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Inhibition of Carcinogenesis

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Preface

This volume has been devoted to examination of aspects of carcinogenesis that are amenable to inhibition and control. It contains a wide range of current approaches varying from the implications of new basic concepts of oncogenesis and the limitations of immunosurveillance to the practical considerations of altering the hostile carcinogenic environment. Between these poles, there are new developments dealing with the mechanism of carcinogen inhibition by chemical antagonists, by enzymes and by alteration of host responses.

While the mechanisms of tumor immunology still remain complex and contradictory, new techniques are becoming available that can make some long-frustrated hopes approach reality. And it is in this area that new and important application are here presented by MORTON *et al.* and GOLD who describe the use of immunological indicators in cancer diagnosis and prognosis.

Two basic levels of carcinogenesis research are discussed by PREHN in the first paper. There is a critical examination of the established dogma of immunosurveillance, exposing the fallacy of applying simplistic immunologic concepts to tumor inhibition. Much of the work presented in the later chapters by GOLD, MORTON *et al.* and RUBIN bear out PREHN's insight into this complex problem.

On the other hand, the nature of the process of oncogenesis also needs a fresh approach. PREHN's comparison of regeneration in lower vertebrates with carcinogenesis provides a novel stimulus to a re-examination of current views. The work of GOLD on the embryonic nature of tumor antigens may establish a practical link to the blastoma concept proposed by PREHN.

There are other types of host defenses, besides immunity, that can affect carcinogenesis. DAO and WATTENBERG have helped to bring some order out

of this complex field, and they begin to show that such knowledge can have application in prophylaxis by the conscious alteration of hormonal and enzymatic mechanisms.

The application of prophylactic techniques to the curbing of environmental carcinogenesis hazards is considered by FALK, who has pioneered the studies of carcinogenic antagonists and here describes their various activities and limitations.

The paradoxical reactions of chemical antagonists are demonstrated in the paper by RUBIN who shows that these agents can also interact with other host factors which can act to *enhance* carcinogenesis, perhaps by distracting the enzymes capable of inactivating the carcinogens. In this final paper, RUBIN shows the intimate relationship between the homograft rejection mechanism and carcinogenesis. Mechanisms that affect one can alter the other in quantitatively similar ways. Chemical mechanisms that stimulate immune response can also inhibit carcinogenesis.

The prospects for the control of carcinogenesis are coming into sight. Several approaches are becoming available. The activities of carcinogens may be minimized by the elimination of environmental hazards, by the inactivation of carcinogens, or by the inhibition of their activity. On the other hand, it may be possible to enhance responses of the host in meaningful ways that will encourage the rejection of disease. And as a by-product of these developments, we may improve the prospect for cancer therapy by the use of new immunological indicators of disease and cure.

F. HOMBURGER, Series Editor
B. A. RUBIN and B. L. VAN DUUREN, Guest Editors
of this volume

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Immunosurveillance, Regeneration and Oncogenesis[1]

R. T. PREHN

The Institute for Cancer Research, Philadelphia, Pa.

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I. Introduction

The tumor-specific immunogenicity of most transplanted neoplasms is now well established. It has also been shown that the very animal in which a particular tumor originated can react immunologically to transplants of that tumor [56, 58]. Under these circumstances it is not surprising that the pendulum of opinion has swung from the immunity-is-theoretically-impossible position of the fifties to the currently prevalent belief that neoplasia may actually be the *raison d'être* for the existence of the homograft type of immune mechanism [40, 111]. It is argued that neoplasia may be largely a vertebrate disease and that this type of immune mechanism evolved to meet this specific threat [15]. Increasingly, the opinion is expressed that were it not for the surveillance function of this mechanism, no vertebrate could long escape death from neoplastic disease [13, 14, 40]. Although this extreme emphasis upon the importance of the immune reac-

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tion is a heuristically appealing position and offers the hope of providing preventive and therapeutic measures, the time has come to question whether or not this extreme position is really justified by the known facts. The issue is no longer whether or not immunity plays a role in the biology of cancer—it does—but rather how important is the role it may play. Therefore, it is my purpose in this chapter to examine the evidence concerning the efficacy and importance of the immune response as a cancer surveillance mechanism. In addition, I will survey another concept that also arises from a consideration of phylogenetic relationships, namely, the concept that a neoplasm may be the counterpart of the blastema of regeneration found in lower forms. I will conclude by considering the possible implications of each concept for the other.

II. Immunosurveillance

If classical immunity is to serve as an efficacious surveillance mechanism, several prerequisites must be largely met. These can be listed as follows and will subsequently be discussed in the same order. Throughout this discussion the antigenicity referred to is always of the cell surface type, which is potentially able to stimulate a cytotoxic immune reaction even in the animal in which the tumor first arose.

1. The cells of most neoplasms must be antigenic in the animal of origin, i.e., they must have cell surfaces with immunogenic potential. Furthermore, the antigenicity must be present at the start or very early in the development of a neoplasm if immunosurveillance is to be maximally effective.

2. The antigen-bearing tumor cells must actually stimulate the immune mechanism and preferably do so early in the course of tumor development. Antigenicity, as demonstrated by transplantation experiments, is not sufficient by and of itself.

3. The host must be immunologically competent. If immunologic surveillance is an important factor in tumor control, the tumor incidence should vary inversely with this degree of competence.

4. The interaction of the tumor cells with the host must not result in significant degrees of immunologic tolerance or enhancement, but rather in immunity.

5. The antigenic specificity must be stable and the quantity of antigen per cell must be and remain fairly high.

6. The tumor cells must be susceptible to immune attack—whether cytotoxic or cytostatic.

Let us now see to what extent these varied prerequisites for immunological surveillance appear to be fulfilled.

It is now widely believed that most, and perhaps all, neoplasms are antigenic in the animal of origin [94]. This viewpoint is certainly a far cry from the attitude of slightly over a decade ago. However, in our enthusiasm we may be guilty of overstating the case. The trouble lies in the fact that it is theoretically impossible to prove the absolute absence of antigenicity and hence we tend to disregard those cases in which antigenicity is not demonstrable. Perhaps antigenicity is really there, but too slight to be detected by current means, or it is masked by some unknown process. This belief is reinforced by the fact that there may be no class of tumors of which all members fail to exhibit antigenicity. Thus, tumors arising by the spontaneous transformation of mouse cells in tissue culture usually have little or no antigenicity, but occasionally such a neoplasm is clearly immunogenic [99]. Lung adenomas induced by urethan are seldom demonstrably antigenic but are in certain instances [90]. Similar results have been obtained with rat mammary carcinomas induced by 2-acetylyaminofluorene [6]. Many but not all spontaneous neoplasms have little or no antigenicity [5, 95]. In all these and other cases, is it correct to assume that our methods are inadequate and that the specimens with good antigenicity are indicative of the real status of the rest? Perhaps rather than emphasizing, as was necessary ten years ago, that most and perhaps all tumors are probably antigenic, we would be better advised to say that most spontaneous and some experimentally induced tumors have little or no measurable antigenicity. Of course, if one assumes *a priori* the efficacy of immunologic surveillance, then the lack of antigenicity of spontaneous tumors is to be expected; these would represent the small minority of neoplasms that were able to survive such surveillance [96]. However, immunoselection cannot account for the lack of antigenicity in the spontaneously transformed tissue cultures. Perhaps most incipient neoplasms, as they occur in nature without the etiologic assist of high concentrations of virus or chemical, are really not very antigenic. This seems as valid an assumption as the converse. Thus, the basic premise upon which the hypothesis of immunologic surveillance depends, namely, the nearly universal existence of effective levels of antigen in tumor cells, is still questionable.

Effective immune surveillance seems to demand, in addition to the antigenicity of most tumors, that the antigenicity be present from the very

start of the process or at least come into existence very early in the course of tumor development. This is so because of the well known fact that large numbers of tumor cells can override levels of immunity that can control small numbers [56]. If a tumor were to reach a large size prior to the formation of antigenicity, the subsequent immunity might often be too little and too late. Fortunately for the surveillance hypothesis, those tumors that appear to be antigenic can, at least in the cases thus far examined, be shown to be antigenic quite early in their formation. Thus, the papillomas that precede hydrocarbon-induced carcinomas of mouse skin were shown by LAPPÉ to be antigenic and each papilloma, while apparently antigenically distinct, shared specificity with the carcinomas that arose from it [62, 63]. A similar situation has been demonstrated in my laboratory by Mr. GLENN SLEMMER with hydrocarbon-induced hyperplastic nodules, the benign growths that precede breast carcinoma in the mouse [93]. A similar situation probably occurs in liver carcinogenesis [34]. These data suggest that antigenicity, when it occurs, occurs early enough to satisfy this requirement of the surveillance hypothesis.

It is of course not sufficient that early tumors be antigenic when transplanted; they must also stimulate the immune mechanism while still small and *in situ*. There is little direct evidence bearing on this point, but what there is suggests that this prerequisite for immunological surveillance may not always be well satisfied.

The first evidence to suggest that a very small incipient neoplasm might not stimulate host immunity was the 'sneaking through' phenomenon first described by HUMPHREYS *et al.* in a tumor homograft context and later confirmed by OLD *et al.* and POTTER *et al.* in the case of tumor-specific immunity [54, 79, 87]. These workers showed that a minimal number of inoculated tumor cells might be able to grow, whereas a slightly larger number would arouse an immune response and be destroyed. Presumably, when the inoculum was very small no immunity was stimulated, at least until after the tumor was too large and well established to be overtaken by the immune reaction.

The second line of evidence concerning the immunogenic properties of very early neoplasms comes from the mouse breast system. As already mentioned, the premalignant lesions (hyperplastic nodules) induced by methylcholanthrene are antigenic. However, it is quite clear that they arouse little immunity while *in situ* in the mammary fat pad. It is only after further tumor progression or transplantation, or when the growth is no longer restricted to the fat pad, that an immune response may occur [108].

Data have been presented by BLAIR *et al.* suggesting that the fat pad is an immunologically privileged site [8]. Thus, lesions arising in it appear, during their early evolution, to be overlooked by the postulated immune surveillance mechanism.

The third system which casts some doubt on the immune mechanism as an important suppressor of incipient neoplasms comes from a consideration of the behavior of hydrocarbon-induced skin papillomas in the mouse. LAPPÉ showed, as already mentioned, that these neoplasms are antigenic. Immunity is capable of causing their regression when the papillomas are produced in hydrocarbon-initiated skin by the promoting stimulus of skin transplantation [62]. However, it is noteworthy that even when the skin grafts were made to immunosuppressed recipient animals many of the papillomas still regressed. Perhaps the immunosuppression was not sufficient and there was still some immune capacity. However, HARAN-GHERA has been unable to influence the incidence of hydrocarbon-induced, croton oil-promoted papillomas by immunosuppression with antilymphocyte serum [47]. Taken together, these 2 sets of data suggest that some mechanism other than classical immunity may be primarily responsible for papilloma regression in the mouse and that an immune response may not be aroused unless the papilloma-bearing skin is transplanted. The work of YOSUHIRA, in contrast to LAPPÉ's studies with transplanted skin, suggests that immunity may operate against the nontransplanted *in situ* lesions only late in their evolution, rather than as a surveillance mechanism against the premalignant papillomas [119].

It should be noted that even advanced tumors may not always maximally immunize. This is suggested in the work of HADDOW and ALEXANDER [46]. These workers were able to increase the sensitivity to X-rays of well established transplanted tumors by further immunization of the tumor-bearing animals. If even relatively large established tumors can fail to immunize maximally, it is perhaps not surprising that small incipient lesions may sometimes be even less efficient in arousing an immune response. On the other hand, there is some evidence that very early chemically-induced premalignancies of the liver may often be suppressed by an immune surveillance mechanism [34].

Obviously, the host must be immunologically highly competent if immune surveillance is to be effective. This prerequisite requires little comment, except to point out that alteration of the immunocompetency of the host should, if immune surveillance is indeed an important anticancer mechanism, have an important influence on tumor incidence. Unfortunate-

ly, it is impossible to alter host immunocompetence without altering numerous other systems, known and unknown. Consequently, the results of such investigations can only be suggestive. Nonetheless, the correlations that have been found are generally consistent with at least some measure of immunological surveillance. These can be listed as follows:

1. Neoplasia is generally easier to induce in newborn or very young, immunologically immature animals than it is in young adults [24].

2. Most and perhaps all of the known oncogenic agents seem to interfere with the immunocompetence of the host [91, 109a]. This includes the chemicals, radiation, and some viral agents. Thus, RUBIN showed that skin allografts enjoyed prolonged survival in mice that had been previously painted with a carcinogenic hydrocarbon [101].

3. Clinical syndromes that are characterized by decreased immunocompetence seem generally to be associated with higher than normal incidences of neoplastic disease. These include kidney transplant patients on immunosuppressive drugs, congenital disorders of the immune mechanism, Down's syndrome and other cytogenetic defects, and last but not least, aging [17, 32, 45, 82, 84, 85]. However, the most prevalent neoplasm accompanying each of these states, with the exception of aging, is some form of lymphoma. Perhaps a clinical condition involving a gross defect in the immune mechanism might be expected to develop leukemia as a result of prolonged compensatory leukocyte proliferation, a mechanism having nothing directly to do with a lack of immunosurveillance. In aging there are so many abnormalities other than a decrease in immunocompetence that it may be unwise to attach much significance to the correlation.

4. In a number of experimental systems it has been possible to increase the tumor incidence or to speed tumor appearance by some measure that interferes with the immune response. Thymectomy of experimental animals as newborns has increased the rate of appearance of several virus-induced tumors and of hydrocarbon-induced skin tumors and sarcomas, and urethan-induced mouse hepatomas [23, 43, 55, 64, 69, 72, 73, 77, 97, 118]. Recently, reports have begun to appear about an increased tumor incidence under the influence of antilymphocyte serum [2, 65].

However, rather disconcertingly, newborn thymectomy decreases the incidence of mouse breast cancer [50, 51, 70, 103, 120]. Also, the results have not been striking in those systems in which the incidence of chemically induced tumors was increased. Possibly this is due to the immunodepressive effects of the carcinogenic agents. These would tend to reduce the immune response in both thymectomized and control mice toward a com-

mon low level regardless of what immunodepressive treatment, such as thymectomy, might be given the experimental group. It was to overcome this problem that the skin transplantation experiments of LAPPÉ, already referred to, were devised [62]. However, while this experimental design eliminated the immunodepression produced by the carcinogen, it did introduce the possible complication of transplantation, as I described in a previous section of this chapter.

In those systems of viral-induced tumors in which thymectomy has had a profound effect, it is difficult to know whether the immunity affected was directed primarily against the viral agents or against the tumor cells. If the former, these successes may have little relevance to the hypothesis of immunosurveillance against tumor cells *per se*.

5. A further item that supports the inverse correlation between the level of immunocompetence and the tumor rate is the lowering of mouse tumor incidences by treatment with an extract of BCG, an immunostimulator [76, 78, 107, 116]. Also, poly I:C interferes with papilloma production in the mouse skin by hydrocarbons [37]. It is doubtful that the antitumor activity of this agent is directly related to its interferon-inducing activity, since poly I:C has been shown to accentuate the graft *versus* host reaction [16]. It seems more probable that it nonspecifically stimulates immune reactions, much as does BCG.

Immunological surveillance cannot be very effective if stimulation of the immune mechanism by the incipient tumor leads to either tolerance or enhancement rather than to tumor immunity. There is little information on this subject, especially in the case of incipient neoplasms where such information would be most meaningful. It is known that in the cases of most experimental solid tumors humoral antibodies interfere with rather than aid immunity [56]. The growth of the tumors thus appears to be enhanceable and a direct demonstration of this has been presented using methylcholanthrene-induced sarcomas of the mouse [74]. STJERNSWÄRD has presented some evidence in favor of the role of tolerance in chemical oncogenesis, but I think the data could as easily be interpreted as supporting enhancement [109]. Tolerance is however an important phenomenon in some viral tumor systems [56, 98]. That enhancement is a real and important condition has been shown by the elegant work of the HELLSTRÖMS and their associates. In a variety of animal and human tumors, autologous sera specifically inhibited the colony-inhibition reaction of the autochthonous lymphoid cells against the tumors [48]. Again, if one wishes to believe in the power of immunosurveillance, perhaps the cases studied by the

HELLSTRÖMS represent only the exceptional tumors that escaped the surveillance mechanism, possibly because of enhancement by humoral antibody.

The antigenic stability of the tumor cells is an obvious prerequisite for competent immunosurveillance. A tumor which could rapidly alter its antigenic potential or specificity might stand a good chance of evading immunosurveillance. Those tumors induced by the oncogenic virus polyoma apparently have quite stable antigenicities. SJÖRGREN was unable to alter significantly the titer of the transplantation antigens associated with polyoma tumors by vigorous attempts at immunoselection [106]. However, the antigenic titer of these viral tumors does vary from case to case. The apparent antigenicity of hydrocarbon-induced sarcomas, as measured by transplantation tests, may be decreased by immunoselection [11, 97]. There is doubt that the tumors ever completely lose all antigenic titer [79]. The specificity, in contrast to the titer, seems to remain constant over a number of transplant generations [38]. Thus, it seems that antigenic stability is the rule in some cases, as exemplified by polyoma tumors, and considerable lability as to titer is the rule with chemically induced lesions. Overall, it seems fair to say that most tumors have a sufficient antigenic stability to permit the possibility of reasonably efficient immunosurveillance.

Immunosurveillance demands as a prerequisite, not only that tumors produce an early immunologic response, but that the cells be susceptible to such attack. It is difficult to separate this susceptibility from the other elements in the total immune reaction. However, it is known that there are variations in this regard. For example, lymphoid tumors and some dissociated cells of other tumors are generally more sensitive to the cytotoxic effects of circulating antibody than are cells in solid tumors. The latter are more likely to be enhanced by such antisera [56]. Furthermore, the result of immunologic attack, even when nonenhancing, may not always be cytotoxic. BAILIFF has presented data suggesting that the Ehrlich ascites tumor may in some instances be modified in its pattern of differentiation rather than completely destroyed [4]. Likewise, DECOSSE has reported that the immune response may reversibly prevent replication in a portion of a tumor cell population [22]. Undoubtedly, many other variations exist in the response of tumor cells to immunity, but there is not sufficient information to permit a judgment as to how important such variation may be in modifying the effectiveness of possible immunosurveillance [29].

In summary, it appears that evidence for several of the prerequisites for immunosurveillance is not yet fully convincing. It is not clear that all tumors are antigenic. It is possible that the great majority of those occur-

ring spontaneously are not. In some systems (in breast and skin tumors in the mouse and in the 'sneaking through' phenomenon) there are data that suggest that very early lesions, even though potentially antigenic, may not always arouse the immune mechanism. Furthermore, immunologic enhancement may be a common phenomenon—and if this is true during early tumor development it might prevent effective immunosurveillance. Additionally, studies designed to test for the presence of immunosurveillance by showing an increase in tumor incidence in immunologically defective animals or humans have yielded only suggestive results. On the other hand, none of the evidence I have cited rules out the possibility of effective immunosurveillance. The evidence does, however, cast some doubt upon its probable general importance.

It is not necessary, *a priori*, to postulate an effective immunosurveillance mechanism to account for the low incidence (considering the numbers of cells at risk) of neoplasia. Other control mechanisms have been demonstrated and more will probably be found. One striking example was discovered by DEOME *et al.* in the mouse breast, and has been repeatedly verified [28]. There is a regulating mechanism which, during the ontogeny of the ductal tree of the breast, determines the spacing of the mammary ducts; normal ducts are prevented from growing closer to other normal ducts than a certain distance (about 0.25 mm). This normal control mechanism is also effective in regulating most early premalignant neoplasms of the breast. These lesions can proliferate in the absence of surrounding normal epithelium, but are inhibited by the near presence of normal ducts. This, then, is nonimmunologic inhibition of the early premalignant growth by surrounding normal tissue of the same tissue type. It does not require cell-to-cell contact.

Numerous other regulating processes may be postulated and perhaps demonstrated. The epithelial chalone described by BULLOUGH *et al.* may be an example [12]. A phenomenon possible requiring cell-to-cell contact has been described by STOKER [110]. He has shown that a population of normal cells can inhibit tumor cells *in vitro*. A striking illustration of the possible general principle that neoplasia may be inhibited by surrounding normal tissues of the same type has been described by LEWIS [66]. He has observed that malignant melanomata of the soles of the feet of negroes in Africa never invade the pigmented areas at the margins of the soles nor do they metastasize to pigmented areas of skin. A further possible nonimmunologic surveillance mechanism is called allogeneic inhibition. It has been well discussed by HELLSTRÖM [49] and need not be described here.