# ADVANCES IN CANCER RESEARCH

*Edited by*

GEORGE KLEIN

SIDNEY WEINHOUSE

# ADVANCES IN CANCER RESEARCH

*Edited by*

GEORGE KLEIN

Department of Tumor Biology
Karolinska Institutet
Stockholm, Sweden

SIDNEY WEINHOUSE

Fels Research Institute
Temple University Medical School
Philadelphia, Pennsylvania

*Volume 45—1985*

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
London Montreal Sydney Tokyo Toronto

ACADEMIC PRESS, INC.
Orlando, Florida 32887

*United Kingdom Edition published by*
ACADEMIC PRESS INC. (LONDON) LTD.
24–28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 52-13360

ISBN 0–12–006645–9

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3 2 1

# CONTENTS

## Down-Regulation of the Antitumor Immune Response

ROBERT J. NORTH



## Cellular Aspects of DNA Repair

BERNARD S. STRAUSS

## The *Blym* Oncogenes

PAUL NEIMAN



## Retrovirus-Induced Acquired Immunodeficiencies

MAURO BENDINELLI, DONATELLA MATTEUCCI, AND HERMAN FRIEDMAN



## The Molecular Action of Platelet-Derived Growth Factor

BRENT H. COCHRAN

## Trichothecenes, Zearalenone, and Other Carcinogenic Metabolites of *Fusarium* and Related Microfungi

R. SCHOENTAL



## Cooperation between Multiple Oncogenes in Rodent Embryo Fibroblasts: An Experimental Model of Tumor Progression?

NICOLAS GLAICHENHAUS, EVELYNE MOUGNEAU, GISÈLE CONNAN, MINOO RASSOULZADEGAN, AND FRANÇOIS CUZIN

# DOWN-REGULATION OF THE ANTITUMOR IMMUNE RESPONSE

Robert J. North

Trudeau Institute, Inc., Saranac Lake, New York



## I. Introduction

This article will discuss the immune response to chemically induced, transplantable tumors in syngeneic mice. Therefore, it will deal only with those tumors that are immunogenic by virtue of their possession of tumor-specific, transplantation rejection antigens. It will avoid dealing with the question of whether spontaneous human tu-



ADVANCES IN CANCER RESEARCH, VOL. 45

mors are immunogenic because it is evident from an ongoing published discussion (Hellström and Hellström, 1983; Mastrangelo *et al.*, 1984) that this question cannot yet be answered. The current view in this laboratory is that the immune response to a progressive immunogenic tumor is a model with which to analyze an unsuccessful immune response to replicating antigens. If some or most human tumors prove to possess tumor-specific transplantation antigens, then the discussion that follows is relevant to the human disease. If not, the discussion still is relevant to the immune response to replicating antigens in general and will help to explain why certain infectious and parasitic diseases become chronic or progressive. It surely would be surprising, however, if it turned out that all human tumors were nonimmunogenic.

The question of whether tumors that have been transplanted repeatedly over a number of years have remained truly syngeneic also will not be discussed. It will suffice to say that there is no evidence to the contrary. Indeed, it is apparent that the immunity that many of these tumors can evoke today is the same, in terms of type and strength, as the immunity they evoked when they were first induced. It is worth pointing out in this connection, moreover, that while there are those who argue (Hewitt, 1979) that tumors that are transplanted over many years are more allogeneic than syngeneic, there are others who argue (Uyttenhove *et al.*, 1983) that the progressive growth of such tumors is possible only because of the survival and emergence of nonimmunogenic, antigen-loss tumor variants. Obviously both points of view cannot be correct. If the first notion were correct it should follow that practically all spontaneous and chemically induced tumors, whether immunogenic or not at the time of their emergence, should become increasingly immunogenic with time. On the other hand, if there were selective pressure for the preservation of nonimmunogenic variants during *in vivo* passage, as described for the P815 mastocytoma (Uyttenhove *et al.*, 1983), then most transplantable tumors should rapidly become nonimmunogenic. In the absence of substantial evidence for either idea, it remains possible that most syngeneic tumors, although probably composed of cells of different antigenicity, may in fact be relatively stable immunogenically (Rogers, 1984).

## II. Tumor Immunogenicity

An immunogenic tumor is one against which a syngeneic host can be immunized. The immunogenicity of chemically induced murine tumors was revealed first by Foley (1953), whose method of immuniz-

ing consisted of removing an established tumor by ligation. He showed that removal of tumor by this means left its host resistant to growth of a subsequent implant of cells of that tumor, but not to the growth of implants of cells of other tumors. The demonstration of the immunogenicity of chemically induced tumors and the specificity of the immunity they engender was soon confirmed by others who immunized against growth of an implant by injection of heavily X-irradiated, nonreplicating tumor cells (Revesz, 1960) or by repeated injection of subtumorigenic doses of replicating tumor cells (Old *et al.*, 1962). It is apparent, however, that the most favored method of testing for tumor immunogenicity remains the one described by Prehn and Main (1957) which consists of testing for immunity to growth of a tumor implant after removing a primary tumor by surgery. This is not to say that this is the best test for immunogenicity, since it is apparent that a proper comparison of the available tests has not been made. On the contrary, it is safe to state that all tests for tumor immunogenicity have been empirically derived and that too much faith is placed in negative results obtained with them (Hewitt *et al.*, 1976). Indeed, results of a recent study of the immunogenicity of two "spontaneous" guinea pig leukemias led Key *et al.* (1984) to stress the importance of employing optimum immunization procedures before concluding that a tumor is devoid of transplantation rejection antigens.

Be this as it may, the demonstrations of immunogenicity of chemically induced tumors were soon followed by attempts to determine whether the immunity they evoke is mediated by cells or antibody. It was shown (Old *et al.*, 1962) that immunity to growth of a tumor implant is cell mediated in nature in that it can be passively transferred from immunized donors to normal recipients with lymphoid cells, but not with serum. It is known from the results of more recent *in vivo* experiments (Fernandez-Cruz *et al.*, 1979; Greenberg *et al.*, 1980; Leclerc and Cantor, 1980; Berendt and North, 1980; North, 1984a) and *in vitro* experiments (Rouse *et al.*, 1973; Plata *et al.*, 1973; Burton *et al.*, 1975; Wagner *et al.*, 1980), moreover, that immunity to syngeneic tumors is mediated by T cells.

Needless to say, the knowledge that syngeneic tumors can possess transplantation rejection antigens presents the problem of explaining how such tumors escape destruction by host immunity. Obviously, the fact that immunogenic tumors exist argues against theories of immunosurveillance, and it is not surprising that there have been a number of attempts to explain how such tumors avoid rejection. It has been suggested, for example, that tumors escape immune destruction by hiding their surface antigens, an explanation based on evidence

(Boyse *et al.*, 1967; Hilgers *et al.*, 1980) that tumor cells can modulate their surface antigens under immunological pressure. This explanation is related to a more recent one based on evidence (Bosslet and Schirrmacher, 1981; Uyttenhove *et al.*, 1983) that progressive tumor growth, in the face of an antitumor immune response, is made possible by the emergence of stable clones of tumor cells that are antigen-loss variants. Yet another suggestion for escape is that tumors avoid confrontation with host effector cells by secreting antiinflammatory factors that function to prevent host cells from migrating across vascular endothelium into the tumor mass (Fauve *et al.*, 1974). This suggestion is supported by the findings (Pike and Snyderman, 1976) that implantation of certain tumors, or injection of small molecular weight extracts from them, can inhibit the entry of mononuclear cells, particularly macrophages, into peritoneal inflammatory exudates. It should be realized, however, that the idea of the secretion of antiinflammatory products by murine tumor cells is difficult to reconcile with the knowledge (Evans, 1973; Eccles and Alexander, 1974; Haskill *et al.*, 1975; Dye and North, 1980) that solid and ascites tumors in mice contain very large numbers of host mononuclear cells, including macrophages.

Another plausible explanation for the escape of immunogenic tumors is that the immunity these tumors evoke is too weak and is generated too late to reject an already established and rapidly growing tumor mass. It has been suggested (Old *et al.*, 1962; Old and Boyse, 1964) that this allows the tumor to "sneak through" immune defenses, an idea in keeping with the knowledge that, whereas implantation of a large number of cells of a given tumor can result in early rejection of the tumor that emerges, implantation of a small number of tumor cells results in progressive tumor growth. Presumably the larger implant provides enough antigen to engender an immune response early enough to be effective.

This "sneak through" hypothesis, because it is based on the weakness and inadequacy of antitumor immunity, is related to the most recent and popular explanation of escape which states that immunogenic tumors are able to escape immune defenses because they induce suppressor T cells. Evidence that suppressor T cells suppress antitumor immunity has been the subject of several articles and reviews (Naor, 1979; Greene, 1980; Schatten *et al.*, 1984b), all of which make it clear that there is ample evidence for the presence of suppressor T cells in a tumor-bearing host. It is apparent, however, that some of the evidence is indirect and that a detailed hypothesis of tumor escape based on the negative regulatory function of suppressor T cells

has not been formally presented. There has been no suggestion in most cases as to the nature of immunity in the tumor-bearing host that suppressor T cells are supposed to suppress. Presumably, if suppressor T cells are responsible for tumor escape, then they must either prevent an antitumor immune response from being generated in the first place or they must function to down-regulate one that is in the process of being generated. Available evidence suggests that the second possibility is the more likely one, because it is well documented that progressive growth of tumors of proved immunogenicity evokes the generation of an immune response in the form of concomitant immunity. Therefore, before discussing the functional significance of suppressor T cells, it is first necessary, by way of introduction, to briefly discuss the evidence that antitumor immunity is generated.

## III. Concomitant Immunity as the Unsuccessful Response to Tumor Growth

Concomitant antitumor immunity is a paradoxical state of acquired immunity that enables a host with a progressive tumor to neutralize the growth of an implant of cells of the same tumor given at a distant site. There is a relatively large literature on the subject of concomitant immunity (reviewed by Vaage, 1971; Gorelik, 1983; Tuttle *et al.*, 1983) going back to the descriptions of it by Ehrlich (1906) and by Bashford *et al.* (1908) at the turn of the century. The interpretation that concomitant immunity serves no purpose, in that the tumor continues to grow unrestrictedly, has been negated by published evidence showing that failure of a host to generate concomitant immunity results in a much shorter survival time because of a faster dissemination of tumor cells and growth of tumor metastases (Milas *et al.*, 1974). This has been observed in animals that fail to generate concomitant immunity because of having been immunodepressed by exposure to X-irradiation (Deodar and Crile, 1969; Yuhas *et al.*, 1975) or by treatment with antilymphocyte serum. Faster development of systemic disease has also been observed in mice that have been made T cell deficient by thymectomy and lethal irradiation and restored with bone marrow (Kearny and Nelson, 1973). Again, there is evidence showing (Gershon and Kondo, 1971) that excision of a primary tumor can result in failure to generate concomitant immunity and consequently in earlier death of the host from the more rapid growth of seeded metastases. This last-mentioned finding presumably depended on the timing of tumor excision. The immunological consequences of tumor excision as it relates to concomitant immunity will be discussed later.

It is necessary to point out at this stage that the majority of published evidence shows, in agreement with the findings about tumor immunogenicity in general, that concomitant immunity is specific for the tumor that evokes its generation. There is some evidence, however, that concomitant immunity can be nonspecific, but only after the primary tumor becomes very large. Kearny and Nelson (1973) have shown, for example, that concomitant immunity to several chemically induced fibrosarcomas of recent origin is specific during early stages of tumor growth, but is nonspecific at later stages. It should be pointed out, however, that even the nonspecific phase of concomitant immunity might be specifically mediated in that it might depend on the immunologically mediated activation of a nonspecific defense mechanism such as activated macrophages. Alternatively, the nonspecific component may not be immunologically mediated, but may represent an additional antitumor mechanism that is superimposed on the specific mechanism. It was demonstrated that lymph node T cells from a tumor-bearing, concomitant immune donor can neutralize, essentially in a specific manner, the growth of an implant of tumor cells in a normal or irradiated recipient, although according only to the Winn neutralization assay (North and Kirstein, 1977). The T cell basis of concomitant immunity is further evidenced by the demonstration (Biddison *et al.*, 1977; Ting *et al.*, 1982; Tuttle *et al.*, 1983) that its generation in response to the growth of certain tumors is associated with the acquisition of T cells that are specifically cytolytic for cells of these tumors *in vitro*. More will be said about cytolytic T cells later when the kinetics of the generation of concomitant immunity are discussed.

It needs to be pointed out at this time, however, that an important aspect of concomitant immunity is that it can undergo rapid decay after the tumor reaches a certain critical size. This eclipse of concomitant immunity has been studied and discussed in some detail by Vaage (1971, 1973, 1977) and by Youn *et al.* (1973). There undoubtedly are some cases where concomitant immunity does not decay (Gorelik, 1983). However, it needs to be determined whether the failure of concomitant immunity to undergo decay is more apparent than real in that the decay is masked by the late development of a mechanism of nonspecific resistance that is not T cell mediated. This would be evidenced by retention of nonspecific resistance to growth of a challenge implant in spite of the loss by the host of T cells capable of passively transferring specific immunity to appropriate recipients (Kearny *et al.*, 1975). Be this as it may, examples of the rapid decay of specific concomitant antitumor immunity are important because they

provide a reason for postulating that suppressor T cells are generated in response to tumor growth.

## IV. Evidence That Tumor Growth Induces Suppressor T Cells

If the generation and subsequent loss of concomitant immunity is a common consequence of the growth of immunogenic tumors, this surely would need to be taken into account in the design of immunotherapeutic modalities because it would mean that any attempt to cause the regression of an immunogenic tumor by active or adoptive immunotherapy would represent an attempt to augment or superimpose an immune response either on an already developing concomitant immune response or on a concomitant immune response that is undergoing decay. It would be highly significant, moreover, if the decay of concomitant immunity proved to be an active process mediated by suppressor T cells, because a mechanism of active suppression of immunity might explain why it has proved so difficult to cause the regression of already established tumors by intralesional injection of immunoadjuvants or by the passive transfer of tumor-sensitized T cells from immunized donors.

Indeed, we considered it highly likely that the presence of suppressor T cells was responsible for documented failures to cause tumor regression by adoptive immunotherapy (Rosenberg and Terry, 1977). It was reasoned, in turn, that if the presence of a mechanism of T cell-mediated immunosuppression is responsible for the refractoriness of an immunogenic tumor to the antitumor function of passively transferred tumor-sensitized T cells, it should be possible to make the tumor susceptible to intravenously infused T cells by growing it in a recipient that has been rendered incapable of generating suppressor T cells.

### A. Ly $1^+,2^-$ Suppressor T Cells Are the Major Obstacle to Adoptive Immunotherapy of Established Tumors

If the presence of tumor-induced suppressor T cells in a recipient animal is responsible for the refractoriness of its established tumor to the action of intravenously infused sensitized T cells from an immune donor, it should be possible to cause the regression of the tumor by preventing the production of suppressor T cells. This prediction was tested (Berendt and North, 1980) by determining whether passive transfer of tumor-sensitized T cells from immune donors would cause the regression of an established tumor growing in recipient mice that

were incapable of generating suppressor T cells because of having been made T cell deficient 6 weeks earlier by thymectomy and lethal γ-radiation and protected with bone marrow (TXB mice). The donors of immune T cells were immunized 3 weeks earlier by the subcutaneous injection of an admixture of living tumor cells and *Corynebacterium parvum* (Dye *et al.*, 1981). This method of immunization is known to leave the host specifically immune to the growth of an implant of tumor cells for many weeks and with splenic T cells capable of transferring this immunity to normal recipients. Experiments were performed with the nonmetastatic, methylcholanthrene-induced Meth A fibrosarcoma, syngeneic in BALB/c mice, and with the P815 mastocytoma syngeneic in DBA/2. It was found (Berendt and North, 1980; Dye and North, 1981), in agreement with the general experience of others, that intravenous infusion of 1 organ equivalent of spleen cells from immune donors failed to have any effect on a tumor growing in immunocompetent recipients. In contrast, infusion of the same number of immune spleen cells caused complete regression of the same-sized tumor growing in TXB mice. It was apparent, therefore, that immunocompetent, tumor-bearing recipients possessed a T cell-dependent mechanism that blocked the capacity of passively transferred immune T cells to express their antitumor function. It was reasoned that if this were true, it should be possible to reveal the presence of this T cell-dependent mechanism of suppression by showing that it can be passively transferred. In other words, it was considered likely that passive transfer of spleen cells from an immunocompetent donor bearing a relatively large tumor should block the expression of antitumor immunity by passively transferred immune T cells in TXB recipients. This prediction proved correct in that passive transfer of 1 organ equivalent of spleen cells from immunocompetent donors bearing a 14- to 16-day (1 cm) Meth A tumor prevented 1 organ equivalent of immune spleen cells infused 3 hr earlier from causing regression of a 4-day tumor in TXB recipients. Moreover, because the same number of spleen cells from normal mice failed to prevent the expression of adoptive immunity in TXB recipients, it was concluded that the suppressor mechanism was tumor induced. The basic suppressor assay is depicted diagrammatically in Fig. 1.

Evidence that the suppressor mechanism is T cell mediated came from experiments that determined whether the ability of suppressor spleen cells to prevent immune T cells from causing tumor regression in TXB recipients is abolished by treating the suppressor cells with monoclonal anti-Thy 1.2 antibody and complement. It was found (Berendt and North, 1980; Dye and North, 1981) that the suppressor

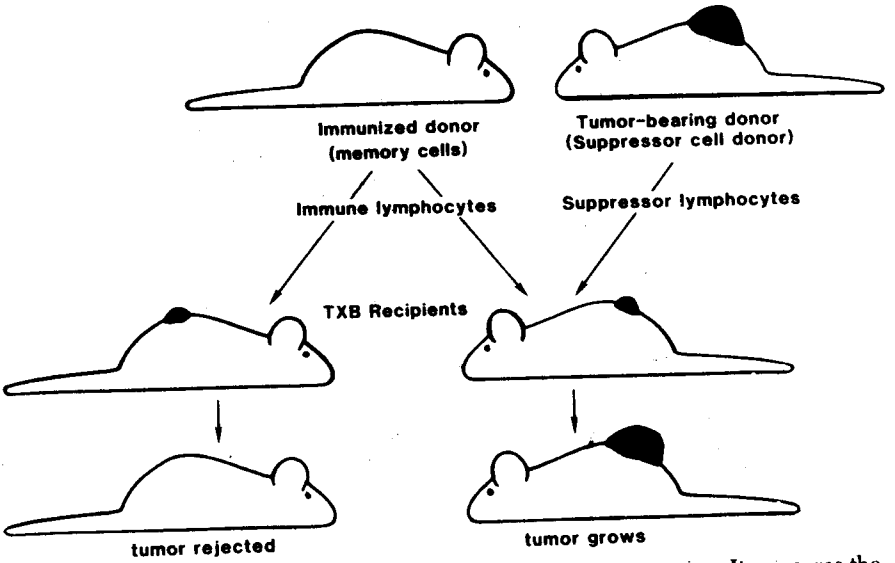


FIG. 1. Diagrammatic representation of the *in vivo* suppressor assay. It measures the capacity of lymphocytes from a tumor-bearing donor to prevent lymphocytes from a preimmunized donor from causing regression of an established tumor in a test recipient made T cell deficient by thymectomy and irradiation.

capacity of the spleen cells was completely eliminated by treatment with anti-Thy 1.2 antibody and complement. These results left little doubt, therefore, that a progressively growing immunogenic tumor eventually evokes in its host the generation of a population of suppressor T cells. These findings were soon confirmed by others (Bonventre *et al.*, 1982) who utilized essentially the same methods, except that athymic nude mice were employed, instead of TXB mice, as tumor-bearing test recipients.

Additional experiments were performed to determine the Ly phenotype of the T cells that passively transfer suppression. The results of these experiments showed (North and Bursuker, 1984) that the ability of splenic T cells from donors with a 16-day Meth A tumor to inhibit the expression of adoptive immunity against an established tumor in TXB test recipients was abolished by treating the suppressor spleen cells with anti-Ly 1 antibody and complement, but not by treating them with anti-Ly 2 antibody and complement. Therefore, the suppressor T cells that function in this model are of the Ly $1^+,2^-$ phenotype, a finding that makes them different from the suppressor T cells that function in other models of suppression of antitumor immunity (Schatten *et al.*, 1984). However, experiments with the P815 masto-

cytoma revealed (North and Dye, 1985) that progressive growth of this tumor also evokes the generation of Ly $1^+,2^-$ suppressor T cells.

## B. T Cell-Mediated Suppression Is Specific

The results of certain published studies have been interpreted as indicating that progressive growth of a tumor can result in a state of generalized immunodepression. This is said to occur, for example, in those cases where tumor growth evokes the production of suppressor macrophages. Indeed, there is evidence (Kirchner *et al.*, 1974; Kruisbeek and Hees, 1977; Mitzushima *et al.*, 1984) that animals bearing large tumors can possess macrophages capable of nonspecifically suppressing T cell responses *in vitro*. However, the interpretation that this represents evidence for nonspecific macrophage-mediated immunosuppression *in vivo* was recently challenged on the basis of results which show (Forni *et al.*, 1982) that tumor-bearing mice that possess macrophages capable of inhibiting immune responses to certain antigens *in vitro* nevertheless are perfectly capable of mounting normal immune responses to antigens *in vivo*. This surely indicates that caution should be exercised in postulating the existence mechanism of immunosuppression purely on the basis of *in vitro* evidence. The same can be said for theories of macrophage-mediated immunosuppression in animals chronically infected with pathogenic bacteria or parasites. Even if the magnitude of immune responses in such animals was greatly decreased according to *in vivo* assays, the onus is on the experimenter to show that the reduced immune responsiveness is not the result of destruction of most of the antigen by a highly activated macrophage system generated in response to infection. There is evidence in this connection that a highly activated macrophage system can be generated in response to growth of immunogenic tumors, including the Meth A fibrosarcoma (North and Kirstein, 1977). However, no evidence was found in this laboratory to indicate that this tumor causes the generation of a state of generalized immunosuppression. For example, mice bearing Meth A tumors large enough to have induced suppressor T cells were shown to have retained a normal capacity to generate and express immunity to a tumor allograft (Berendt and North, 1980). Such mice also retained a normal ability to generate T cell-mediated immunity to infection with bacterial and viral pathogens (Bonventre *et al.*, 1982). Indeed, reciprocal passive transfer experiments with the P815 mastocytoma and the syngeneic P388 lymphoma showed that T cell-mediated suppression of adoptive immunity to these tumors is specific (Dye and North, 1984). Thus,