Mechanisms of Tumor Immunity

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STANLEY COHEN

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Preface

For many years it has been suspected that the immune system may be involved in host defense against neoplastic disease. This notion was based in part on early speculations as to the role of immunologic reactions in maintaining cellular homeostasis, in part on the rare but documented phenomenon of spontaneous regression of tumors, and in part on analogies with situations involving the response to infectious agents or organ transplants. It received support from the discovery of a wide variety of tumor-specific antigens and the development of a number of animal models in which immunization against such antigens modified the behavior of transplanted, induced, or spontaneous neoplasms. A large amount of clinical data has accumulated as well. It has become apparent in these studies that although all the known forms of immunologic reactivity may play a role, cell-mediated immunity seems to be of central importance in many cases, especially those involving solid tumors.

Thus, there is a wealth of evidence that the immune system can play a protective role in neoplastic disease. Nevertheless, in the natural state, tumors frequently grow in an unrelenting, lethal manner, often in the face of a demonstrable immune response against the tumor. This has had three major consequences, all potentially beneficial. First, it has prompted a reexploration and critical reevaluation of the concept of immune surveillance itself. Second, it has led to the investigation of a multiplicity of factors which serve to modify, limit, or regulate the immune response in tumor-bearing animals and man. Third, it has led to various procedures for increasing immunologic reactivity, either by specific immunization or by nonspecific methods of augmentation. Although these clinical approaches have had limited success to date, they provide a framework upon which our increasing knowledge of basic immunologic mechanisms can be applied in the future. Also, they have provided data which have given new insight into fundamental processes.

Although the above subjects have been discussed in reviews and workshops, no single comprehensive text has covered all these major advances. The present volume has been designed to provide such a treatise. It begins with a general overview of cell-mediated immunity and a critical discussion of the evidence for and against immune surveillance. Next, various mechanisms by which the immune system can destroy tumors are described. Following is a review of im-

munologic enhancement as a category of reactions that may limit the in vivo effectiveness of tumor immunity. With this as background, the evidence for the existence of tumor immunity in man is presented, followed by definitive reviews of the current status of immunotherapy, both in experimental models and in human disease. Subsequent chapters deal with the known neoplasms of the immune system and with the interactions of lymphocytes with oncogenic viruses. The final chapter reemphasizes the links between the immune and inflammatory systems and discusses in a speculative manner possible ways by which such interactions might be spontaneously inhibited in tumor-bearing subjects. Experimental circumvention of such inhibitory mechanisms might lead to new kinds of therapeutic approaches.

A certain amount of repetition has accompanied the attempt for broad coverage. This is unavoidable and in fact desirable. Although the available information in the literature is vast, it is finite. Each author has dipped into the same pool of published data, and it is not surprising that the cited references often overlap. Each has organized and interpreted a subset of these data to focus on his or her assigned topic, and the multiplicity of views that emerge provide a well-balanced summary of our present state of knowledge. We believe that this text should therefore prove of value to immunologists, students, and clinicians who have interest in any of the basic or applied aspects of tumor immunity.

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Contents

Chapter One

Cell-Mediated Reactions in vivo

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The acquisition of solid knowledge concerning tumor immunity began about two decades ago, when it was shown that inbred strains of mice were capable of rejecting syngeneic or autologous neoplasms against which they had previously been immunized (1). These and subsequent experiments led to the widely held concept that effective tumor immunity usually depends upon recognition of tumor-associated antigens on the surface of neoplastic cells and that tumor destruction is effected by mechanisms similar to those responsible for the destruction of solid tissue allografts—that is, primarily through cell-mediated mechanisms. Indeed, Thomas proposed that the "purpose" of the allograft rejection mechanism is to recognize and destroy autochthonous neoplasms at an early stage (2). However, the validity of this concept has been seriously challenged in recent years (3), and the possibility that the mechanisms of cell-mediated destruction observed in tumor models are of little relevance to most spontaneously arising tumors cannot be dismissed at present (see Chapter 2).

Although the first definitive studies on tumor immunity were carried out in vivo, in the past decade most investigations concerning cell-mediated destruction of neoplastic cells have been performed in vitro, which has resulted in the delineation of several distinct mechanisms (see Chapters 3 and 4). However, as noted by numerous authors, the relevance of many of these observations remains to be established.

This chapter will review the salient cellular events occurring in several forms of cell-mediated reactions (delayed hypersensitivity, cutaneous basophil hypersensitivity reactions (CBH), allograft reactions, and certain autoimmune diseases) and attempt to relate these findings to observations made in tumor immunity.

Cell-mediated reactions in vivo are by no means homogeneous in terms of the nature of the infiltrating cells, a fact that has not been sufficiently appreciated until recently. The most important factors known to influence the character of the reaction are: the species studied; the route and method of immunization (espe-

cially the use and type of adjuvant); the physical properties of the antigen; the site of challenge; the presence or absence of humoral antibodies; and the capacity of the host to mount an appropriate inflammatory response, in large part by virtue of possessing sufficient circulating leukocytes and by having the capacity to produce or release adequate mediator substances. (For more extensive discussion of these factors see refs. 4,5,6.)

The first form of cell-mediated reaction to be studied was delayed hypersensitivity response to bacterial antigens, in particular the tuberculin reaction (7). In the 1920s Dienes showed that similar reactions could be produced by purified proteins, such as ovalbumin (8). The criteria for defining these prototype delayed hypersensitivity reactions are relatively precise and well known. They are immunologically specific responses that develop in appropriately sensitized hosts following local (usually intradermal) challenge with antigen. Typical reactions occur in animals without demonstrable antibodies. The reactions appear several hours after challenge and then exhibit gradually increasing erythema and induration. Histologically a predominantly mononuclear infiltrate is seen. Reactivity can be transferred with lymphoid cells, but not with serum. The antigens used for elicitation of delayed reactions generally must possess carrier specificity, in contrast with most antibody-mediated reactions, which can be elicited with hapten-specific antigens (9). Lymphocytes from animals with delayed sensitivity can be stimulated by antigen in vitro to produce a variety of mediator substances (lymphokines). The carrier specificity requirements for such stimulation parallel those required for in vivo elicitation of delayed reactions (10).

Within the past two decades the concept of delayed hypersensitivity (or cell-mediated immune reactivity) has expanded to include responses to a variety of antigens other than bacterial products and purified proteins, including simple reactive chemicals (contact sensitivity), viruses, allografts, autologous antigens (in certain autoimmune diseases), and tumor-associated antigens (11). In most of these instances it has been possible to obtain convincing evidence for cell-mediated mechanisms. Nevertheless, it is not always possible to be certain that a given inflammatory reaction is cell mediated, especially when all criteria cannot be examined (as is true of studies in man). The histologic features, although characteristic, are not pathognomonic. Indeed, even certain lesions that are mediated by humoral antibodies can be characterized by an almost exclusively mononuclear cell infiltrate (12). Further, certain reactions with no known immunologic mechanisms can show mononuclear infiltrates. Perhaps the most conclusive evidence for a cell-mediated mechanism is the demonstration that typical reactivity can be transferred with lymphocytes but not with serum. Obviously, this type of evidence can be obtained only in experimental animals. The demonstration of in vitro correlates of cell-mediated immunity, employing the host's lymphocytes and the antigen in question, indicates that the host possesses cell-mediated reactivity against that antigen but does not prove that this accounts for reactions observed in vivo. Clearly, new methods are needed for the recognition of cell-mediated reactions in vivo.

CELLULAR EVENTS IN PATHOGENESIS OF CELL-MEDIATED REACTIONS

The principal cellular events occurring in experimental animals developing cell-mediated reactions of a variety of types can be summarized as follows. The

immunizing antigen, administered in the form of a local injection (usually with adjuvant), or as a graft, stimulates the proliferation of T lymphocytes, including some with specificity against the immunizing antigen, especially in the draining lymph nodes. Many of these newly formed T lymphocytes enter the circulation, which provides them with the opportunity to come in contact with the immunizing antigen at the site of challenging injection of antigen, tumor, or graft implantation or in an organ containing a tissue-specific antigen. Contact between a few specifically sensitized cells and antigen causes these cells to produce and release a group of lymphokines. These mediators bring about an inflammatory reaction, in which most of the leukocytes exhibit no specificity toward the responsible antigen.

Published descriptions of the kinds of cells present in cell-mediated reactions vary widely; this appears to stem not only from the heterogeneity of these reactions but also differences in terminology, differences in emphasis and bias of the authors, variations in techniques used to study the cells, and the growth of knowledge (which is still far from complete) on the nature and identifying characteristics of subpopulations of mononuclear cells.

Knowledge concerning important aspects of cell-mediated reactions—changes in draining lymph nodes, effector mechanisms, and the nature and immunologic specificity of the mononuclear cells in infiltrates—are best considered in the light of experiments carried out with several types of reactions. These topics will be discussed separately after brief descriptions of the major morphologic features of the individual types of reactions.

DELAYED HYPERSENSITIVITY REACTIONS

Delayed hypersensitivity reactions have been most thoroughly studied in human beings, guinea pigs, and rats. In experimental animals immunization usually involves local injections of microgram amounts of antigen (often modified in some way so as to reduce antigenicity) (9) in complete Freund's adjuvant. The reactions are generally elicited by intradermal injection, although they can be evoked in other sites. The rate of development varies in different species; it is fastest in guinea pigs and slowest in man. The most consistent microscopic feature, as seen at 24–48 hours, is perivascular accumulation of mononuclear cells, some of which extend into the dermis as a more diffuse infiltrate. Dermal lymphatics are often dilated and packed with mononuclear cells. Mononuclear cells can also be seen invading the epidermis, a feature which is much more conspicuous in contact reactions than in reactions elicited by intracutaneous injections. In severe reactions necrosis may be found. Two additional features have recently been recognized as characteristic of delayed reactions: one, the accumulation of substantial amounts of fibrin in the interstitial tissue, which accounts for the induration typical of these reactions (13); two, changes in the microvasculature, characterized by endothelial cell damage with lumenal narrowing and later by irregular basement membrane thickening (14).

Identification of many of the mononuclear cells is not possible on morphologic grounds. In particular, large lymphocytes cannot always be distinguished from monocytes. Small lymphocytes have been estimated to account for about 20–25% of the cells in the guinea pig and rat (15). The remaining mononuclear cells have been considered to be mostly either mononuclear phagocytes (monocytes and

macrophages), especially in the rat, or large ("activated") lymphoctyes (15). However, in man only a small percentage of the mononuclear cells in tuberculin or contact reactions can be identified on morphologic grounds as mononuclear phagocytes (16). Additional information concerning the nature of the infiltrating cells in experimental animals has been obtained in transfer studies; as will be discussed, these findings indicate that a large proportion of the cells are mononuclear phagocytes.

It is worth noting that delayed reactions elicited by ordinary protein antigens do not usually exhibit features generally considered to be characteristic of granulomatous reactions—i.e., lesions with nodular accumulation of epitheloid cells and giant cells—although such changes have been described in late tuberculin reactions (17). However, it has recently been shown that delayed sensitivity to purified proteins *can* express itself to some extent in the form of a granulomatous response, if the antigen is coupled to sepharose beads prior to challenging injection (18,19), probably because the antigen is rendered nondiffusible and nondegradable. In addition, granulomas can be induced by poorly understood immunologic mechanisms, even in situations where no immunologic factors appear to be operating.

Neutrophils are present to some degree in most delayed reactions. Although generally seen only in small numbers, they are sometimes quite numerous, especially in the rat and the mouse. Their presence can sometimes be attributed to irritating properties of the challenging material, to a response to necrosis, or to an antibody-mediated component of reaction. Nonetheless, they cannot all be explained away in this fashion, and they must be considered an intrinsic part of some reactions.

Eosinophils are sparse or lacking in most delayed reactions but in some instances are quite numerous, especially after several days. Moreover, eosinophils often appear in abundance in reactions elicited by repeated injections of antigen in the same skin site in guinea pigs with delayed sensitivity (retest reaction) (20). One mechanism that could account for eosinophil accumulation involves a combined effect of cell-mediated and humoral immunity. Thus, it has been shown that lymphocytes from sensitized guinea pigs can be stimulated by antigen to produce a substance that interacts with immune complexes of the same specificity to generate a potent eosinophil chemotactic factor (21). Production of eosinophil chemotactic factors may also be mediated by mechanisms related to anaphylaxis (22).

Basophils are found in only small numbers or are lacking in "classical" delayed hypersensitivity reactions in guinea pigs but are quite numerous in so-called cutaneous basophil hypersensitivity reactions (CBH). The participation of basophils and mast cells in cell-mediated reactions is discussed below.

A few plasma cells may be seen after several days in tuberculin reactions, or in delayed reactions elicited by purified proteins, obviously reflecting the stimulation of antibody production. However, plasma cells are never numerous, as they are in the later stages of active Arthus reactions (23).

Thus, all kinds of leukocytes participate in delayed hypersensitivity reactions. The percentage of cell types varies in different reactions and can be modified by several factors, including the concomitant presence of antibodies.

CUTANEOUS BASOPHIL HYPERSENSITIVITY IN THE GUINEA PIG

In the past several years Dvorak and his associates have recognized and analyzed the important participation of basophils in cell-mediated reactions to a variety of antigens and have coined the term cutaneous basophil hypersensitivity (CBH)* to describe basophil-rich reactivity seen in guinea pigs (24).

CBH can be reproducibly induced in guinea pigs by immunization with a variety of soluble proteins administered locally in microgram amounts in saline or in *incomplete* Freund's adjuvant. Reactivity is maximal at 6 or 7 days, before the appearance of detectable antibody. The reactions begin several hours after challenge, with perivascular accumulations of mononuclear cells. However, basophils soon appear in considerable numbers, and at 24–48 hours generally comprise 20–60% of the infiltrating cells. It is important to realize that basophils cannot be reliably identified in ordinary histologic preparations; their recognition in CBH reactions depended upon improved morphologic techniques, involving fixation and embedding of the type used in electron microscopy (25).

The possibility that appreciable numbers of basophils in the infiltrate exhibit specificity toward the eliciting antigen, which might occur as the result of coating by homocytotrophic γ_1 antibody, has been excluded (26).

Some of the basophils in the infiltrate exhibit degranulation. Using tracers that can be followed by electron microscopy, Dvorak et al. (27) demonstrated that basophils can release their granule contents by means of a previously unrecognized vesicular transport mechanism, which permits prolonged release of mediators over a span of hours or days, rather than in the explosive fashion seen with anaphylactic reactions.

CBH reactions lack the deposits of fibrin in the intervascular dermis that are characteristic of delayed reactions and for this reason do not exhibit induration (13).

Basophil-rich reactivity can be induced in guinea pigs not only against soluble protein antigens but also against a variety of antigens of greater biologic importance, such as contact allergens, vaccinia virus, allogeneic tumor cells, schistosomes, and skin allografts (28). Indeed, in guinea pigs the usual type of cell-mediated reactivity against these agents appears to be basophil-rich reactivity, rather then the classical type of delayed sensitivity. Moreover, in contrast to the reactivity induced against soluble protein antigens, which is evanescent and typically wanes when humoral antibodies appear, basophil-rich reactivity persists indefinitely following immunization with contact-sensitizing antigens and allogeneic tumors (28).

Basophil-rich hypersensitivity is considered to be a form of cell-mediated immunity, since reactivity can be transferred with lymph node cells (29). However, the exact nature of the responsible lymphocytes and their relation to those that mediate "classical" delayed hypersensitivity have not been established.

Moreover, several reports of transfer of reactivity with serum have appeared (30,31,32). Although further confirmation of these reports would be desirable,

*This was perhaps an unfortunate choice, since similar reactions can be elicited in sites other than the skin.

they are sufficiently provocative to suggest that mechanisms other than direct initiation of CBH reactions by sensitized cells is possible. The claim that 7s γ_1 antibody is responsible awaits confirmation (32).

BASOPHIL-RICH CELL-MEDIATED REACTIONS IN MAN

The distinction between CBH and delayed hypersensitivity reactivity in man is not as clear-cut as in the guinea pig. Thus, although a variety of cell-mediated reactions in man show appreciable numbers of basophils (16), their numbers are quite variable, even in similar types of reactions. Moreover, they are never seen in such large numbers as may be found in guinea pigs. Nearly all contact reactions studied in man have been found to contain abundant basophils. They are seen in smaller numbers in many tuberculin reactions. Basophils have also been seen in early renal allografts (34).

Mast cells appear to play an active role in cell-mediated reactions in man. In early reactions degranulated cells are often found. In later stages hyperplasia may occur (14,16). It has been postulated that basophils and mast cells are supplementary cells with similar or identical functions and that participation of these cells in delayed reactions is governed by their relative frequency in a given species and by the duration of the reaction, with mast cells assuming a greater role late in the reaction (35).

ALLOGRAFT REACTIONS

Rejection of first-set allografts of solid tissues or organs is believed to result from cell-mediated mechanisms. The composition of the infiltrate varies, depending on such factors as the nature of the graft, the species studied, the state of sensitization of the recipient, and the time of examination of the graft. The infiltrate is best studied in experimental animals, where the reactions need not be modified by immunosuppressive agents.

The most extensive studies of the infiltrating cells have been carried out in skin, kidney, and cardiac allografts. Interpretation of skin grafts is complicated to some extent because of the nonspecific inflammatory component that occurs during vascularization. However, the cells invading the graft itself and especially the epidermis can be considered to be the result of the rejection process. The majority of these cells have been described as lymphocytes, or as cells intermediate in morphology between lymphocytes and monocytes (36). Early first-set renal allografts are characterized by infiltration of the interstitium, tubules, and blood vessel walls by mononuclear cells, the great majority of which appear to be small or large lymphocytes, as judged in histologic sections or by electron microscopy (37). In this respect the infiltrates differ from those of cutaneous delayed reactions, where other leukocytes, especially mononuclear phagocytes, are often prominent. However, some macrophages are seen in early renal allografts, as well as appreciable numbers of basophils in some instances. In older grafts increasing numbers of plasma cells and mast cells may be found. With the onset of necrosis, the number of macrophages increases and numerous neutrophils appear.

Tilney et al. (38,39) have performed careful and revealing studies on the cells infiltrating recently transplanted cardiac heterotopic allografts in rats. On the basis of examination of histologic preparations and of cells isolated from 4- to 5-day-old grafts they estimated that about 75% were lymphocytes, 15–20% macrophages, and the rest neutrophils. The time of appearance and distribution of macrophages were studied after intravenous injections of India ink. Lymphocytes appeared early, followed by increasing numbers of carbon-bearing macrophages. At day 4, many of the macrophages were seen in groups in areas of frank necrosis, although scattered isolated cells were also seen. With complete rejection, which occurred at 6 to 7 days, macrophages and neutrophils appeared in very large numbers.

AUTOIMMUNE LESIONS

Certain tissue-specific autoimmune diseases (adrenalitis, thyroiditis, encephalitis) are characterized by a predominantly mononuclear cell infiltrate. Since in some instances these diseases can be transferred with lymph node cells but not with serum, the lesions are presumed to result from cell-mediated mechanisms. However, it also appears that at least in some cases humoral antibody is responsible for such lesions, since they can be transferred with serum, if it is collected at the appropriate time after immunization or after removal of the target organ (40,41). Furthermore, in certain tissue-specific autoimmune lesions, plasma cells and germinal centers are conspicuous, especially in the later stages (42), providing evidence for an antibody-mediated component. Moreover, eosinophils are numerous in some lesions and probably result from a combined effect of cell-mediated immunity and humoral antibodies (43). Accordingly, the conclusion that a given tissue-specific autoimmune lesion results principally or entirely from cell-mediated mechanisms can be considered fairly secure only if transfer can be accomplished with cells and not with serum. These criteria are met in certain models, including autoimmune encephalitis and adrenalitis induced in rats by immunization with tissue-specific antigen in Freund's adjuvant plus pertussis (44). The infiltrates in these lesions typically contain very high percentages (up to 80%) of lymphocytes, as judged morphologically, and thus resemble early allografts but differ from cutaneous delayed reactions.

Basophils have apparently not been described in autoimmune lesions, but it is not known whether they have been looked for by appropriate techniques.

NATURE AND SPECIFICITY OF THE MONONUCLEAR CELLS IN CELL-MEDIATED REACTIONS

Because of the limitations of morphologic criteria applied to tissue sections, other methods have been used to investigate the nature of the cells in infiltrates. Autoradiographic tracer studies have been of value, especially in transfer studies, and in experiments in which populations of lymphoid cells were labeled in situ (45,46). As described in Chapter 11, techniques are available that permit identification of mononuclear phagocytes and B lymphocytes in tissue sections.

Although these techniques have been used to study a variety of infiltrates (including those in and around neoplasms), they apparently have not been systematically used to study the prototype delayed hypersensitivity reactions.

Another method is to prepare suspensions of cells present in the infiltrate and to study their properties in vitro. This offers the advantage of permitting identification of certain surface markers that cannot be detected in tissue sections and makes it possible to study certain functional properties of the cells in vitro. There is at present no way of identifying various subpopulations of T or B cells in inflammatory infiltrates except by isolating cells from the lesions and studying their functional properties.

This type of investigation presents several problems, however. For one thing, with scanty infiltrates and small lesions it may not be possible to obtain sufficient cells. Furthermore, it is possible that the recovered cells are selected populations, rather than representative of all the infiltrating cells. In addition, some of the recovered cells may be circulating cells or cells from lymphoid tissue normally present in the region (as in the alimentary tract), rather than cells that have emigrated. In addition, treatment with enzymes, which are often used in the preparations of cell suspensions, may modify surface markers. Moreover, topographic relationships between cells are destroyed.

It has been clearly shown in autoradiographic studies that the majority of mononuclear cells in delayed reactions are not specifically sensitized to the eliciting antigen. Thus, when delayed sensitivity is transferred from immunized to normal guinea pigs with labeled lymph node cells, only small numbers of labeled cells are found in reactions in the recipient (47). In contrast, when prospective recipients are given repeated injections of ^{3}H thymidine for several days prior to transfer of unlabeled lymph node cells, the great majority of cells at the test site are labeled.

These findings show that most of the cells are of recipient origin and that they are derived from precursors that are rapidly and continuously dividing in the absence of specific antigenic stimulation. Mononuclear phagocytes are probably the most numerous circulating mononuclear cells with these properites, although a certain percentage of T cells and even of B cells possess these attributes. Studies by Lubaroff and Waksman have provided evidence that the majority of mononuclear cells in tuberculin reactions in rats are derived from bone marrow and are therefore probably monocytes (48,49). It is also possible, however, that some of the bone-marrow-derived cells are B lymphocytes. In a study of cells recovered from acutely rejecting cardiac allografts in rats, Tilney et al. concluded that about 15–25% of cells were macrophages and about 75% were lymphocytes (38,39).

Even among the lymphocytes in cell-mediated reactions, the great majority do not appear to be sensitized to the eliciting antigen. This has been shown in experiments in which labeled lymph node cells (almost all of which are lymphocytes) obtained from donors stimulated with a particular antigen have been traced into reactions elicited by the same antigen and by unrelated antigens. In most such studies no difference in the percentage of labeled cells was found between the two sites (4). However, in some reports more labeled cells were found in the appropriate site, although usually not to an impressive degree. Studies of this sort have been performed in animals with delayed or contact reactions, allografts, and autoimmune lesions (4). This lack of consistent or marked preferential accumula-