David W. Weiss

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An Outline

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David W. Weiss
Lautenberg Center for General and Tumor Immunology
Hebrew University - Hadassah Medical School
Jerusalem, Israel

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Introduction

This volume is not intended as review of the large literature on tumor antigenicity and efforts at tumor immunotherapy. Its purpose, rather, is to present discursively an outline of the likely approaches to immunological intervention in neoplastic diseases which present themselves today, in light of the probable antigenic properties of cancer cells. References are cited only selectively, in illustration of some of the major considerations to which allusion is made and of some of the supportive evidence. No attempt is made at inclusiveness in the citation of concepts and findings. If undue emphasis appears to be given to some aspects of the literature and only sparse documentation to others, the grounds do not lie necessarily with a critical estimation of the extent or quality of reported work, but rather with the bias of the writer who considers stress on some facets of the field more appropriate than on others for elaboration of his arguments. The references brought in support of a given point are often intentionally varied, including both reports of original work and reviews, very recent observations and contributions that gave initial impetus to investigations, in an attempt to exemplify the pertinent literature; and reference is made both to data presented and to concepts advanced. The accent placed on studies conducted by the writer and his present and former associates is motivated not by any attribution of exaggerated significance to this work, but rather by an intimacy of familiarity and by the consideration that our own efforts in the field over the past two decades have been representative of many of its fluctuating developments.

Contents

1 Tumor-Associated Antigenicity and Host Responsiveness: Basic Questions and Considerations

The central hypothesis underlying all attempts at immunological intervention in neoplastic diseases is today in danger of falling. The expectations rife only a few years ago that most, if not all, neoplasms would be found to express immunogenic determinants characteristic of the transformation event, or of the neoplastic condition per se, have come to be viewed by many investigators as the hopeful but probably unwarranted extrapolations of findings with a limited spectrum of experimental tumors in laboratory rodents [164, 213, 217, 218, 292, 424, 430], test models of very questionable relevance to neoplasia in nature [164, 425, 428].

Although there is now developing a body of more carefully adduced evidence for the distinctive antigenic properties of some spontaneously arising growths of man as well as of laboratory animals [13, 39, 65, 116, 136, 137, 158, 242, 278, 279, 336, 337, 402, 403], in addition to those of experimental neoplasms [88, 274, 443], past failures to establish the specificity of immunological responses directed against cancer cells leave standing the question whether neoplasm-unique anitgenicity is a universal, or even common, manifestation of spontaneously transformed cells. Still greater doubt has been cast on the capability of any distinguishing tumor antigens to evoke *protective* immunological reactions by the host. At least some spontaneous cancers of mice and rats do not present antigens which readily elicit protective immunity [164, 165], and even tumors experimentally induced by powerful carcinogens do not perforce possess such constituents [15, 16].

It is necessary, moreover, to hold in abeyance the surmise of a qualitative tumor-*specific* antigenicity (TSA) even with regard to neoplasms which do appear to display peculiar antigenic attributes. The biological nature and immunological behavior of antigens particularly associated with the neoplastic state remain very uncertain. Where such antigens are presumably demonstrated, they have often been discovered to be entities exhibited as well on normal cells, but primarily early in life ("fetal" or "embryonic" antigens), perhaps fleetingly and in minute quantities; or only in other tissues, sometimes highly specialized ("organ-specific" antigens); or in cells of the same type but there in categorically smaller amounts or different molecular configurations [6, 18, 19, 35, 54, 77, 138, 226, 238, 241, 253, 307, 351, 391, 223]. It is virtually impossible, accordingly, to disprove the eventuality that a seeming TSA is not, in fact, such a normal, "displacement" antigen. Even where the occurrence of such markers on normal cells is minimal, dissimilar, only transient, or restricted to specialized, perhaps immunologically sequestered, tissues, their representation (within the lifetime of the organism) among normal self-structures consigns them to a category other than that of any hypothetical antigens wholly unique to the circumstances of neoplasia, and places large constraints on their operative immunogenicity. Tumor-*associated* antigen (TAA) thus appears a far more appropriate term than tumor-specific antigen [35].

It must be said at once, however, that the decisive question to be asked from the perspective of immunological intervention in malignant disease is not whether spontaneous tumors are, on the whole, de facto immunogenic as they exist in nature, but rather whether they are *potentially* immunogenic. A clear differentiation must be made between antigenicity and immunogenicity. In the context of tumor immunology, we may define as antigen any structure of a neoplastic cell variant which is sufficiently different in kind, organization, or amount from the composition of analogous normal cells to be capable of inciting an immunological response *under appropriate conditions.* The

designation "distinctive tumor antigenicity" should thus imply no more than the *potential* for immunological reactivity, or potential immunogenicity. The term immunogen, in contrast, should be restricted to tumor cell constituents whose potential immunological reactivity is realized, which by reason of their characteristics and of the suitability of host and environment actively evoke immunological responses.

Many factors impinge on the realization of immunogenic potential. In addition to the alterations in cell structure which accompany or follow neoplastic transformation, they include host genotype, age, sex, and previous experience with related antigens; external environmental variables which influence host immunological capacity; and the tissue environment in which the initial and subsequent confrontations between host and neoplastic parasite take place [423]. Some of these determinants are labile and inherently variable, and instead of static descriptions of tumor cell immunogenicity there are demanded definitions formulated precisely as a function of time and flux in the course of given host–tumor associations. This is borne out by the consideration that the amount of antigen presented [98, 161] as well as the method and route of presentation and its persistence in host tissues can cetermine whether TAAs reach the threshold of immunogenicity, quantitatively [161].

The range of factors which actuate the translation of antigenicity to immunogenicity can be extended by the investigator. Spontaneous alterations in the molecular constituents and arrangements of a tumor cell may be too small to bring the cell to the threshold of immunogenicity in a host of ordinary immunological ability. Artificial modification of TAAs can, however, bring about host responses which will be directed ultimately at the unmodified structure as well; and, nonspecific potentiation and modulation of the immunological apparatus can make for responsiveness of which the unstimulated organism is incapable. Such manipulations of antigen and host must be included within an operative perspective of the potentials of immunological reactivity toward tumor cell components.

Autoimmune reactions directed at normal cells are a not uncommon event, moreover, and evidence has been brought forward for the constant presence among the immunocyte complement in healthy subjects of clones programmed against ordinary self-components, prevented from exerting cytotoxic effects in vivo only by circulating blocking substances and perhaps also by suppressor cells [80, 371]. The proclivity for autochthonous recognition of some or many of the components of normal tissue may thus be, in fact, a pedestrian immunological reality, whith only the final cytotoxic consequences being the exceptional, pathological happening. The argument is further advanced by the proposition that the cytotoxic T lymphocyte receptor repertoire is basically directed at variants of autologous major histocompatibility complex (MHC) products [58, 140], a possibility supported by the finding that a large proportion of cytotoxic T cell precursors are programmed against such antigens [38]. Persuasive evidence that at least some TAAs may be closely related to normal MHC-coded structures has been advanced by some investigators, although the conclusion is questioned by others [64, 68, 117, 222, 257, 287, 453].

Although immunological reactivity against fetal, organ-specific, and other normal-cell antigens is likely to be compromised within the framework of self-tolerance, a degree of cognizance of tumor cells as "alien" because of the exposure of such markers at a deviant time or place or in variant amounts and arrangement is not, accordingly, improbable. Sensitization against normally occurring self-markers may result from in situ recognition of their aberrant expression by relevant lymphoid centers, as well as from

systemic responsiveness to changes in antigen makeup and presentation that accompany abnormal tissue growth and damage. It is known that organ-specific antigens can be reacted to as markedly alien when they are transposed to distal body compartments, especially where the transposition is from or to sites not normally in full systemic immunological communication within the organism. Adult capacity to react toward antigens which characterize structures in early development, then eclipse, and are reintroduced experimentally in maturity has indeed been demonstrated in models not pertaining to neoplasia [389]. "Fetal" or "embryonic" antigens may persist on certain normal cells (stem cells?) throughout life, albeit at very low levels, and could in fact thereby reinforce a measure of immunological awareness of their presence, albeit normally suppressed and quiescent. Despite the tendency of some investigators to the contrary, one cannot discount the possibility that some fetal antigens on tumor cells can incite and be the targets of cytotoxic immunological reactions [18, 19, 253].

It would thus seem that host responsiveness to neoplastic cells is not conditioned wholly on their presentation of truly unique antigens, and that it may suffice if our immunotherapeutic intentions center on the detection and maneuverability of antigens merely *associated* with the neoplastic state – antigens that may be deviant from normal only in ontogeny, steric configuration, quantity, or tissue localization.

A generally assumed qualification has been that the TAAs of interest in host defensive responses are those located on the cell surface, there providing targets for immune attack, i.e., tumor-associated transplantation antigens (TATAs). This view may be an unnecessarily restricted one, however. Recent experiments conducted by *J. Vaage* (1978, personal communication; 398), suggest that immunological reactions expressed against some tumors may be manifested by a process of walling off and necrosis of the neoplastic focus akin to tubercle formation [306], rather than by direct cytotoxicity against the living transformed cells; as has also been suggested by other workers (*G.J. Svet-Moldavsky* 1978, personal communication; 459), nonlymphoid cells of stromal origin may play a large role in attracting other cell types to participate in such indirect attack or serve themselves as "natural killers." Although these findings are preliminary, they point to the possibility that TAAs other than those located on the cell surface may be involved in the elicitation of host defenses, by triggering a sequence of responses leading to tumor destruction mechanically and by change in the local tissue environment.

We can then rephrase our central question, operatively: Are the molecular changes which characterize tumor cells sufficient in themselves to allow for host immunological responsiveness, or do they at least provide a handle for extrinsic activation to immunogenictiy and host reactivity?

Host-tumor interactions in which immunological reactivity to the neoplastic variants does develop, naturally or in consequence of extrinsic intervention, present a second key question: Do the immunological mechanisms brought into play lead to inhibition or destruction of the tumor cells, are they without appreciable import for tumor cell growth, or do they cause tumor growth stimulation, directly [293, 294] or by affording protection against other, damaging facets of the response? This question, too, must be posed of each individual neoplastic process, repeatedly in the course of its evolution; and the likelihood must be entertained that the multifaceted immunological responses to antigenic stimulation have varied, changeable, and mixed implications for the fate of tumor cells [118, 451]. Even where cellular immune responses are of defensive value, distinct immunocyte populations may be responsible for cytotoxic and for cytostatic action [1, 356]. Conversely, the possibility cannot be excluded that a host cell characterized in

given tests to have reactivity of given import may also have other, even contradictory, capacities, that come to light by other appropriate measurements. It is not inconceivable that the same cell can execute, under different circumstances or even simultaneously, cytotoxic, suppressor, and stromal functions toward the same or another tumor (63, 459, *E.M. Fenyö* 1979, personal communication; *F. Vánky* 1979, personal communication). This eventuality is not ruled out by findings of different surface markers on immunocytes showing distinct behavior in defined test systems; lymphoreticular cells may be flexible in the phenotypic expression of membrane determinants, which may come to the fore differentially as the cell experiences changing physiological conditions and excitation.

A third core question to which answers must be sought as basis for the construction of rational pathways to immunotherapy is addressed to tumor escape from immunological control, and to idiosyncratic host immune failure. Where tumor antigenicity, host genotype, and both external and internal environmental variables are such as to permit the mounting of tumor inhibitory immunological responses, what are the means by which clones of neoplastic cells can avoid or abort immunological attack, and what are the epigenetic circumstances that can produce precipitous host failure at effective responsiveness, systemically or in the immediate vicinity of the tumor? The earlier conception of host immunological dyscrasia and tumor "sneaking through" as primary causes for progressive neoplastic disease is no longer given prominence by many investigators; inadequate immunogenicity of those tumors that constitute the clinical problem is deemed an adequate reason for their occurrence. This view seems insufficient, however. Both direct and indirect evidence for immunological capacity of at least some organisms against at least some spontaneous neoplasms is accumulating; phenotypic immunodeficiency does appear to contribute to host susceptivility in certain cases, although the contribution may not be cardinal [228, 281, 282; 283, 347, 366, 368]; and numerous avenues of possible tumor cell escape from immune attack have been shown, albeit many in artifactual test systems [178, 195, 212, 215, 218]. The contributory roles to progressive neoplastic disease of host failure and tumor cell circumvention must be weighed, even though our attention now centers on the immunogenic paucity of TATAs; and the opportunities for prevention or reversal of individual host deficiency and of tumor cell evasion can be explored only against a background of comprehension of the vagaries of host and tumor cell conduct and interaction [423].

It is self-evident that the development of effectively inhibitory, escape-route-sealed immunological responses against tumor cells that have initiated progressive growth in an organism is very much more the exception than the rule in the natural history of neoplastic diseases. Can we then attain sufficient mastery over the immunological interplay, actual and potential, between tumor cells and host immunocytes to magnify and direct immune reactivity toward definitive therapeutic ends?

Much of what has been essayed so far has been an approach of trial and error, with error and only marginal success, at best, the prominent features [377, 378]. The aura of crisis which permeates the attitude to treatment of malignancies has made acceptable the introduction of immunotherapeutic procedures with only scanty foundations of rationality and laboratory experience. Many of the immunotherapeutic agents employed until now have been taken to clinical trial peremptorily, with threadbare foreknowledge of the range, conditions, and modes of their hoped-for activities. The philosophy of their use has often been based on that of other treatment modalities, and not infrequently in frontal defiance of recognized immunological principles. Thus, for instance, design of dosage and schedule of treatment with nonspecific immunomodulators has commonly

ignored patent hazards of suppressor cell activation and of other eventualities of tolerance induction, as well as of the nullifying of immunological stimulation by inappropriately spaced chemo- and radiation therapy [426, 428]

It must be admitted, on the other hand, that the plight of many cancer patients gives grounds for the grasping at straws and for leaps from drawing board to clinic that would be condemned as wild acrobatics in many other areas of medicine. Moreover, the intimations of some success with immunotherapeutic intervention that have come from several programs of investigation [176, 378], albeit still tentative and limited, provide some reason to believe that further exploration is warranted. Future investigations in the clinic must be predicated, however, on a much broadened understanding of the immunology of neoplasia, and on full appreciation of the individuality and fluidity of host-tumor relationships.

The brief survey of tumor antigenicity in the light of etiology here presented is proposed as a point of departure for the examination of several major directions of immunotherapeutic attempts, currently and in the immediate future.

2 Tumor Etiology and Antigenicity

2.1 Tumors Known to be Induced by Viruses

Several distinct classes of antigens can appear on tumor cells in outcome of the presence and activities of oncogenic viruses: components of the virion itself; structures made under the genetic control of the viral genome; structures for which the host cell carries the genetic coding, with the virus acting to derepress repressed information; and configurations appearing as secondary manifestations of the disturbances taking place in the morphology and physiology of a virally infected cell [23, 59, 90, 173, 188, 307, 342].

Virus-dependent antigens may be group-specific for the agent, and may appear in common on tumors induced in different hosts [408]; they may also have degrees of specificity associated with the particular host in which the virus induced transformation, and perhaps even with individual tumors [409, 410]. Some passenger viruses infecting neoplastic cells can probably give rise to a similar diversity of antigens [222].

Although the focus of our discussion on the antigenicity of virus-induced neoplasms and of tumors secondarily infected with viruses is here on structures attending the presence and functions of the agents, it must be noted that a variety of other TAAs – fetal, organ-specific, perhaps even TSTA – may also occur on such tumors, in some instances specific for individual growths, and may compete for full expression with those directly associated with the virus [392, 400, 418, 443]. It would be erroneous, therefore, to make automatic and limited assumptions as to the range of antigens borne by neoplastic cells of viral etiology or viral superinfection; it could indeed be that antigenic markers of particular interest as resistance-inducing antigens are at times concomitants of the transformed state per-se, not directly of its origin.

Similarly, there is indication that TAAs may also compete for expression on the cell surface with normal histocompatibility antigens [76, 216, 307, 367]. Such competition can bring about dilution of relevant tumor target epitopes. It may also be of interest with view to the concept that cellular immunological responsiveness is derivative of, and to an extent circumscribed by, recognition of normal self-MHC antigens [58], and that reactivity to tumor-associated membrane configurations can be affected by the arrangement of MHC locus products with other determinants [334, 456]. Clark et al. have suggested that

"the particular arrangement of specific tumor antigen, organ-specific antigen, HL-A (or H-2) antigen and nonantigenic glycoprotein present on tumor membranes and in sera within a single molecular entity may explain the apparent nonimmunogenicity of metastasizing tumors . . ." [76A].

The active immunogenicity of virus-dependent antigens on tumor cells is problematic, and perhaps especially their elicitation of cytotoxic responses in the tumor host. Where the agents are transmitted vertically, or infect horizontally soon after birth, or enter the organism repeatedly in adulthood, reaction to the virus-associated antigens can lead readily to prolonged specific (partial) tolerance [90, 91, 234, 235]. This is most probable where such antigens appear early in ontogeny, expressed on infected cells for long periods prior to their final neoplastic transformation; it may also be the case where oncogenic virus infection occurs later in life and the associated antigens appear and are maintained on still-normal cells for some time preceding frank neoplasia. It could be argued indeed that the development of such tolerance is an evolutionary necessity, to facilitate survival of the organism into reproductive maturity. This consideration applies as well to immunological deportment vis-à-vis fetal and tissue-specific antigens, translocated in time and tissue geography with the neoplastic condition and expressed exaggeratedly on cells whose transformation was precipitated by different oncogenic stimuli. "Negative" acknowledgment by the immunological mechanism of such determinants, i.e., specific immunological unresponsiveness, may be a fundamental requisite of self-acceptance, alternative or in addition to a steering of active responsiveness in directions innocuous of cytotoxic consequences.

In addition, some viruses, oncogenic and incidental, can suppress immunological ability broadly [46, 168, 366]. Moreover, the mutability of many viral agents can lead to the generation of different antigens on cells transformed by the same family of viruses, with successive clonal variability even in a given neoplastic event, and thereby with resultant impediment to the development of effective acquired immunity in the course of neoplastic progression.

Certain ubiquitous oncogenic viruses pose a serious threat to survival of host populations in nature [217, 218]. Where this is the case, existing host species may indeed have evolved, for all these difficulties, highly effective immune surveillance mechanisms directed at virus dependent antigens, mechanisms perhaps already operative vis-à-vis *pre*neoplastic cell variants. Multiple lines of immune defense may well have arisen to guarantee protection against such tumors. These neoplastic diseases may take place only in organisms whose immunological capacity has not yet matured or has been severely injured; polyoma tumors of mice are a classic example of neoplasms induced by a virus which occur naturally only in animals incapable of normal immunological reactivity [144, 217].

It may be argued, then, that many species, including man, would be subject to a very high incidence of virus-induced neoplastic disease, were it not for effective immune surveillance. Although certainly of theoretical interest, this argument does not address itself directly to the problem of those neoplastic diseases of viral, or other, etiology which *are* prevalent today in man and in animals, i.e., those tumors against which selective processes for immunological, or other, mechanisms of resistance have not (yet) developed to categorical efficacy. Nonetheless, analysis of the modes of resistance which in normal organisms prevent forms of progressive neoplasia that are prominent in immunologically impoverished ones may pave the way to immunotherapeutic (and perhaps also immu-

noprophylactic) intervention designed to replicate evolutionarily successful immune defenses.

In some oncogen-host relationships in nature, the evolution of host resistance appears to have been only partially effective so far, some members of a species being incapable of offering decisive opposition to oncogenesis or progressive neoplastic growth, others able to stand up to the challenge. Feline leukemia and Burkitt's lymphoma of man may be cases in point [213, 218], and it may well be that other tumors, too, including some that are rather common in populations at their present stage in phylogeny, constitute the exceptions to a generally accomplished evolution of host refractoriness. The differential virulence of different types of neoplasms may also be related in part to the relative success of ongoing selective pressures for defense. It would not seem unreasonable to hope that insight into the circumscribed and imperfect defense reactions of tumor hosts in the field, including the cancer patient, could lead to a focusing of immunotherapeutic efforts toward strengthening those safeguards which are evolving in nature but which are still incomplete or breachable.

Laboratory investigations into virus-induced neoplasia have been confined largely to tumors occurring with a high incidence in inbred animals, in many instances inbred at multiple genetic loci making for prevalence of tumors with a viral etiology. Leukemia in AKR mice is a prototype example [217]; mammary carcinomas in mice carrying the mammary tumor viruses (MTV and NIV) another [417]. Studies in outbred animals have usually been with viral variants selected for high tumorigenic potential, and propagated under artificial laboratory conditions. It is doubtful whether such models of neoplasia, including tumors appearing "spontaneously" in inbred subjects infected with oncogenic agents, bear much relevance to neoplastic disease of viral origin in nature. Selection for viral oncogenic potency and host susceptibility (or resistance), the probability that isogenicity between long-transplanted tumors and current test animals is often incomplete [412], and the relative facility of immunological manipulation in genetically homogeneous organisms remove most of the test systems employed from the realities of neoplasia in clinic and field.

Nonetheless, the wealth of data which has come from laboratory investigations of viral neoplasia cannot be disregarded as devoid of all illustrative value for the advancement of immunotherapy. It *is* of importance that cancer cure and prevention can be effected by immunological means in test systems where native host resistance is, in fact, low and where oncogenic agents or tumor isografts produce rapidly fatal disease in control animals. It is of no less interest that some degree of antitumor immunological and resistance reactivity can be demonstrated even where neoplastic disease is triggered by viruses under conditions strongly favoring immunological unresponsiveness to virus-associated antigens [10, 44, 45, 167, 367, 375, 376, 399, 416, 434].

Elucidation of the immunological interactions between experimental hosts and tumors also provides guidelines, for all the artifactuality of the models, to analysis of host-tumor equilibria in nature. To cite only one instance, recent studies in our laboratories with Rous sarcomas have revealed a consistent autochthonous preference of tumor cell recognition by murine and avian hosts despite the dominant expression of shared group-specific viral antigenicities on the transformed cells [409, 410]; this indicates that even against a background of immunological reactions manifested against antigens directly associated with an oncogenic virus, the host can mount responses distinct for the neoplasm that poses the individual challenge. Cognizance of this phenomenon in the la-

boratory now prescribes a search for its occurrence, and therapeutic exploitation, in human cancer. Encouragement for this search is provided by the possibility that some TATAs appearing on neoplasms under the influence of oncogenic viruses may be compound structures composed of virion- or virus-dependent and normal host cell membrane constituents [230, 325]. Such TATAs are likely to bear a considerable degree of host-associated as well as of agent-associated specificity. Evidence is also developing for the view that some virus-associated TATAs may be polymolecular entities composed of virion constituents and virus-coded products [196]. The creation of constellations of histocompatibility, virion and virus linked, and individual tumor associated antigens may well favor immunological recognition and responsiveness [58, 334, 456, 457], and facilitate "autochthonous preference"; it might also prevent the induction of specific immunological unresponsiveness, to markers determined exclusively by the presence or activities of the agent.

At the present, viral agents are incriminated clearly in the etiology of only a few types of human neoplasia. It is not out of the question, however, that further investigation will implicate viruses more widely in the causation of human tumors [5A, 168, 256]. Efforts at heightening immunity to malignant diseases of man would be buoyed, in that event, by the analogy of successful immunological intervention in many virus-initiated neoplasms of animals.

2.2 Tumors Known to be Induced by Chemical Agents and by Other Carcinogenic Stimuli

The transplantation immunogenicity of tumors produced experimentally with a variety of carcinogenic chemicals, irradiation, hormones, and certain physical irritants varies from pronounced to marginal or nonexistent, and where antigenicity is apparent, variable degrees of uniqueness associated with individual tumors, histological type, and carcinogenic stimulus have been noted [15, 147, 211, 219, 229, 290, 341, 435].

The antigens fall into different categories. Fetal, organ-associated, and other determinants not appearing in similar amounts on analogous normal cells have been detected [15, 351]. Some carcinogenic agents may activate latent, or masked, viruses either with oncogenic activity or capable of superinfection, and the TAAs of tumors so initiated or infected may include the spectrum of virus-related entities. Some antigens may reflect the consequences of other morphologic alterations, causal or incidental of the neoplastic condition, which are effected by the carcinogenic stimulus on target cells. As with virus-induced neoplasms, the TAAs of tumors produced by chemical and physical agents are often expressed to different extents by similar growths, and perhaps even by subpopulations of transformed cells originating from the same primary neoplasm; and the phenotypic expression of some TAAs can be, like that of normal histocompatibility antigens [238], an expendable concomitant of the neoplastic state, to a variable extent for different tumors [76, 210, 214].

Recent studies in our laboratories support the findings of others that strong antitumor reactivity can be manifested by immunocytes derived even from hosts with large tumor burdens [264], although, as has been the common experience of investigators, with uncertain and wavering lines of specificity. (It is apparent, moreover, that the presence of a first tumor can have important regulatory effects on concomitant immunity: removal of a primary growth is sometimes followed shortly by explosive metastatic involvement, and the suggestion has been offered, in line with not infrequent clinical observation, that

"surgical excision of a tumor may not be, in all instances, in the long-term interest of the tumor-bearing host" [118].) Current experiments by our group also reemphasize the variability of immunological responses often evoked against the same tumor by different means of contact – neoplasms implanted and remaining in situ; ligation of a limb bearing the implant; surgical extirpation of the growth – and the variability of heightened responsiveness as assessed by different assays [449]. Attention is clearly mandatory to the totality of a tumor process, however initiated, and to the methodology of sensitization and testing, if an accurate picture of the immunological facets of the interaction is to be obtained.

Widely distributed oncogenic factors other than viruses also threaten species in nature, and selective pressures for resistance are likely to be operative with regard to susceptibility both to carcinogenesis and to progressive tumor development. The evolutionary considerations discussed for viral oncogens probably apply as well in many respects to other cancer-causing stimuli.

Etiological participation in at least some human cancers of chemical and radiological excitants similar to those blatantly carcinogenic in laboratory animals appears to be beyond dispute. It may be anticipated, accordingly, that the antigenic behavior of many human neoplasms is not entirely dissimilar to that of experimental neoplasms intentionally induced by chemical and physical means. The qualifications and reservations that limit the validity of laboratory tumors of viral causality as models of neoplasia in nature hold true for all experimentally incited neoplasms. Nonetheless, the marked success of immunological intervention against some chemically and physically, as well as against virally, induced experimental neoplasms affords grounds for hope that intelligent manipulation of host immune mechanisms can aid patients with many forms of malignant disease.

2.3 "Spontaneous" Tumors, with No Obvious Viral Etiology[1] (Animal and Human)

Designation of a tumor as "spontaneous" is a declaration of uncertainty as to its etiology, and conveys no substantive information beyond the nonintrusion of the observer in its immediate causation. In referring to spontaneous neoplasia, the qualification must also be made clearly that tumors arising unprovoked in inbred laboratory animals may fall far short, despite their "spontaneity" and even where the hosts do not carry viruses of known oncogenic activity, of representing veracious analogs of growths appearing in outbred organisms, and especially of neoplasms that develop under the normal ecologic circumstances of the species.

As indicated above, many neoplasms occurring in the absence of any intentional manipulation by the investigator are undoubtedly triggered into being, at one or another step in the progressive deviation from normal, by chemical, viral, and other factors similar to those used experimentally as carcinogens. The conditions of experimental and spontaneous carcinogenesis effected by the same agents may indeed differ pronouncedly. It is likely, for one, that carcinogenic stimuli are experienced in nature in much lower amounts than are employed experimentally, and there is persuasive indication that protective immunogenicity of TAAs is proportional to the inducing dose of carcinogen [19]. Nonetheless, the proven immunogenicity of many laboratory neoplasms suggests that at

[1] Tumors arising with high frequency in experimental animals bearing viruses of demonstrated oncogenic potency are here excluded from the category of spontaneous neoplasms

least some spontaneous cancers may be studied within the same immunological frame of reference, even though their antigenic potency may be far lower. Other spontaneously occurring tumors may be the final outcome of somatic mutations or selective gene activation, and of epigenetic malfunctions which disturb normal growth regulation or lead to the formation of tissue environments particularly favorable to aberrant growth, without the impelling force of specific external agents; the defects in cell structure and deportment, probably cumulative, may occur accidentally, at random, or as foreseeable stochastic concomitants of certain physiological functions, aging, and degeneration. Mutational changes, regardless of what has triggered them, are often accompanied by altered cell antigenicities, as may be nongenetic deviations in the composition and assembly of cell constituents. It would be incorrect, in light of these considerations, to view spontaneously arising neoplasms as lying intrinsically beyond the pale of immunological reactivity.

Although some spontaneous tumors of animals seem incapable of evoking sensitization expressed by acquired immunity to re-challenge, and give no other ready evidence of immunogenic properties [164, 165], other do provide indication of tumor-associated immunogenicity [15A]. In some instances, this immunogenicity is evinced by the manifestation of specifically heightened resistance in the autochthonous or in syngeneic hosts, accompanied by the production of detectable effector cells or antibodies specifically cytotoxic to the tumor; in others, there is evident only an excitation of humoral or cellular responses apparently directed at TAAs, without attestation of a defensive value. For an increasing number of human neoplasms as well, production by the patient of antibodies and lymphoreticular cells with specific reactivity against the autochthonous growth has been documented (see citations above). Although the *protective* immunogenicity of the corresponding antigens on such human tumors remains to be defined, there are seen, not infrequently, persuasive ancillary indications of the import of immunological reactions for host refractoriness.

The commonly observed patterns of fluctuating opposition and susceptibility to neoplastic advances in the patient; the correlations sometimes ostensible between propitious clinical status and a histological complexion of the tumor site suggestive of active immunocyte attack [41, 43, 183, 232, 293, 406]; the occasional spontaneous regression of advanced neoplastic lesions, often following severe infections with agents known to potentiate immunological responsiveness [270, 354]; and the therapeutic efficacy, albeit still largely anecdotal and limited, of immunological intervention, all betoken the existence of host immunological and immune potentiality against spontaneous neoplasia. The evidence is, admittedly, indirect and circumstantial, and no single set of observations hinting at immune reactivity resolves the question of causality. Cumulatively, however, the natural history of at least some neoplastic processes in man as well as in animals speaks for active host defenses of immunological kind. The impression is borne out experimentally. The early observations of Brunschwig et al. [57] on neoplastic auto- and homotransplants in advanced cancer patients are a case in point. Although questioned on grounds of propriety of such human experimentation, the findings of these workers, supported and extended subsequently by other, unobjectionable techniques [42, 43], remain as premise for the operation of immunological resistance factors to a variety of human neoplasms, even in individuals with extensive tumor involvement.

It may be concluded tentatively that the data now available, although still scattered, intimate that spontaneous tumors are, on the whole, less likely to be strongly immuno-

genic than many artificially induced ones, but do not negate the ability of the host to mount some immunological and immune reactions against them. That these reactions often fall short – the progressively growing tumors presented to us clinically – does not vitiate the possibilities of bringing immunological resistance to a higher level by felicitous intervention.

Theoretical deliberation, too, favors the probability that most tumors, regardless of causation, express at least a measure of surface antigenicity associated with the neoplastic state [421, 423, 424]. Neoplastic cells generally differ to some extent from analogous normal ones in morphology and function, and many of the changes that come with neoplastic transformation involve the cell surface. Differences in architecture and behavior must be assumed to be rooted in alterations of the composition and arrangement of cell components; and even small changes in molecular structure, configuration, and topography readily confer new antigenic qualities. As long as our expectations remain reserved and focus on antigenic "handles" accompanying neoplastic transformations, rather than on prevalent, frank immunogenicities [161], we have before us an open field of investigation directed at immunotherapeutic goals – provided that the transformed cells possessing an earmark of altered antigenicity are also intrinsically susceptible to immunological attack.

It is evident, however, that the obstacles on the way toward these goals are very large. Efforts to benefit the cancer patient by immunological means start from a negative point of departure: the fact that he is a patient declares that the balance of interaction with the neoplasm which confronts him has already tilted against him. The factors responsible for the defeat of the host may be single or many. Genetic determinants, age, life experience, environmental circumstances at the moment, and the specific carcinogenic stimulus may act, separately or jointly, to create syndromes of immunological and other resistance insufficiency, systemically or at the site of tumor incipience; the dyscrasia may be aggravated by the developing tumor burden and by the consequences of conventional therapy. In some instances, rapidity of tumor growth may lead to an overwhelming, quantitatively, of host defenses that might have halted a slower-growing neoplasm. Host selective pressures on the population of neoplastic variants must always be suspected as bringing to the fore clones with evasive capabilities: in order to survive in a hostile tissue environment, tumor cells must "learn" rapidly to take advantage of lacunae in host resistance, to seek "staging sites" in localities partially sequestered from systemic immunological attack [419], to lose or modulate surface antigens which serve as targets for cytotoxic immune attack, to actively neutralize damaging antibodies and cellular elements of the host, and by any other means to escape potential immune surveillance. The occurrence of many such mechanisms of evasion has been described [212, 214, 442], and others may well be discovered; they are likely to include both adaptive adjustments to host hostility and the selection, probably sequential, of capable mutants.

On the other hand, no selective pressure is operative for host recognition and effective responsiveness vis-à-vis tumors that, at the present stage of phylogeny, develop late in life, beyond the period of peak reproductive activity; and selection for immunological, and any other, mechanisms of refractoriness is likely to be tenuous with regard to neoplasias that do occur earlier in life but only sporadically. Thus, for the majority of cancers that pose the clinical problem in man, exceptional idiosyncratic host immunological and immune failure indeed need not be invoked as a primary contribution to etiology. Rather, recognition and reaction faculties to TATAs characteristic of many neoplasms