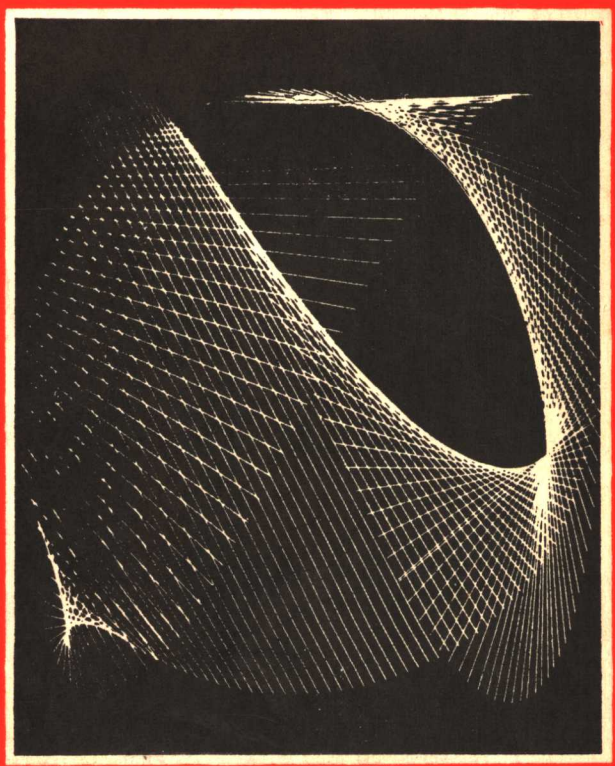
Immunity and Cancer in Man

AN INTRODUCTION



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IMMUNITY AND CANCER IN MAN

An Introduction

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PREFACE

Today, approximately 35% of all cancer patients survive more than 5 years after the diagnosis is made.* This percentage is almost double what it was 40 years ago.† This improvement is the result of at least four separate factors: (a) more accurately informed use of the established methods of therapy, surgery and x-ray treatment; (b) the use of hormones and chemotherapy to lengthen and improve the quality of life for the cancer patient and, in certain types of cancer, even to produce cures, as in Burkitt's and Hodgkin's lymphomas, choriocarcinoma, embryonal carcinoma of the testis, Wilms' tumor, and acute lymphocytic leukemia in children; (c) widespread use of Papanicolaou's method of exfoliative cytology for early diagnosis of cervical and other types of epithelial cancers; and (d) a gradual change in society's attitude toward cancer, with the concept that cancer represents an unmentionable, dread scourge giving way to the much healthier belief that cancer can often be cured if discovered sufficiently early.

While the end of the line in developments due to human ingenuity is never reached, radically new findings are more difficult to make in well-established areas such as surgery, x-ray, and hormone treatment of cancer. In the last decade, it has become increasingly apparent that the body's ability to recognize a tumor as foreign to the genetic blueprint, and to prevent or at

*R. L. Clark, Progress in cancer research: Diagnosis and treatment. *Proceedings, Seventh National Cancer Conference*, Philadelphia, Lippincott, 1973, pp. 11-15.

†American Cancer Society, *Cancer Facts and Figures*, Washington, D.C., 1971.

least limit its growth and spread, lies in the area of immunology. For these reasons, the most exciting frontier in cancer detection and treatment lies in the area of immunology and its allied science, virology.

Despite the wide differences in the areas of expertise of the authors who have contributed to this volume, all are vitally concerned with understanding the mechanisms by which tumor cells manage to evade the body's defense systems. Because we do not yet have definitive answers, it is valuable to have different authors give us their various views. This will enable the reader to decide which explanation is most rational in the light of present knowledge.

Strong evidence is presented by all of the authors for the premise that at least some of the body's defenses against the initiation and spread of tumors are immunological in nature. Present data indicate that these defenses are effective only when the mass of tumor tissue in the body is quite small. Nevertheless, by attempting to first understand and secondly strengthen these defenses, we may well be on the right track to success in immunotherapy of those types of tumors that are "antigenic," and therefore amenable to this type of treatment.

Perhaps the greatest challenge of a new area is that little is known accurately, much remains to be charted and understood. Unless newcomers (whatever their age, training, or status) are willing to take up the challenge of cancer, the promise of future advances will remain unfulfilled. Now that farsighted legislation has increased grant support in this vital area, it is easier than ever before to enter this fascinating field. If training is lacking, that can be remedied by hard work and time: All over the world, there now exist many excellent cancer research laboratories eager to accept trainees. This book will have served its purpose if it helps an undecided reader to dedicate him- or herself with single-mindedness to work on cancer.

A final note from a wider perspective seems in order. This book deals with the impact that the recent extensive discoveries in immunology have had on our outlook on cancer and on the care and treatment of cancer patients. Nevertheless, the most effective attack on the problem of cancer is certainly likely to be prevention rather than cure. In the area of immunology and virology, a search for human cancer viruses and vaccines to provide immunity is already under way. In the area of chemical carcinogenesis, a surprisingly large number of industrial and environmental carcinogens have been identified and some have been banned.* Perhaps most important, dramatic differences

*R. W. Raven and F. J. C. Roe, *The Prevention of Cancer,* London, Butterworths, 1967.

exist in the geographical distribution of certain types of tumors, which may well hold the key to their elimination. For instance, the incidence of cancer of the breast, prostate, and colon is far lower in Japan than in the United States, while the opposite is true for stomach cancer, and native Africans only rarely develop cancer of the colon. The study of cancer incidence in relocated ethnic groups, such as Americans of Japanese or Negro descent, has served to eliminate genetic factors as mainly responsible for these (and certain other) differences in cancer incidence; instead, therefore, environmental factors, or social habits, or type and method of preparation of food, or food contamination by fungal products still to be proven carcinogenic must be responsible. It follows that prevention is possible once the relevant factors have been discovered. Given this scientific success, it has been estimated that 80% of all human cancer may ultimately be preventable.* Unfortunately, in order to translate scientific knowledge into lives saved from cancer, modification of popular habits may be essential. Thus, the scientific "breakthrough" that resulted in official recognition of the hazards of cigarette smoking had already been made well before 1964, the year of publication of the *Surgeon General's Report on Smoking and Health.* Since then, the annual consumption of cigarettes has decreased only slightly on a per person basis, even though cigarette smoking is presently responsible for 30% of *all* cancer in American men.† To realize the relatively vast "paper" profits that should accrue from this and future discoveries, research workers may have to learn how to participate meaningfully in public education on the results of the research, especially when these require the modification of popular customs or habits. Otherwise, given man's propensity to place enjoyment before health, cancer will continue to claim nearly one-fifth of our population for many years to come, and the therapy of cancer will continue to be a vital endeavor.

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*R. M. Taylor, The international campaign against cancer. *Cancer 24*:368 (1974).
†P. H. Abelson, Prevention of cancer. *Science 182*:973 (1973).

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INTRODUCTION

Tumor immunology has had a troubled history, brief as history goes, but long enough to have undergone decay and resurrection. It's germination at the close of the nineteenth century was coeval with the immunology of infectious diseases: a branch of knowledge that blossomed into brilliant science and progressed to fruitfulness in the diagnosis, prevention and treatment of the infections which were then the leading causes of death. At a time when almost every year saw the revelation of a new microbial pathogen and the conquering of a new disease, at a time when the science of immunology (with its handmaiden serology) was proving its worth in the contro of these diseases, it was inevitable that optimism for the application of immunology should extend to the neoplastic diseases. Cancer was not recognized yet as differing basically from the more chronic infectious diseases. In fact, bacteria were quite commonly recovered from tumors, and it seemed reasonable to expect that the postulates of Koch soon would be fulfilled, and the application of immunological methods would conquer this scourge, just as tetanus and diphtheria had been controlled by toxoid prophylaxis and antitoxin treatment.

The appropriate studies were carried out tirelessly by men of great competence, but with no success. None of the microbes could be shown to cause cancer. Vaccines and antisera, and microbial products, which are now known to be nonspecific immunostimulants, generally failed in the treatment of cancer patients. It is true that patients, occasionally, did seem to respond, but so rarely that only such inveterate optimists as Berkley, Coca, Coley,

Vaughn, and Vidal were impressed.[1] Soon the enthusiasm waned. Finally, it was the pathologists, focusing their lenses on cancer, who quashed the very concept of tumor immunology by the same observations that brought new order and understanding into the still uninformed field of tumor pathology. The histopathologists recognized that cancer differed from most other diseases in that the patients' cells were not being destroyed by foreign pathogens, but were growing exuberantly in tumor masses, while destroying normal body tissues. Then the immunologists concluded that there could be no tumor immunology and that they had been pursuing the impossible because, obviously, the body would not destroy itself. The body would avoid self-destruction, as promulgated by Paul Ehrlich in the doctrine of *horror autotoxicus*,[2] which was accepted as axiomatic for half a century.

Without doubt, the concept of *horror autotoxicus* is generally valid. As restated by Burnet, in explaining his term *self and not-self*, "the need and the capacity to distinguish between what is acceptable as self, and what must be rejected as alien, is the evolutionary basis of immunology".[3] The absoluteness of the doctrine gradually required modification, as clinicians described diseases like rheumatoid arthritis, glomerular nephritis, sympathetic ophthalmitis and Hashimoto's thyroiditis, and as immunologists demonstrated that the tissue damage was due to reactions between the patient's immune mechanisms and his own tissue. Although these autoimmune diseases had no direct relation to cancer, their recognition suggested to a few unorthodox cancer researchers that if under exceptional circumstances the body would indeed react immunologically against its own normal tissues, there was no evident reason why it should not react against its own neoplastic tissues.*

*Parenthetically, Ehrlich himself recognized this truism and suggested that if autoimmunity (he spoke of "autolysins") could occur it might be expected when an organism was exposed to its own broken down tissues as, for example, following contusion and ecchymosis or when "spontaneously or under the influence of arsenic, large lymph gland tumors are absorbed . . ."[3a] However, he and his collaborators tried again and again to produce autolysins to erythrocytes with consistently negative results and, reasoning inductively from these data, he did not exclude neoplastic tissue from the generalization than an organism could not react immunologically against its own cells.

[1] C. M. Southam: *Applications of immunology to clinical cancer:* Past attempts and future possibilities. *Cancer Res.* 21:1302-1316 (1961).

[2] P. Ehrlich and his collaborators: *Studies in Immunity*. Collected and translated by C. Bolduan, 2nd ed. New York, John Wiley, 1910, p. 712.

[3] F. M. Burnet: *Self and Non Self*. Victoria, Australia, Melbourne, Univ. Press, and London and New York, Cambridge Univ. Press, 1969, p. 318.

[3a] P. Ehrlich, and his collaborators: *Studies in Immunity*, 2d ed. (C. Boldman, ed.). New York, John Wiley, 1910, p. 24.

Thus, the concept of antitumor immunity was reborn, but the jump from concept to evidence was long. There was a durable resistance to the resurrection of tumor immunology because previous disappointments had not only engendered a strong and appropriate skepticism, and, more logically, because it was now recognized that the serological and transplantation techniques, previously used to study tumor immunity, could not be expected to reveal tumor-specific immune reactions since they could not discriminate between the hoped for tumor-specific immune response, and immune homograft reactions directed against normal but genetically foreign tissue antigens. Vindication of the new gospel came from an unexpected quarter, from studies of mouse genetics, which at that time seemed remote indeed to problems of tumor immunology. This work (notably by Snell[4] and his colleagues at the Jackson Laboratory in Bar Harbor, Maine) yielded strains of mice so closely inbred that those of the same strain were genetically identical (syngeneic) and, therefore, had antigenically identical tissues. As in identical twins, normal tissues transplanted from one to another would grow permanently without exciting any immunologic reaction. In such genetically and antigenically identical animals, if a tumor which arises in one cannot be transplanted to another because of immunologic resistance to its growth, or if any other indications of immunologic reaction to the tumor can be detected, it follows that the tumor contains antigens which are not normally present in such animals. If it can then be shown that these new antigens are intrinsic to the tumor cells (not due to contaminating extraneous organisms), the existence of tumor-specific neoantigens has been demonstrated. Such antigens are the targets against which immunologic attack upon the tumor might be directed.

Utilizing sarcomas that are induced by chemical carcinogens and are transplanted only two or three times (to reduce the chance of subsequent antigenic mutation) in syngeneic mice, the existence of tumor-specific antigens was convincingly demonstrated by transplantation resistance in experiments by Foley,[5] Prehn,[6] Klein,[7] and others, in experiments performed less than 25 years ago, but which are already classics.

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[4]G. D. Snell: *Methods for the study of histocompatibility genes. Genetics* 49:87-108 (1948).

[5]E. J. Foley: *Autogenic properties of methylcholanthrene-induced tumors in mice of the strain of origin. Cancer Res.* 13:835-837 (1953).

[6]R. T. Prehn and J. M. Main: *Immunity to methylcholanthrene-induced sarcomas. J. Nat. Cancer Inst.* 18:769-778 (1957).

[7]G. Klein, H. O. Sjogren, E. Klein, and K. E. Hellstrom: *Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. Cancer Res.* 20:1561-1572 (1960).

Of course a viral cause of chicken neoplasms had been demonstrated early in this century[8] and immune reactions were shown to be effective in neutralizing the virus, thereby preventing tumor formation.[9] This knowledge had failed to influence the prevailing negative attitude toward tumor immunology because tumor virology, itself, encountered a stifling skepticism. Neither chickens nor their lymphomatous diseases were considered relevant to human cancer. The few examples of virus-caused tumors in mammals were nonmalignant, or if they became malignant, the causal virus was no longer demonstrable;[10,11] and although antiviral immunity could be demonstrated, there was no evidence of antitumor immunity, as such.

A turnabout in this attitude toward viral oncogenesis awaited new conceptual and technical developments: invention of more sensitive and discriminating serologic techniques and the advent of a new discipline, molecular biology. Application of these new methods and concepts, as well as the syngeneic transplantation techniques which had been so useful in studies of chemically-induced sarcomas, was soon fruitful. Evidence was obtained for the presence of tumor-specific antigens and of immunologically specific resistance to tumor-cell implantation in tumors produced by both DNA[12,13] and RNA[14] oncogenic viruses of mice, and even in tumors induced in experimental animals by viruses which can infect man (EBV, SV40, adenoviruses).[15,16] It was found that the genome of the oncogenic viruses directed the synthesis of tumor-specific neoantigens that were distinct from the antigens

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[8] P. Rous: *A Sarcoma of the foul transmissible by an agent separable from the tumor cells. J. Exper. Med.* 13:397-411 (1911).

[9] P. Rous, O. H. Robertson, and J. Oliver: *Experiments on the production of specific antisera for infections of unknown cause.* II. *The production of a serum effective against the agent causing a chicken sarcoma. J. Exper. Med.* 29:305-320 (1919).

[10] J. T. Syverton, E. B. Wells, H. Koomen, H. E. Dascomb, and G. P. Berry: *The virus induced rabbit papilloma-to-carcinoma sequence.* III. *Immunological tests for papilloma virus in cottontail rabbits. Cancer Res.* 10:474-482 (1950).

[11] R. Dulbecco: *Viral carcinogenesis. Cancer Res.* 21:975-980 (1961).

[12] K. Habel: *Immunological determinants of polyoma virus oncogenesis. JEM* 115:181-193, 1962.

[13] F. Rapp, J. L. Melnick, and T. Kitahara: Tumor and virus antigens of Simian virus 40: Differential inhibition of synthesis by cytosine arabinoside. *Science* 147:625-627, 1965.

[14] H. Bauer: Virion and tumor cell antigens of C-type RNA tumor viruses. *Advances Cancer Res.* 20:275-341, 1974.

[15] F. Rapp: Herpesviruses and cancer. *Advances Cancer Res.* 19:265-302, 1974.

[16] J. Ankerst and H. O. Sjogren: Cross-reacting tumor-specific transplantation antigens in tumors induced by adenoviruses 3, 14 and 12. *Cancer Res.* 30:1499-1505 (1970).

of the virus particles.[17] The newly developed labeled antibody techniques using fluorescent tags,[18,19] or electron dense tags,[20] further revealed that some of the neoantigens were, in fact, localized at the cell surface where they would be accessible to immunological attack.

Thus, the basic questions of the existence of tumor-specific antigens and the response of the tumor-bearing host to such antigens, have been resolved. Now, the focus of research in tumor immunology has turned to the mechanisms of that immune response, and how it can be enhanced so as to inhibit more effectively the growth of established tumors. The chapters of this book are primarily directed to these questions. As will be evident, the answers are often ambiguous and always incomplete. Particularly on the question of the practical application of tumor immunology to the therapy of cancer patients, few definitive answers will be found. In short, we have not yet entered a new era in tumor immunology; rather, the present represents a continuation of the renaissance.

If I am allowed to use an extended metaphor to look into the future of this field of medical science, I will compare the broad subject of tumor immunology to a high range of mountains. I will welcome Dr. Arnold Reif as the leader of a group of mountaineers, who undertakes in this book to describe their efforts to reach the highest pinnacle of that range, the achievement of clinical cancer immunotherapy.

The leader is well-qualified and he has assembled a competent team. Dr. Reif has spent two decades in the foothills of basic immunochemistry and has a magnificant "first" to his credit in which he led the way to the twin peaks of T&B lymphocyte discrimination by his discovery of *theta*, the distinctive antigen of thymic lymphocytes.[21] His well-balanced team has a surgeon and an internist, as would any well-organized high mountain expedition, but (straining my metaphor) it also includes a radiotherapist and pathologist. Each climber brings to the team effort his broad but individual skills and experience

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[17]H. M. Temin: The cellular and molecular biology of RNA tumor viruses, especially avian leukosis-sarcoma viruses, and their relatives. *Advan. Cancer Res.* 19:48-104 (1974).

[18]S. S. Tevethia, M. Katz, and F. Rapp: New surface antigens in cells transformed by Simian papovavirus SV40. *Proc. Soc. Exp. Biol. Med.* 119:896-901 (1965).

[19]J. S. Irlin: Immunofluorescent demonstration of a specific surface antigen in cells infected or transformed by polyoma virus. *Virology* 32:725-728 (1967).

[20]T. Aoki: Murine type-C RNA viruses: A proposed reclassification, other possible pathogenicity, and a new immunologic function. *J. Nat. Cancer Inst.* 52:1029-1034 (1974).

[21]A. E. Reif and J. M. V. Allen: The AKR thymic antigen and its distribution in leukemias and nervous tissues. *J. Exptl. Med.* 120:413-433 (1964).

and perspective. Dr. Kaiser serves on this team as the able "second" on the leader's rope. Dr. Berman, as pathologist, must analyze the unique problems of each climb so that the team is forewarned of the nature and complexity of their endeavor. Certainly, the surgeon, Dr. Fisher, can be likened to the accomplished and confident climber, who tackles his pitch head-on by the clean, technically brilliant, hardware-encumbered *directissima* approach that so often pays off. As radiotherapist, Dr. Order might be likened to the expert in ice-climbing, whose special techniques are ideal for certain climbs but which, more often, serve as an indispensible adjunct at some stages of the journey. Dr. Biano, as medical oncologist, represents the broadly knowledgeable but less spectacular climber who can sometimes succeed by utilizing the corners and cracks and chimneys with endurance and determination, but more often plays the role of organizer and manager of the team.

Solo climbs are rare indeed in the mountains of oncology. Each climb requires team effort—the *directissima*—most of all. Our routes are tortuous. Each leg presents new challenges, which require a pooling of talents and constant cooperation. The good leader will recognize that he is not always number one on the rope, but must relinquish that honor and responsibility to the specialist who is most capable of conquering the pitch which lies immediately ahead. The team, but no individual, will have the breadth of experience and the imagination to use all available methods and to devise new ones to attack the overhangs that so often block our progress.

How do our mountaineers fare on their climb? That story will unfold as the reader accompanies them chapter by chapter. We will note that no chapter recounts the experiences of its individual writer, but they rather relate the achievements, near misses, and missions still being planned, of scores of climbers who are contributing the best of their abilities to the seemingly disorganized but magnificent effort. It is no secret that the present team, and those for whom they report, have not conquered Mt. Everest. Some of these adventurers will eventually recognize that their chosen or assigned route will not "go." And to acknowledge that a route is wrong takes much more courage than to arrive by a route that is easily negotiated. Others will have to be content with the role of anonymous bearers who merely contribute a bit to the establishment of base camps, unglamorous back-breaking work, but essential to further the assault. A few will conquer lesser peaks and will have a day of public glory, but will be remembered only by their most intimate peers. A few will gloriously fail after seeming about to attain their goal, and will wonder for a lifetime whether their efforts were worthwhile.

Climbing and looking ever upward is the only way to conquer mountains, but it can give a distressing sense of getting nowhere. As each peak flattens before the slow advance and reveals another higher one ahead, a sense of discouragement may prevail. Then it is necessary that we pause for an instant and look about us, to look back down the treacherous pitches that have been negotiated, to look beyond the detours and false starts that led not to our goal but nevertheless opened the way to other interesting prominences, to look far below into the valleys to the base camps which supply our needs. Then we realize that we have indeed advanced high up the mountain side. We do not know how far ahead the peak may be, for this mountain has never been charted, but this much is sure; we have come much much farther up the mountain than the teams that preceded us just a few years ago, and we can rest assured that if we do not gain our Everest, we may retreat from Annapurna with confidence that others will continue the effort to gain the real summit. Good climbing!

Chester M. Southam, M.D.

CONTENTS

ONE

Immune Defenses Against Initiation of Tumors

Arnold E. Reif

INTRODUCTION

It is now 30 years since Ludwig Gross showed that tumors induced by a chemical carcinogen could be antigenic and stimulate a rejection reaction in their host [1]. This is the cornerstone of modern cancer immunology, for only if tumors are recognized as nonself can the host react against them. The purpose of this chapter is to present a sufficient amount of the evidence that has accumulated in the interim to enable the reader to decide whether immune defenses exist against the development of cancer in man.

If immunity played a role in the initiation of tumors, one would expect to find an increased incidence of tumors in immunosuppressed individuals. In contrast, immune stimulation should delay or reduce tumor development. First, some recent and striking data that bear on these questions are presented. Thereafter, we