Mechanisms of Tumor Immunity

An Overview

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The involvement of the immune system in the host response to tumors is complex and involves both lymphocytes and lymphocyte-derived mediators, as well as inflammatory cells and various other agents such as complement. These activities are not confined to cytodestructive processes; recent studies demonstrate that the migration characteristics of tumor cells may be modified by immunologically derived substances. The multiplicity of possible immune system–neoplastic cell interaction is unfortunately balanced by the multiplicity of mechanisms that serve to interfere with an effective immune response to tumors. These mechanisms may reflect pathologic derangements of normal immunoregulatory processes or may involve the production of suppressive substances by the tumors themselves. In either case, a number of genetic and other predisposing factors must contribute to the ultimate resolution of the battle between host and tumor. Successful strategies for immunologic intervention must take all these parameters into account. (Am J Pathol 93:449–458, 1978)

THE INVOLVEMENT of the immune system in the destruction of tumor cells has been an important theme in immunologic research since the early speculations of Ehrlich.[1] This concept found support in the rare but documented phenomenon of spontaneous regression of tumors and, in part, in analogies with situations involving the response to infectious agents or organ transplants. It received further 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.

In all this work, attention has been focused on the destruction of tumor cells by immune mechanisms. Such mechanisms fall conveniently into two categories: those that involve cells as effector agents and those that do not. A summary of various categories of immunologic killing mechanisms is presented in Table 1. As can be seen, the pathways involving cells far outnumber those which are not cell-dependent. This is an important generalization; although all of the known forms of immunologic reactivity

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Table 1—Immune Mechanisms of Tumor Cell Killing

Cell-mediated	Non-cell-mediated
Cytolysis by T cells	Complement
Antibody-dependent lymphocytes, neutrophils, macrophages, fetal liver, platelets	Lymphotoxin
Lymphokine-dependent macrophages	
"Natural killer" (NK) cells	

may play a role in tumor immunity, cell-mediated immunity seems to be of central importance in many cases, especially those involving solid tumors. This, however, does not mean that only mononuclear cells are important. Various other inflammatory cells, and, in unusual situations, certain noninflammatory cells, can participate in these reactions.

In contrast to the situation with respect to cytotoxic mechanisms, very little is known about ways the immune system can modify the biologic behavior of tumor cells short of killing them. One obvious candidate for such attention involves the migration properties of neoplastic cells. In a later section, we will discuss in detail some experiments relating to this.

In spite of our success with experimental models, dramatically successful modes of tumor immunotherapy still largely elude us. There are obviously a number of factors that serve to modify, limit, or regulate the immune response in tumor-bearing animals and humans. Some of these are genetic. Other factors involve the overall immunologic status of the host. One important interrelationship here involves a concomitant state of autoimmunity, and there are a number of experimental models dealing with tumor resistance in relation to autoimmunity. Finally, a variety of soluble suppressor substances and cellular suppressive systems have been described in tumor models. These various factors will be discussed in detail during the course of this symposium.

In this presentation, we will touch briefly and in general terms on some of these aspects of tumor immunity. Only a limited number of references will be cited and only to illustrate certain points.

Effector Cells in Tumor Cell Destruction

Lymphocytes

Lymphocytes are directly involved in three kinds of cytotoxic reactions; they are indirectly involved in tumor cell destruction through the elaboration of soluble mediators such as lymphotoxin and activating factors. T cells themselves may become cytotoxic. Other lymphocytes may participate in antibody-dependent reactions. Here the subpopulation is not

totally defined (eg, K cells, null cells), but the cytotoxic event involves effector-cell–target-cell interaction.[2] The antibody molecule is the key to cell bridging and is the agent which confers specificity to the system. The third mechanism by which lymphocytes destroy tumor cells involves the activation or induction of "natural killer" (NK) cells. Once activated, these lack specificity and can exert their effects on a wide variety of target cells. These aspects of lymphocyte-mediated cytolysis are described in detail in a recent review.[3]

As stated above, lymphocytes also are involved in tumor cell destruction through the elaboration of soluble mediators. This will be covered in a later section.

Macrophages

It has long been recognized that tumors frequently evoke a mononuclear cell infiltrate. However, only a relatively small number of studies have dealt specifically with this point in *in vivo* systems. Russel and Cochrane[4] investigated the relationship between the progressive or regressive behavior of a murine (Moloney) tumor and the extent and nature of the inflammatory infiltrate. Tumors were induced in adult and neonatal mice by intramuscular injections of a cultured Moloney sarcoma line. In adults with tumors that regressed, a macrophage-rich infiltrate extended throughout the neoplastic tissue, whereas in progressive tumors the mononuclear infiltrate remained confined to peripheral portions of the tumor and later disappeared. In either progressing or regressing tumors, neoplastic cells in close association with mononuclear cells often showed evidence of damage. Tumors in neonates, which almost always progressed, never developed an appreciable mononuclear infiltrate.

These experiments provide good evidence for a role of macrophages in this model. This is consistent with a large number of observations in *in vitro* systems involving participation of appropriately activated macrophages. Also, as can be seen in Table 1, macrophages can serve as the effector cell in certain antibody-dependent reactions.

The participation of macrophages in tumor immunity may be specific or nonspecific. Cytotoxicity by macrophages armed with a product of activated lymphocytes called "specific macrophage arming factor (SMAF)" was described by Evans and Alexander.[5] SMAF is a product of thymus-dependent lymphocytes stimulated by antigen. The SMAF produced by stimulating specifically immune lymphocytes with one tumor specifically arms macrophages to kill that tumor but not others. The arming factor is cytophilic and can be absorbed by the macrophages. It can also be absorbed by the specific tumor used to produce it. It may be a cytotoxic receptor shed into the culture medium by activated lymphocytes or a

cytophilic antibody. Although there is no direct evidence, it is also possible that SMAF is another version of the antigen-specific MIF reported by Amos et al.[6]

Churchill et al have shown that supernatants from cultures of lymphocytes stimulated by an antigen (ortho-chlorobenzoyl-bovine gamma globulin, OCB–BGG) activate normal macrophages, either as monolayers[7] or in suspension culture.[8] The responsible lymphokine was called "macrophage activating factor" (MAF). These "activated" macrophages exhibit enhanced cytotoxic capacity against syngeneic strain 2 hepatoma and MCA–25 sarcoma cells. Compared with SMAF, MAF may thus be induced by unrelated antigens, and it enhances the cytotoxicity of macrophages in the absence of the specific eliciting antigen.

Granulocytes

The role of granulocytes in various manifestations of cell-mediated immunity is now well documented.[9] With respect to tumor immunity, the studies of the Dvoraks and their associates have demonstrated the participation of basophils in certain animal tumor models.[10] Also, as seen in Table 1 and described in Reference 3, neutrophils can participate in antibody-dependent cytotoxic reactions. We are not aware of similar studies on the role of eosinophils in this regard.

Noncellular Mechanisms of Tumor Cell Destruction

Lymphotoxin

Evidence to support a role of lymphotoxins in lymphocyte-mediated cell killing in tumor immunity has been obtained in many laboratories, and this topic has been critically reviewed.[11] It has been shown that lymphotoxins produced by lymphocytes stimulated with specific antigen or with nonspecific mitogens could kill or at least damage various cell lines in culture. Like other lymphokines, lymphotoxins, although induced by specific immunologic or mitogenic stimuli, are themselves capable of acting in a nonspecific manner. Although lymphotoxins are thus usually cytotoxic to a variety of both normal and malignant cultured cells, a few reports show that tumor cells are more susceptible to lymphotoxins than normal cells. Thus, Meltzer and Bartlett [12] have shown that supernatants obtained from PPD-stimulated spleen cells of BCG-immunized mice could destroy tumor cell monolayers but not normal cell monolayers. Weedon et al [13] have shown that lymphotoxins obtained by PHA stimulation of human lymphocytes could be cytotoxic to various tumor cells as well as their normal counterparts, although, for example, glioma was more susceptible than normal nervous tissue. Although studies by other investi-

gators in addition to those mentioned above confirm the nonspecific tumor cell destruction by lymphotoxins which are produced by reacting lymphocytes with a variety of antigens (or mitogens), there are almost no data available on the production of lymphotoxins by immune lymphocytes stimulated with tumor-associated antigen.

Most studies on lymphocyte-mediated cytotoxicity for tumor cells seem to have excluded the possibility of involving any nonspecific factors (such as lymphotoxins) in the mechanism of cytotoxicity. Thus, the majority of results suggest that cytotoxic effects of lymphocytes are highly specific and that adjacent nonspecific target cells remain intact. As stated previously, close contact between attacking and target cells is necessary for the effect. However, some studies have demonstrated that innocent bystander cells can also be affected by a specific interaction between immune lymphocytes and target cells.

In any event, it has been almost impossible to recover cell-free lymphotoxins after the specific reactions between immune lymphocytes and target cells. Therefore, one has to assume that lymphotoxins, if generated by such interactions, must be in smaller quantity than that usually generated by antigen- or mitogen-stimulated lymphocytes or that the production and consumption of lymphotoxin must occur at the limited area adjacent to the area of contact.

Complement-Mediated Lysis

The lysis of cells by antibody in the presence of complement is one of the best studied examples of cytodestruction by the immune system. Much of our knowledge of complement-induced lysis has been derived from studies on the destruction of erythrocytes, although there is evidence that the lysis of nucleated cells, including tumor cells, proceeds similarly. Thus, Kalfayan and Kidd showed in 1953 that tumor cells swelled in the presence of antibody and complement.[14] Subsequently, it was found by Green et al [15] that in the presence of rabbit antibody and complement, Krebs ascites cells leaked ions such as potassium before they lost intracellular protein. In solutions of high osmolarity, cell membranes did not disrupt or leak protein. The authors concluded that complement induced "holes" of a discrete size in the cell membrane. Later, electron microscopic studies produced direct experimental evidence that antibody and complement produced lesions, resembling "holes," in erythrocyte membranes, as well as in nucleated cells, including tumor cells.[16]

Noncytotoxic Effects of the Immune System

The bulk of the experimental work dealing with mechanisms of tumor immunity has focused on cell killing. Other effects of the immune system

on tumor cells have not been explored in detail. One candidate for such an effect involves modification of cell mobility or migration properties. It has been thought for many years that the mobility of individual tumor cells may be important in local tumor spread and in the establishment of metastasis. More recently, a relationship between the loss of contact inhibition, as a specific characteristic of malignant cells, and the motility of *in vitro* tumor cells has been suggested.[17] It has, however, proved difficult to examine the mobility of tumor cells in *in vitro* systems. Recently, murine lymphoma cells, mastocytoma cells, and various human tumor cells have been shown to be capable of migration from a capillary tube.[18-20] This is a simple system which allows a quantitative approach to the examination of the motility of tumor cells *en masse*. The technique is similar to that used in studies on macrophage migration *in vitro* as a model for *in vivo* delayed hypersensitivity.

Most murine and human tumor cells obtained from *in vivo* tumors or from tissue culture cells so far studied have been shown to migrate well in this system, although some of the cells from solid and ascitic tumors have been reported not to be motile.[18] The migration of these tumor cells out of capillary tubes is inhibited by various biologic and chemical substances such as concanavalin A or serums from tumor-bearing mice or humans.[21-23] In addition to these agents, we have recently shown that lymphokines can be inhibitory to the migration of tumor cells. Cohen et al demonstrated that the migration of P815 mastocytoma cells out of capillary tubes was inhibited by the MIF-rich supernatants of antigen-stimulated human lymphocytes and of SV40-infected monkey kidney cells.[20] Antigen-stimulated guinea pig lymphocyte supernatant was not effective. This fact may suggest limited species specificity of the responsible factor as in the case of the MIF effect on macrophages.

More recently,[23a] we have demonstrated that supernatants from human lymphoid cell lines and from antigen- or mitogen-activated murine lymphocytes can also inhibit *in vitro* migration of a variety of neoplastic cells derived from mouse or rat tumors. The factor, tentatively named "TMIF," is distinct from MIF, and, on the basis of current physicochemical data, appears to represent a new lymphokine. Extension of these findings to *in vivo* systems is of obvious importance.

Regulatory Mechanisms

Although there is a wealth of data to suggest that the immune system can play a protective role in neoplastic disease, in the natural state tumors frequently grow in an unrelenting, lethal manner. This often occurs in the face of a demonstrable immune response against the tumor. Thus, there

must exist factors which serve to modify, limit, or regulate the immune response in tumor-bearing animals or humans. Much of the work relating to these matters is outside the scope of the present discussion and will be covered in detail by the various participants in this symposium. Here we wish to call attention to the existence of factors which do not interfere with the state of immunologic sensitization per se but which limit the consequences of this state. Thus, Snyderman and his associates [24] have reported that extracts of various tumors contain a low molecular weight factor that can impair the chemotactic responses on monocytes. Another example is a factor defined as "chemotactic factor inactivator" (CFI) by Ward et al,[25,26] which is also extractable from tumors but appears distinct from the factor described by Snyderman. CFI inhibits the chemotactic responses of neutrophils.

Some recent observations point to an *in vivo* role for CFI. Thus, following intraperitoneal administration, the P815 mastocytoma can grow in ascitic form in DBA/2 mice and kill the host within 1 to 2 weeks. In other strains, such as C57BL/6, the tumor is rejected and the animal survives. The intraperitoneal inflammatory infiltrate associated with this process consists mainly of neutrophils. In the DBA/2 mouse, which cannot reject the tumor, there is only a transient neutrophil response, which rapidly disappears. Cohen et al [27] have shown that this disappearance correlates precisely with the appearance of detectable CFI in the peritoneal fluid. It is not yet known whether the mechanism for neutrophil accumulation in this model involves a lymphokine. However, the histocompatibility requirements between tumor and host which determine survival or death strongly suggest that the mechanisms involved are immunologic.

Concluding Remarks

In this brief overview, we have described some of the ways in which the immune system can exert an effect on tumor cells. The various mechanisms that have been shown to exist are ultimately dependent on intact lymphocytes or lymphocyte products. However, the final reaction with the tumor frequently involves other participants such as various inflammatory cells or complement components.

These mechanisms are clearly imperfect in the sense that many individuals with intact immunologic machinery succumb to neoplastic disease. The modulating factors that are responsible for this are largely outside the scope of the present discussion. However, attention should be drawn to the fact that inhibition can occur not only at the stage of sensitization but also at any point in the effector sequence. It is only through manipulations

of both the basic immunologic state and the general biologic milieu of the individual that we can hope to effectively control neoplastic disease through immunologic intervention.

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