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Escape from Immune Destruction by the Host through Shedding of Surface Antigens: Is This a Characteristic Shared by Malignant and Embryonic Cells?[1]

Peter Alexander

Chester Beatty Research Institute, Sutton, Surrey, England

Summary

The hypothesis is advanced that macromolecules normally found only in embryonic and fetal cells are also found in tumors because malignant cells, like the fetus, must develop mechanisms to avoid immunological destruction by the host. While anatomical factors play an important role in the "escape" of the fetus as well as the tumor, they are not by themselves adequate. In tumors, the shedding of antigens in a soluble form provides powerful protection because such antigens compete with the tumor for the effector processes of the immune response. Soluble antigens form adducts with antibodies as well as cytotoxic cells, which are then no longer capable of killing the tumor cells. Evidence is presented that the rate of spontaneous shedding of antigen may determine in part the growth pattern of the tumor *in vivo*. Sarcoma cells, which shed antigen rapidly, metastasize more readily than those with a slow spontaneous release of antigen. It is proposed that rapid shedding of transplantation antigens is a characteristic of embryonic cells and tumors.

Relationship between Fetal Characteristics and Cancer

The evidence that most, and possibly all, malignant cells contain macromolecules that are characteristic of early embryonic and fetal life[2] has been brought together in the 3 symposia of this series. Three possibilities can be envisaged to explain their association with the malignant transformation. (*a*) The synthesis of fetal macromolecules by tumor cells is fortuitous and merely reflects the unprogrammed switching on of silent genes (*cf.* Ref. 2). This property of tumor cells was first recognized by the production by some tumors of hormones such as adrenocorticotrophic hormone and insulin which give rise to clinical symptoms; it is now known to be a characteristic feature of cancer which leads to the synthesis of many types of "untoward" proteins by tumors. (*b*) Fetal antigens are present in tumor cells because cancer is a disease of differentiation and there is, therefore, an obligate association between the malignant transformation and embryogenesis. This viewpoint has been reviewed in detail by Coggin and Anderson (4). (*c*) Cancer requires 2 types of cellular changes, one of which involves a degree of differentiation, and this leads to the acquisition of fetal proteins by tumors. One can hypothesize that at the cellular level the transformation of cells to the malignant state (*i.e.*, the loss of the capacity to respond to the normal growth stimuli that control growth) is not associated with fetal characteristics but that the proliferation of such transformed cells *in vivo* can occur only if they possess in addition some characteristics of embryonic cells.

This last model would explain the presence on the surface of tumor cells of several neoantigens, only some of which can be detected in early embryos, the others apparently represented solely in tumor cells. This situation has been clearly documented for chemically induced sarcomas in the rat (25).

Escape of the Conceptus and of Tumors

In this paper I will discuss the idea that the fetal characteristic needed for a transformed cell to produce tumors is the means to avoid destruction by the different effector arms of the immune response.

The most successful of all natural allografts is mammalian pregnancy; the evolutionary ascendancy of mammals required the development of mechanisms to ensure that the fetus not be rejected by the mother as non-self. Embryonic (18) and fetal (22) cells contain transplantation antigens to which the pregnant female is capable of reacting. Also, she has not developed tolerance to paternal transplantation antigens (16). While chorionic gonadotrophin and other hormones are immunosuppressive (19), immune reactivity is not ablated (*cf.* Ref. 5). In other words, the fetus is antigenic, and the mother can and does respond to these antigens.

The success of tumors also requires effective escape mechanisms from the host, which recognizes the neoantigens on the tumor membrane and evokes effector mechanisms, both cellular and humoral; these host responses are

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[2] Some of these evoke an immune reaction in the tumor-bearing host, presumably because they disappear early in fetal life and before tolerance to them has become established. When such molecules reappear in tumors, they are accurately described as oncofetal antigens (*cf.* Ref. 2). To other oncofetal substances there is no immune reaction in the adult host, and they are referred to only as "antigens" because they are frequently detected with antisera raised in hetero species.

potentially capable of killing the autochthonous tumor cells, but they obviously fail *in vivo*, because in the absence of treatment tumors grow relentlessly, spread, and kill. Experiments that appeared to indicate that escape is due to specific tolerance to the neoantigens have not been sustained (*cf.* Ref. 17), and it now seems clear that in cases in which the lymphoid organs are not involved or the disease is not very advanced, the tumor-bearing host reacts briskly against the autochthonous tumor. The mechanisms of escape are clearly multifactorial. The role of anatomical factors that limit the physiological expression of different effector arms of the immune response directed against tumors has already been discussed (1). Such factors are illustrated by circulating antibody, which contributes to the control of blood-borne metastatic spread (21) but appears to have little consequence in controlling the i.m. growth of sarcomas.

In the escape of the conceptus, the anatomical separation of the mother and the fetus plays a key role, and in the hemochorial placenta the maternal surface of the trophoblast presents a powerful barrier (14) to cellular traffic involved in both the afferent and the efferent arm of the immune response of the mother. However, the protection conferred by the fetomaternal barrier is not complete; there is evidence for cellular traffic from the fetus to the mother. Antibodies capable of reacting with the fetus that are produced in response to conception can be detected in the circulation of interstrain matings (20). Moreover, there is no fetomaternal barrier to such antibodies, so they readily gain access to the fetus. At least in the later stages of pregnancy the placenta seems to be leaky and may be penetrated by cells in both directions (28). Consequently, it would seem that, in addition to avoiding damage by antibodies, the fetus also needs mechanisms to interdict the cell-mediated response mounted by the mother.

For tumors also, anatomical factors must be reinforced by other mechanisms to provide for the effectiveness of the escape that is observed. Considerations such as these led to the search for systemic circulating factors that protect the target, be it tumor or conceptus (*cf.* Ref. 5), in a specific way against immune destruction. A theory promulgated by Hellström and Hellström (10), which has received much attention, is that tumors as well as the fetus are coated by circulating "blocking" factors, which are defined as having a specific affinity for the antigens on the surface of the target cells and which can be removed from the serum of the host in a specific way by absorption with target cells. Initially, the blocking factor was identified (10) as a 7*S* non-complement-binding antibody which covered the target cells and shielded them against attack by cytotoxic cells. To accommodate other data, a so-called "deblocking" antibody which antagonized the hypothetical "blocking" antibody had to be evoked (11). Subsequent failure to detect such blocking antibodies led to a further modification of the theory in which it was claimed that the serum factor that specifically bound to the target was an antigen-antibody complex (23). In fact (12), there is no evidence to support the view that escape is brought about by an antibody (or complex) that combines with or "blocks" the target, and the theory that the formation of antibody in one way or another facilitates escape *in vivo* should now be abandoned. Indeed, there is every indication that stimulation of antibody formation aids the host defenses against tumors and in no way facilitates escape (*cf.* Refs. 6, 13, and 21). A decisive blow to the blocking antibody hypothesis in all its various guises was also provided by Thompson and Linna (15, 24), who showed that bursectomy and the consequent impairment of antibody formation did not affect tumor growth in birds.

Interference by Circulating Antigen with the Efferent Arm of the Immune Response to Tumors

The 1st indication that antigen shed by a growing tumor may assist in "escape" came from studies that showed that the nodes draining a tumor, while highly stimulated, did not function normally. The observed impairment was ascribed to the combination of stimulated lymphocytes with antigen that was continually being released from the tumor (3). Since then, evidence has been accumulating that the tumor-specific membrane antigens that constitute the point of attack by the host to the autochthonous tumor are present in a soluble form in body fluids (*e.g.*, blood, lymph, and urine) of the tumor bearer (for references, see Table 1). They are effective in providing escape because they combine with the effector arms (antibodies as well as specifically cytotoxic cells) and thereby neutralize them. Moreover, in a soluble form the tumor antigens are very poor immunogens, at least in the absence of adjuvants. In other words, when soluble, these antigens do not stimulate the immune defenses but nonetheless inhibit the antibodies and cytotoxic cells that are produced when the same antigens are presented to the host as part of a plasma membrane.

Table 1 summarizes the methods that have been used to demonstrate the presence in the blood, lymph, and urine of both man and rats with tumors of soluble substances in the molecular weight range of 50,000 daltons and with the immunological properties to be expected of tumor antigens. Materials with the same immunological properties as these serum components have been isolated by papain digestion of membranes derived from melanomas and hypernephromas (G. A. Currie, unpublished information). One of the tumor antigens was obtained from a rat sarcoma in a high state of purity by affinity chromatography (26); by radioimmunological criteria (Chart 1), the antigen in the serum could not be distinguished from the circulating antigen found in the sera of tumor-bearing rats.

Table 1

Methods for detecting soluble tumor-specific transplantation antigen in body fluids

Inhibition of cytotoxicity of mononuclear cells from blood or lymph nodes of tumor bearers:
In man (8).
In rats (9).
Serological:
Neutralization of syngeneic antiserum obtained from rats after excision of tumor (27).
Radioimmunoassay using ^{125}I-labeled tumor-specific transplantation antigen isolated from tumor by affinity chromatography (26).

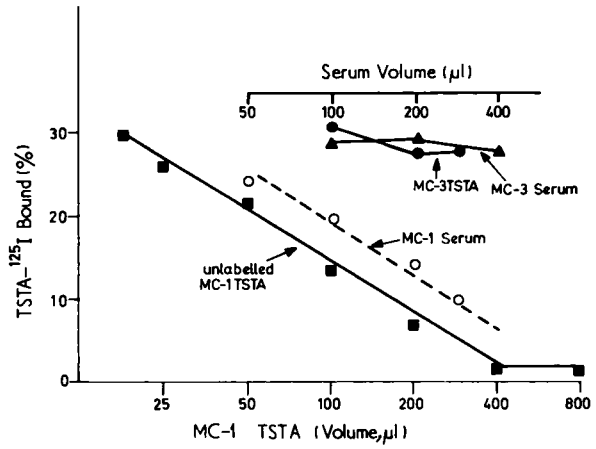

Chart 1. Inhibition curve for radioimmunoassay of the tumor-specific transplantation antigen (*TSTA*) unique to the MC-1 rat sarcoma by serum of tumor-bearing rats (O, ▲) and by extracts from tumors (■, ●). The MC-1 and MC-3 tumors do not cross-react. (From Ref. 26.)

Mechanisms of Release of Tumor-specific Antigens into the Circulation

Detailed studies showed that there are 3 distinct mechanisms by which antigen gains access to the circulation in a soluble form.

By Autolysis of Tumor Cells. When tumor cells are injected s.c. into nonimmune rats, circulating antigens appear within 24 hr in the blood of both normal and immune-suppressed rats (Chart 2). It is well established that the majority of cells administered as a s.c. inoculum undergo rapid autolysis and that only a minority survive to give rise to the transplantable tumor. Antigen introduced in this way is cleared within 3 days (Chart 2).

As a Consequence of Immune Attack. As shown in Chart 2, with some tumors like the MC-1 rat sarcoma, soluble antigen cannot be detected in the serum of immunosuppressed (*i.e.*, after whole-body X-irradiation) tumor-bearing rats (26, 27). We interpret this as showing that the soluble antigens appear because tumor cells are being killed and lysed by the immune reactions of the host. This phenomenon can be clearly observed *in vivo* only in those tumors that do not shed their antigens spontaneously at a high rate. Circulating antigen can be detected in the serum of immune-suppressed rats bearing another tumor, the MC-3 sarcoma (G. A. Currie, unpublished information).

By Spontaneous Release. Table 2 illustrates a series of experiments in which the presence of soluble antigen in serum and tissue culture supernatants was determined by measuring the capacity of these fluids to inhibit the cytotoxic action of immune lymph node cells to specific tumor cells *in vitro*, as described by Currie and Gage (9). The immune lymphoid cells were obtained from nodes draining growing tumors. It is evident that the serum of tumor-bearing rats exerted a specific inhibitory activity towards cells cytotoxic to either the MC-1 or MC-3 sarcoma cells. However, while the supernatant from tissue cultures of the MC-3 sarcoma had an inhibitory activity similar to that of the serum from MC-3 tumor bearers, only minimal activity was found in the supernatant of MC-1 sarcoma cultures (7). We conclude that the rate of spontaneous shedding of tumor antigens by growing tumor cells is much higher for the MC-3 than for the MC-1 sarcoma.

Relationship between the Rate of Spontaneous Shedding of Surface Antigen and the Capacity to Metastasize

The MC-1 and MC-3 sarcomas differ not only in the rate with which they shed antigen *in vitro* but also in their growth patterns *in vivo* (Table 3). The MC-1 tumor does not metastasize spontaneously in normal (*i.e.*, not immunosuppressed) rats, and rats can be cured of both i.m. and s.c. tumors by surgical excision, even when these are quite large. The MC-3 tumor, however, metastasizes rapidly both by blood-borne and lymphatic spread; local surgery as early as 7 days after an i.m. implant of tumor is unsuccessful, and 100% of all the rats thus treated die of distant metastases. Initially, we were inclined to attribute the metastatic behavior of the MC-3 to its nonimmunogenicity, because it proved impossible to immunize rats even against a challenge with as few as 10^2 cells by repeated immunization with irradiated MC-3 cells. Immunity to the MC-1 sarcoma, on the other hand, could readily be induced by this procedure (J. W. Proctor, C. M. Rudenstam, and P. Alexander. Spontaneously Metastasizing Rat Sarcoma Evokes Concomitant Immunity but after Irradiation Such Tumor Cells Fail to Immunize, in preparation). This is not, however, the correct interpretation, since *in vitro* tests have shown that the growing MC-3 tumor invokes a powerful immune response when measured by the *in vitro* cytotoxic activity of the cells in the draining node (9). Indeed, by this test the antigenicity of the MC-3 is not markedly inferior to or quantitatively different from that of the MC-1 tumor (Table 2). The fact that the growing MC-3 sarcoma elicits an immune response in the syngeneic host is further demon-

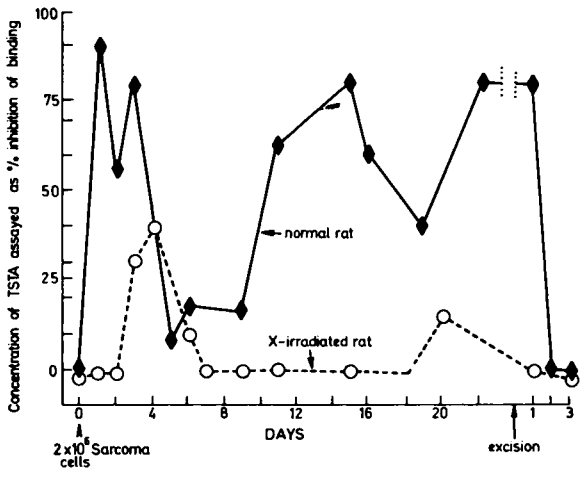

Chart 2. Time course for the appearance in the serum of rats of tumor-specific transplantation antigen during growth of tumor following injection of MC-1 sarcoma cells and subsequent surgical excision in normal rats and rats that had been immunosuppressed by whole-body radiation with 500 R of X-rays. (From Ref. 26.)

Table 2

Detection of soluble tumor-specific transplantation antigen in tumor-bearing serum and in tissue culture supernatants of sarcoma cell cultures by inhibition of specific cytotoxicity of lymph node cells (From Ref. 7)

Test system[a]	Inhibitor added from either tumor-bearing serum or tissue culture supernatant	Target cells killed (%)	Inhibition of killing (%)
MC-1 tumor cells confronted with lymph node cells from rats with MC-1 tumor	None	65	
	MC-1 serum	0 (-3)	100
	MC-3 serum	68	0
	HSN serum	63	2
	MC-1 supernatant	59	9.5
	MC-3 supernatant	67	0
	HSN supernatant	60	9.0
MC-3 tumor cells confronted with lymph node cells from rats with MC-3 tumor	None	47	
	MC-3 serum	8	84
	MC-1 serum	44	6
	HSN serum	45	5
	MC-3 supernatant	1	98
	MC-1 supernatant	41	12
	HSN supernatant	39	17

[a] The tumors used were transplantable rat sarcomas syngeneic to the hooded strain. The MC-1 and MC-3 tumors were induced by methylcholanthrene and the HSN by benzpyrene. In these 3 tumors, individually specific transplantation antigens were demonstrated by grafting techniques.

Table 3

Comparison of 2 chemically induced transplantable rat sarcomas

	MC-1 sarcoma	MC-3 sarcoma
Growth pattern *in vivo*	Local only; curable by surgery	Metastasizes to nodes and lung
Resistance to challenge following hyperimmunization with irradiated sarcoma cells	Powerful protection	No protection
Specific *in vitro* cytotoxicity of lymphoid cells from nodes draining the tumor	+++	+++
Soluble tumor-specific transplantation antigen in serum of tumor bearers		
Normal	++	++
Immunosuppressed	*-ve*	++
Soluble tumor-specific transplantation antigen detected in tissue culture supernatant	*-ve*	++

strated by the fact that a rat with a growing MC-3 tumor shows specific concomitant immunity if challenged at a distant site with MC-3 cells (J. W. Proctor, C. M. Rudenstam, and P. Alexander. Spontaneously Metastasizing Rat Sarcoma Evokes Concomitant Immunity but after Irradiation Such Tumor Cells Fail to Immunize, in preparation).

The capacity of the MC-3 sarcoma to metastasize and the failure to induce immunity to it with irradiated cells cannot, therefore, be attributed to the absence of tumor-specific antigens. It is tempting to associate these properties of MC-3 sarcoma cells with their ability to shed antigens in a soluble form. A metastatic deposit of MC-3 cells may avoid destruction by the host because it is enveloped by soluble antigen which intercepts the host defenses. The rate of shedding of antigen may determine the size of the tumor that can escape from the immune defenses. If the amount of antigen that is shed is low, then only relatively large tumor masses will be protected, and metastases will be eliminated by the host; this is presumed to be the case for the MC-1 sarcoma. If shedding is intense, then even small tumor foci will escape, hence the high rate of spontaneous metastatic spread of the MC-3 and its capacity to be transplanted with a very few cells.

The inability to immunize with MC-3 cells that have been exposed to 10^4 rads of X-rays may also be related to a high rate of antigen shedding. This dose of radiation, needed to sterilize a high inoculum of cells, will interfere with protein synthesis, and the synthesis of antigen may thus be stopped. In the case of the MC-1, the "half-life" of antigen within the membrane may be relatively long so that, even in the absence of further synthesis, radiated cells are immunogenic (*i.e.*, can present the host with antigen bound to membrane). Rapid shedding of antigen may imply that the "half-life" of

an antigen molecule within the membrane is short; this may not interfere with the immunogenicity of growing tumor cells, since the antigens within the membrane will be continuously replaced by new synthesis. However, after radiation the membrane of the MC-3 cells may quickly become deficient in antigen and thus fail to immunize.

Antigen Shedding and the "Fetal Connection" of Tumors

In the preceding sections I have summarized the evidence for the view that the release of soluble antigens from the membrane of tumor cells presents a potent means of escape from the immune defenses of the host. Tumors are not unique in this respect; the virulence of different strains of pneumococci has long been known to be determined by the rate at which they release the cell surface polysaccharide antigen to which the host reacts. Similarly, the successful growth of some metazoan infections has been attributed to the release of membrane constituents. Does the fetus also make use of this mechanism to guard against the hazards of immunological attack by the mother? The rate of shedding of normal transplantation antigens from adult cells must be low, since such antigens can be found only in very small amounts in normal serum. Indeed, it has only been within the last 3 years that such substances have been detected in human serum. The hypothesis that my colleagues Currie and Sime are currently testing is that the cells of the embryo and fetus differ from those of the adult in that, like some tumor cells, they shed transplantation antigens at a high rate from their surface. If this were the case, one should find transplantation antigens, either free or as antigen-antibody complexes, in both the maternal and fetal circulation. *In vitro* experiments with embryonic cells are unlikely to be decisive, since the cells may rapidly undergo differentiation. As yet, there is no decisive evidence for this hypothesis, but preliminary experiments are promising. In earlier experiments Currie (5) had shown that pregnant mice rejected paternal strain tumor allografts much less readily than unrelated tumor grafts. This could be explained by antigen shedding from the fetus, since the molecular size of transplantation antigen is sufficiently small for it to pass into the maternal circulation. Currie was, in fact, able adoptively to transfer specific unresponsiveness into virgin females with serum from pregnant mice.

Whatever the mechanisms, it is clear that escape processes must be highly developed in the fetomaternal system and that they may be linked to physiological processes present in fetal but not in adult cells. Similarly, a cell that has undergone malignant transformation must acquire the capacity to escape if it is to be manifest as a tumor. I suggest that the malignant cell may mimic the escape mechanisms that evolved in the fetus. Antigens in tumors may thus be a consequence of the tumor's having acquired some of the fetal escape mechanisms.

One attribute of the malignant transformation is the capacity to switch on silent genes to make products otherwise formed only by differentiated cells or cells in a defined stage of development. The production of these new products is not susceptible to the normal feedback control. Thus hormone-synthesizing tumors continue to make hormone when the normal endocrine organs are totally repressed. Hence the paraendocrine syndromes associated with some forms of cancer. Once a malignant cell has become derepressed with regard to the production of a particular protein or set of proteins, it continues to produce these whatever the host signals. One would expect the same situation to arise if a malignant cell switches on to make a phase-specific protein. In normal cells, the genes responsible would be derepressed only during a specific phase of development, but a malignant cell would not be so limited. The existence of tumor-specific antigens could, of course, represent the inappropriate synthesis of such fetal phase-specific proteins.

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