis patients, a correlation also appears to exist between disease activity and IL-8 level in the synovial fluid. Whether or not IL-8 antibodies are able to play a role in the treatment of chronic inflammatory diseases is yet to be determined.

EPIDERMAL GROWTH FACTOR (EGF) AND TGF-α

According to the hypothesis of autocrine tumour growth, malignant cells may express, synthesise and secrete growth factors in higher amounts than normal cells and trigger these cells into proliferation. Clear data, however, regarding a relationship between growth factor levels in the serum or urine of patients and their clinical outcome are lacking (R. Grosse, Berlin).

A polypeptide with epidermal growth factor (EGF)-like activity was studied in the urine of breast cancer patients. This molecule was shown to be clearly different from EGF and TGF-α. As it is immunoprecipitated by an EGF antiserum and its

activity is neutralised by an EGF antiserum and not by a TGF-α antiserum, the polypeptide is immunologically related to EGF but not to TGF-α.

When the relationship of this EGF-like activity and clinical factors is studied by analysing urinary fractions from breast cancer patients, the patients can be divided into two groups with low and high activity and a correlation with the tumour load is found. EGF-like activity is significantly enhanced in the oestrogen receptor negative group of breast cancer patients. In a clinical trial with 31 patients the activity was controlled before and after neoadjuvant chemotherapy. Before initiation of therapy, no significant difference could be determined among the patients, while after termination of chemotherapy a clear statistical correlation between progressive patients and increase of EGF-like activity was found. A significant decrease of activity was measured in patients responding to chemotherapy.

Long-term follow-up of EGF-like activity in 25 patients during their disease demonstrated a clear relationship between growth factor activity and long-term survival. Long-term remissions never demonstrated an increased level, while most progressive patients showed an increase in activity up to 2–5-fold the initial value.

In a number of different tumours, it was recognised that EGF receptor can be a prognostic index (R. Leake, Glasgow) and, for instance in breast and bladder cancer, a higher number of EGF receptors means a poorer prognosis. However, this is not true for all tissues, as in ovarian cancer a high EGF receptor expression correlates with good response to chemotherapy and longer survival. Embryonic and developing tissues usually contain very high levels of TGF-α, and the levels decline when the tissue differentiates. Thus, TGF-α is a normal growth promoting factor not only associated with neoplastic cell growth but also involved in normal cell proliferation.

As TGF-α does not act through its own receptor but by binding to the EGF receptor, TGF-α levels in gynaecological tumours, particularly in ovarian cancer, were studied. In these tumours, paracrine mechanisms might promote growth and thus have a future theapeutic impact by trying to reduce tumour growth by blocking either the factor or its receptor. The vast majority of ovarian cancers contain detectable levels of TGF-α and the values are rather identical for all histological subentities. No conclusion, however, has been drawn until now from an ongoing study of whether high levels of TGF-α correlate with prognosis. There are also tumours with a high level of TGF-α, which are EGF receptor negative.

Endometrium cancer also shows a trend to an extremely high TGF-α concentration in patients who die within a rather short time. Thus, in this tumour TGF-α activity might be an index for prognosis.

TGF-β activity, which can be induced by IFN-α, also influences tumour growth by very efficiently diminishing growth of breast cancer cells. Thus, on the one hand, TGF-β decreases growth while, on the other, TGF-α induces proliferation of breast cancer cells, provided there are enough receptors present.

MOTILITY FACTORS

Scatter factor (SF) purified from the supernatant of fibroblasts belongs to the group of the so-called motility factors (W. Birchmeier, Essen). This group of cytokines also includes "migration stimulating factor" or "autocrine motility factor". The receptors for all these factors are unknown.

As tumours can grow to an enormous size without metastases, the growth of epithelial cells is not the only factor of major importance in the prognosis of malignant diseases. The potential to invade and to metastasise is even more important and is often correlated with dedifferentiated phenotype.

SF, a 92 kD protein, is involved in cellular invasion. It is produced by mesenchymal cells, fibroblasts and smooth muscle cells in a paracrine fashion and acts on epithelial cells, where it induces a dedifferentiated stage. When SF is added to the medium of epithelial cells, they no longer grow in colonies but become scattered, stop interacting with each other and take on the appearance of fibroblasts.

SF is identical to hepatocyte growth factor (HGF), which was recently cloned, and can be detected in the blood of patients with fulminant hepatic failure. As the motility and dissociation promoting activity is not shown in the hepatocyte system, HGF and SF have an identical structure but show very different activities in various systems. It is not known whether factors like EGF or TGF-α, which are able to promote tumour growth, induce SF production.

PLATELET-DERIVED GROWTH FACTOR (PDGF)

Platelet-derived growth factor (PDGF) was originally found to be released by the α-granules of platelets during blood clotting at the site of a wound, and it is thought to promote wound repair by stimulating growth of fibroblasts. Two distinct receptors (α and β) exist for the molecule and demonstrate a very similar structure, with certain domains resembling part of immunoglobulin molecules (L. Claesson-Welsh, Uppsala). The intracellular structure of the receptor is similar to tyrosine kinase receptors, except that the tyrosine kinase domain is split into two parts by the insertion of a so-called kinase insert sequence. This sequence might be responsible for the association between receptors and substrates.

At least in culture, PDGF is expressed by almost all cells including epithelial and connective tissue cells. Expression occurs in a differentiation-dependent manner and is regulated by cytokines and growth factors. As PDGF is found in tropoblastic and embryonal cells, it seems to be involved in embryonal development. Furthermore, PDGF is a differentiation factor for glial cell development and is involved in bone healing.

In cell and tissue culture, the PDGF receptor is expressed on mesenchymally derived cells like fibroblasts and glial cells, but can also be found on epithelial or endothelial cells. Binding of PDGF to the receptor molecule induces three types of specific dimerisation of the receptor, possibly representing unique functional abilities. PDGF itself leads to an upregulation of mRNA expression for both its receptors and, when added to fibroblasts, they begin to express PDGF receptors as they do after stimulation with IL-1, TNF or EGF. Cells involved in inflammation generally show an upregulation of PDGF receptor expression, as can be found in rheumatoid arthritis or inflammation of the skin or kidney.

CONCLUSIONS

The aim of the Task Force Cytokines of the EORTC Research Branch was to coordinate clinical trials with scientific evaluation and to develop a close relationship not only among the EORTC Groups but also with other groups involved in cytokine research.

At the meeting, current knowledge on cytokines was updated in presentations and extensive discussions and it became apparent that common ground existed between the participants. It was felt that the effectiveness of the clinical studies conducted by the EORTC as well as by other groups could be improved by coordinating scientific programs around these trials. On the

other hand, discussion between basic scientists and clinicians concerning the therapeutic potential of at the moment purely experimental cytokines is very important for the development of cytokines for future clinical application.

All the participants agreed that there is an urgent need to establish formal research centres in order to understand the mechanisms involved in the regulation of cytokines and their effects. Such research projects, requiring basic scientists as well as clinicians, will be defined within the next months in order to determine the important aims of clinical development.

A circular will be sent out to all chairmen of Treatment and Research Groups of the EORTC as well as to other European groups involved in cytokine research. This circular will provide information on the activities of the Task Force Cytokines and request information on all running or planned clinical trials with cytokines in order to build up an accompanying scientific program. Research groups will be asked to give recommendations for these accompanying programs.

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Recent Advances in the Biology of Lymphomas

P.G. Isaacson

IN TERMS of lymphoma biology, the 1970s can only be described as the decade of classification. As more powerful chemotherapeutic agents became available, clinicians began to demand a more rational and reproducible histological classification of lymphoma which gave some indication of the biological behaviour of the disease and permitted comparison of the results of clinical trials. In this respect the Rye classification of Hodgkin's disease (HD), formulated in 1966 [1], was already a success but something of equal value was urgently needed for the non-Hodgkin lymphomas. Starting with the discovery by Lennert [2] that a significant number of lymphomas was derived from follicle centre cells, new classifications, each claiming both biological significance and reproducibility, flowered; this resulted in more rather than less confusion in both pathological and clinical circles and a louder than ever cry for a single meaningful classification. Pathologists responded with a complex and exhaustive multicentre study which resulted in the compromise, and to many wholly unsatisfactory, morphologically based classification of non-Hodgkin lymphoma known as the "working formulation for clinical use" [3]. By the time this classification appeared, however, exciting new techniques were already having a significant impact on lymphoma research. Immunocytochemistry, coupled with the availability of an ever increasing number of monoclonal antibodies, permitted identification of cell phenotype and other characteristics in histological sections providing opportunities for better and more scientifically based classifications. Even before the advances permitted by these techniques could be properly consolidated the principles of cell and molecular biology were finding ready application to lymphomas and this effectively ended the obsession with classification. The impact of these newer biological sciences on the study of lymphoma has been tumultuous, leading even to a blurring of the almost sacrosanct division between HD and the non-Hodgkin lymphomas. There is no doubt that at some time in the not too distant future the thorny problem of classification will have to be addressed again in the light of all these advances. Meanwhile, those engaged in lymphoma research continue to enjoy a rich harvest as the seemingly unending flow of new techniques continues to refine our knowledge of lymphoma biology and leads to the discovery of new entities.

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NEW TECHNIQUES IN LYMPHOMA BIOLOGY

Immunophenotyping

Before 1980 the methods for identifying the phenotype of lymphoma cells were confined to the study of cell suspensions. Because of the large reactive cell population present in lymphomas this technique was not entirely satisfactory. In 1980 Stein *et al.* [4] described successful immunophenotyping of lymphomas by applying monoclonal antibodies to cryostat sections using the immunoperoxidase technique and this, together with the development of newer techniques for the study of cell suspensions using the fluorescent activation cell sorter (FACS), has had a profound effect on lymphoma research and diagnosis. The vast number of monoclonal anti-leucocyte antibodies produced has led to the institution of special international workshops for their classification and standardisation and the introduction of the CD (cluster of differentiation) nomenclature. The antigens recognised by leucocyte differentiation antibodies not only assist in identifying the lineage and differentiation state of the cell or cells in question but also represent functionally important cell surface bound ligands and membrane associated enzymes together with both cytoplasmic and nuclear antigens. Thus, with improved optical resolution now afforded by antibodies that recognise formalin resistant epitopes in paraffin sections, the biology of cell to cell interactions can be studied. The addition of *in situ* molecular hybridisation techniques (see below) has served further to enhance the value of both current and archival histological material in lymphoma studies.

Molecular genetic analysis

In most instances immunocytochemistry permits determination of the lineage (i.e. either B or T cell) in a case of lymphoproliferative disease and in B cell lymphoproliferation