CLINICAL TUMOR IMMUNOLOGY

EORTC Monographs on Cancer

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INTRODUCTION

THE IMMUNOLOGICAL DREAM OF AN ONCOLOGIST

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This book is the Proceedings of the Symposium of Clinical Tumor Immunology held in Brussels (May 26–29, 1975) and organized jointly by the EORTC and the Department of Immunology of the University Hospital of Brussels. This Conference was mainly educational and developed in a few days most of the general topics and some special subjects related to this new field of Cancer Medicine.

The importance of immunology is, indeed, growing not only in oncology but in most sectors of medicine. This can easily be explained. First, the basic mechanisms of the immune reactions are better understood and immune phenomenon in diseases appear numerous. Secondly, immunological reactions in biological and medical sciences are widely used. This is mainly due to the fact that they offer both the specificity and the possibility of measuring trace amounts of substances (e.g. radioimmunoassay of hormones, blood proteins). Finally, and therefore, the immunologist has left his ivory tower to join the glorious life of the day to day medicine. Immunology is no more dealing only with animals but has wide human applications. It is frequently possible to determine the specific defect in patients with immunological diseases and sometimes completely correct it. The oncologist asks four basic questions of the immunologist. Their answer should provide a clue to the diagnosis, treatment and prevention of cancer.

(a) Is there an immunological defect in cancer patients? If yes, is it primary or secondary? A primary failure is not easy to determine since only a prospective study of the normal population by a battery of tests could possibly detect those subjects who have perhaps only subtle immune defects and who will develop a malignancy. Also, if this study could theoretically be done, such a defect, if present, may be transient which implies frequent testing. Finally, the tests should be rapid, reliable, reproducible and done on a small blood sample. These are difficult criteria. Nothing, as yet, suggests that this type of test is known. Therefore the question of a primary defect in cancer remains unanswered.

(b) The second question asked of immunologists is to provide a way of making an early diagnosis of cancer. Some hopes, in this field, were raised by the discovery of fetal-linked tumor specific antigens. It appears that their determination is not yet helpful in making a clear-cut diagnosis. This is, quite often, due to their non-specificity, for both the diseased organ and the type of disease. A lot of non-malignant diseases can be associated with measurable levels of these foeto-proteins. Similarly, malignant diseases

may not be associated with such foeto-proteins. These antigens are, however, of value in determining the recurrence or the cure of disease. Since this area is being intensively investigated, a good chance exists to find a battery of foeto-antigens, organ and disease specific. For instance, alpha-protein is very interesting. It appears to be a rather specific marker for hepatoma and some testicular carcinoma. Recently, it was discovered that it has immunosuppressive properties. This finding should open a new avenue of investigation and, perhaps, bring some exciting new concepts regarding the question of immune defect in cancer.

(c) A third important question asked of immunologists concerns the field of therapy. According to classical data of cell kinetics, chemotherapy will not kill the last tumor cell. It has been proposed that the role of immunotherapy is to eradicate these last tumor cells. Some preliminary data also suggest that immunotherapy could act in combination with chemotherapy or other forms of classical therapy.

Of interest is that cytotoxic drugs given on an intermittent basis are not, or are less, immunosuppressive than the one given on a chronic basis. Recent data also appear to indicate that immunotherapy can be helpful even in the presence of a relative number of tumor cells. Current clinical applications, fully discussed in this book, indeed suggest that immunotherapy can be a useful adjunct to classical therapy. Finally, since so few tests correlate with the clinical status of the patients, who do or do not respond to therapy, one could heretically think that non-specific immunotherapy works, in fact, through non-immune mechanisms.

(d) The final and more important question concerns the hope of an immune prevention of cancer. For instance, conflicting data suggest that BCG vaccination, in neonates, decreases the incidence of acute leukemia. Active immunization by a vaccine against cancer seems now a dream, but progress in the fields of basic science, virology, immunochemistry and cellular immunology should eventually materialize such a dream and kill the nightmare of cancer.

EORTC, AIM, STRUCTURE AND ACTIVITY

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The aims of the European Organization for Research on Treatment of Cancer (EORTC) are to conduct, develop, coordinate and stimulate research in Europe on the experimental and clinical bases of treatment of cancer and related problems. Extensive and comprehensive research in this wide field is often beyond the means of the individual European laboratories and hospitals and can be best accomplished through the joint efforts of the clinical and basic research groups of several countries.

The organization was founded in 1962 by research workers in the main cancer research institutes of the Common Market countries and Switzerland. It was named "Groupe Européen de Chimiothérapie Anticancéreuse" (GECA). Representatives from Great Britain and from Austria joined the organization in 1966 and 1970 respectively. EORTC has a Council which plans and supervises the activities within the organization. The Administrative Board of the Council prepares the meetings of the Council and handles the daily work involved with administrative and financial matters. Within the EORTC clinical and laboratory research is carried out by clinical cooperative groups, cooperative research project groups, working parties and research information exchange clubs. The Council meets six to eight times per year in the various European institutes associated with EORTC to discuss scientific as well as administrative matters. At regular intervals it receives the results of the drug screening programs and those of the clinical cooperative groups and the research project groups. Cooperative groups, project groups, working parties and clubs meet as often as their activity requires. An annual plenary meeting is organized in June for all members of EORTC on one or more subjects related to the treatment of cancer.

EORTC has initiated four major efforts in the field of cancer treatment:

- a screening program of potential anti-cancer agents;
- the organization of clinical and preclinical cooperative groups aimed at carrying out controlled clinical trials with new therapeutic agents and regimens;
- the organization of collaborative research programs;
- the initiation of symposia, courses and publications on the subject of cancer research and treatment.

The clinical cooperative groups have organized themselves under the auspices of EORTC to carry out collaborative work, mostly aimed at the clinical evaluation of new drugs and treatments for cancer. Through this cooperation, data on large numbers of

patients treated according to one or more accepted protocols can be collected at a much faster rate than within any of the clinical centers alone. To assist the groups in their administrative and scientific work, the EORTC Coordinating Office was established in 1969 and expanded in a EORTC Coordinating Office and Data Center in 1974. The purpose of the Coordinating and Data Center is to provide central coordination for the activities of the EORTC groups and to make available to the groups a wide range of statistical and data processing expertise, including advice on study design as well as analysis and reporting of results, at a central, and hence economical, location.

EORTC is not a scientific society, but a transnational institution consisting of laboratories and clinical services situated in different countries: 15 laboratories, 1500 research personnel, 200 clinical services and several hundreds of clinicians are associated with EORTC. This structure permits an increasing number of patients to be treated every year, according to the highest international standards and in such a way that the results of the treatment can be evaluated within a short time. It also provides opportunities for the organization and integration of research programs on cancer treatment on a level that could never be attained by individual institutes or services.

Table 1. *EORTC Trials Involving Immunotherapy*

		Trial's secretary
ACUTE MYELOCYTIC	two arms protocol comparing chemo-immuno-therapy to immunotherapy during maintenance	M. HAYAT (Villejuif)
EPIDERMOID BRONCHIAL CARCINOMA	four arms protocol comparing chemo-immuno-therapy to chemotherapy to immunotherapy and to no treatment in postsurgical patients	L. ISRAEL (Paris)
EPIDERMOID BRONCHIAL CARCINOMA	same as above in inoperable irradiated patients	L. ISRAEL (Paris)
MALIGNANT MELANOMA	two arms protocol comparing immunotherapy to no treatment in residual primary melanoma of the skin	J.P. CESARINI (Paris)
MALIGNANT MELANOMA	four arms protocol comparing immunochemothe-rapy to chemotherapy to immunotherapy and to no treatment in postoperative melanoma of the skin with positive lymph nodes	J.P. CESARINI (Paris)
ACUTE LYMPHOBLASTIC LEUKEMIA	two arms protocol comparing chemotherapy to immunotherapy in maintenance	P. STRYCKMANS (Brussels)

Numerous attempts at modifying the course of cancer by inducing changes of the immunologic response of the host have been tried in the last 10 years. Although a rational approach to the problem is still missing because of the lack of knowledge of fundamental mechanisms, several empirical trials have indicated the possible usefulness of immunological treatment. Many authors have shown that immunotherapy alone is unable to inhibit the progressive growth of a tumor, and that it will be best used in conjunction with surgery, chemotherapy and irradiation. This, in turn, explains the very complex clinical trials involving strict prognosis stratification. Because of the large number of patients which will be necessary to detect small changes in responses to

therapy, very few clinical institutions will be able to conduct phase III trials alone. Therefore, they have to resort to multi-center trials and complex statistical techniques. EORTC has initiated six cooperative clinical trials (Table 1) in the field of immunotherapy, all using live BCG.

The symposium reported in this issue is part of a collaborative effort aiming to inform oncologists in the recent developments in cancer research and to increase collaboration between the researchers in the field of immunology.

EORTC is grateful to the chairmen of the sessions and to the lecturers for their collaboration.

CONTENTS

GENERAL MECHANISMS IN TUMOR IMMUNITY

BIOLOGICAL ACTIVITIES OF THE CIRCULATING THYMIC HORMONE

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The mechanisms of T-cell maturation are still a matter of speculation and controversy. There are several arguments in favour of a direct differentiation of stem cells at the contact of the thymic epithelium microenvironment. The seeding of T-cells from the thymus, as proved by experiments using chromosomal markers [1] and the presence itself of large numbers of lymphocytes within the thymus, are among the best of these arguments. Conversely, the restoration of immunocompetence in neonatally thymectomized (NTx) mice by thymus grafts in cell-impermeable Millipore chambers [2], and especially by cell-free thymic extracts, argue in favour of humoral mechanisms [3, 4]. In fact these two theories are not mutually exclusive since, as is known for erythrocytes or granulocytes, there might be a first microenvironmental stage of differentiation followed by a hormone-dependent phase.

Our approach to the problem was perceptibly different from that mentioned above. We had demonstrated that a large percentage of rosette-forming cells (RFC) are thymus-dependent and bear the theta (θ) antigen, specific to T-cells [5–7]. Conversely, other RFC, thymus-independent, do not bear the θ antigen and include both B-cells and T-cell precursors. We first showed that thymic extracts (given by Goldstein and White, and Trainin) induced the appearance of the θ antigen on certain RFC from the bone marrow [6, 8, 9]. Similarly, spleen cells from adult thymectomized (ATx) mice, which had lost the θ antigen [8], recovered their sensitivity to anti-θ serum after *in vivo* or *in vitro* treatment by thymic extracts. These experiments, perhaps more analytical then the previous ones by Goldstein and White, and Trainin, were, however, submitted to the same specificity criticism, even if spleen extracts were essentially inactive. The demonstration that there was a substance in normal mouse serum, with the same activities as thymic extracts, that was absent in the serum of Tx mice brought the definitive proof of the specificity of the phenomenon [10, 11]. Besides the action of the hormone on RFC provided a simple and reproducible bioassay that allowed us to isolate and characterize the serum thymic hormone. The hormone appears to be a peptide with a molecular weight (MW) of about 1000; i.e. 10 times lower than that of the thymosin of Goldstein and White, but also 10 to 1000 times more active for the same molarity. We have temporarily called this peptide "thymic factor" (TF) and not "thymosin" because the thymic factor and thymosin are obviously two distinct entities, even if they may be biochemically related.

I shall not give details, in this article, on TF biochemistry nor on the physiological conditions of its secretion (such as age-dependence, thymectomy and thymus grafting experiments published elsewhere [12]), limiting myself to the TF biological activities. This is a particularly important matter since the assay in which TF has been isolated and characterized is an *in vitro* assay and as such always submitted to a number of possible artefacts and to pharmacological effects of biologically irrelevant substances.

The effects of pig TF have been studied in various *in vivo* and *in vitro* systems. It should be emphasized that all the studies which will be mentioned have been performed with fairly purified preparations, active *in vivo* and *in vitro* at ng levels at variance with experiments reported by other authors, using rather unfractionated preparations (even if purified materials had been made available for biochemical studies) at μg or even mg levels. One should also stress that in *in vivo* studies TF was bound to carboxymethyl-cellulose (CMC) in order to increase its half-life [12].

THETA CONVERSION

Purified TF can induce the appearance of the θ antigen on previously θ-negative RFC. However, with some very active AθS batches, it may be shown that there is in fact some inhibition on the TF-sensitive RFC before TF treatment, as seen in normal bone marrow or in spleen from ATx or nude mice. In that case, TF increases θ antigen expression rather than provokes its appearance [11, 13].

Similar data have been obtained in normal spleen cells at ng levels using a cytotoxic assay after cell fractionation on a BSA gradient, as described by Komuro and Boyse [14]. These results [13] must, however, be interpreted with caution since some θ conversion can be obtained by incubating the cells at 37°C without any added material, suggesting a possible fragilization of cells with small amounts of θ antigen, making them more sensitive to otherwise non-cytotoxic concentrations. This criticism does not apply to the sheep cell rosette assay which does not involve BSA fractionation and long-term incubation.

The mechanism of action of TF on RFC θ conversion has been investigated with regard to the cAMP system. In brief, TF effects are mimicked by low (and physiological) concentrations of cAMP, theophylline and prostaglandins [13]. One can even show a synergy between cAMP and TF, both used at subliminal levels. Lastly, indomethacin, a prostaglandin synthetase inhibitor, blocks TF effect [13] as well; injected into normal mice it decreases transiently the number of θ-positive cells in the spleen (but interestingly not in lymph nodes) [15]. Finally, it appears that θ antigen expression has a rapid turnover and is probably regulated by agents altering cAMP synthesis, TF possibly acting in this system through stimulation of cAMP synthesis, eventually itself through prostaglandin stimulation in view of indomethacin data.

MITOGEN RESPONSES

TF has been shown to induce some responsiveness to Concanavalin A (Con A) and, to a lesser degree, to PHA in nude mouse spleen cells and in low density normal spleen cells separated on a Ficoll gradient (P. Hayry and J.F. Bach, in preparation). However, one should emphasize that the increase, although significant, was not dramatic (×2–×4).

Similarly, a 2-week treatment with TF increases significantly Con A and PHA responses in the spleen of NZB mice at an age when they show decreased mitogen responses. Interestingly, a similar restoration is obtained by grafting a thymus eventually in a Millipore chamber (to be published).

AUTOLOGOUS ROSETTES [16, 17]

A small percentage of normal mouse lymphocytes form rosettes when mixed with autologous (or syngeneic) erythrocytes. These autologous RFC (ARFC) normally predominate in the thymus. Interestingly, their number increases with age and especially after ATx within a few days after the operation, ARFC level reaching its peak 30–45 days after the operation (x 20 increase). TF normalized ARFC level in ATx mice injected *in vivo* or after *in vitro* incubation. ARFC characteristics indicate that they belong to the pool of post-thymic or To cells. The significance of the ARFC phenomenon is still obscure but might have some relevance to autoantigen recognition. Anyhow, autologous rosette formation appears to be a marker of To cells and the effects of TF on this marker can be utilized as a useful TF assay.

ANTIGEN-INDUCED CAPPING

It has been reported that RFC (like radio-labelled antigen-binding cells) redistribute their antigen-binding receptors after 15 min incubation at 37°C with the antigen in question. We have shown that such "capping" could be observed at the T-RFC level in the spleen and that ATx depleted the capacity of these T-spleen RFC to cap. Interestingly, *in vitro* treatment with purified TF rapidly restored normal capping characteristics when added to the spleen cells simultaneously with SRBC. Whereas normal mouse serum also showed that effect, serum from ATx mice and nude mice did not exhibit it [18].

STEROID RECEPTORS

Thymocytes include two cell subpopulations, one mainly present in the cortex which is sensitive to the *in vivo* and *in vitro* lytic action of steroids, the other predominant in the thymus medulla which is corticoresistant. It has been reported by Trainin [19] that *in vitro* incubation of thymus cells with the dialysable thymus humoral factor decreased the sensitivity of thymocytes to *in vitro* cortisol-induced lysis. We have investigated this experimental system by studying the receptors for steroids present on the surface of thymocytes in collaboration with D. Duval. These receptors, which are detected by their property of binding triated dexamethasone, are specific for cortisol. They are located in the cell cytosol from which they are extracted. We have examined the effect of *in vitro* TF treatment of thymocytes on the expression of steroid receptors. Purified TF was shown to induce a 70% inhibition of steroid-specific uptake by thymus cells, suggesting that decrease in the number or in the availability of receptors for steroids is one of the mechanisms by which T-cells lose their high steroid sensitivity as they mature. Our experiments also indicate that such changes might be due to TF action (to be published).