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Immune surveillance against virus-induced tumors and nonrejectability of spontaneous tumors: Contrasting consequences of host *versus* tumor evolution

(progression of tumors/immune responsiveness)

GEORGE KLEIN AND EVA KLEIN

Department of Tumor Biology, Karolinska Institutet, S 104 01 Stockholm 60, Sweden

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ABSTRACT **Spontaneous tumors are defined as tumors that develop in the absence of all experimental interference. In contrast to the widely documented, strong rejection reactions against most virus-induced tumors, spontaneous tumors evoke little or no detectable rejection reaction in intact or preimmunized syngeneic hosts. The difference can be viewed in relation to the contrasting natural history of the two conditions. Spontaneous tumors evolve in several steps, as a rule. "Tumor progression" is a microevolutionary process at the level of the somatic tissue where successive clonal variants replace each other. Each new variant gains the upper hand due to its greater independence of some restricting host mechanism. Independence of immune restrictions must be part of this process. Host selection for immune resistance apparently plays no major role here, presumably because most of the naturally occurring tumors arise after the host has passed the peak of its reproductive period.**

Protection against the oncogenic effects of ubiquitous tumor viruses is, on the other hand, the result of host selection for immune mechanisms favoring prompt rejection of virus-transformed cells. This is neither synonymous with nor related to protection against the viral infection *per se*, which is frequently successful and usually quite harmless. A certain relationship can be perceived between the degree of viral ubiquity and the strength of immune protection against the corresponding tumor cells.

Natural selection for host recognition of commonly occurring, virally induced changes in neoplastic cell membranes can be surmised to occur, at least in part, by the fixation of appropriate immune responsiveness (*Ir*) genes. The role of *Ir* genes for tumor recognition can be approached by the genetic analysis of the F_1 hybrid resistance effect. Unresponsiveness to spontaneous tumors may be overcome by target-cell modification, e.g., by chemical coupling, somatic cell hybridization, or viral "xenogenization."

In its original, generalized form, the immune surveillance theory has postulated that (*i*) tumor cells arise in the normal organism at an "enormous frequency" and (*ii*) they are regularly eliminated by immune mechanisms (1). Most of the recent experimental work in tumor immunology is based on this assumption, with or without modifications, consciously or by implication. As a result, attempts are focused on the demonstration and characterization of the "immunological failure" that is assumed to bear the primary responsibility for tumor development in the cancer patient.

Recently, the surveillance concept has been criticized by several authors. The relative rarity of primary tumors in thymusless (*nude*) mice is often used as the main argument. Even if this observation is true and not merely an artefact due to the short survival and poor health of these animals in most laboratories, the argument completely overlooks the well-documented fact that *nude* mice possess powerful non-T-cell-mediated surveillance mechanisms (2–4). A more serious case can be made on the basis of the well-demonstrated but frequently overlooked inability of spontaneous tumors to immunize autochthonous or syngeneic hosts.

In this article, the word *spontaneous* refers to tumors that have arisen without any experimental interference in the laboratory or in nature. In this context, inbreeding with selection for high tumor incidence must be regarded as very serious experimental interference; we shall therefore avoid considering tumors of this category as far as possible.

Baldwin (5) tested the ability of spontaneous rat tumors to immunize syngeneic hosts against viable grafts. In contrast to the good rejection in the corresponding experiment with certain chemically induced tumors, "preimmunized" hosts showed no detectable rejection, in comparison with untreated controls. Prehn (6, 7) obtained similarly negative results with spontaneous fibrosarcomas that had arisen in old mice. The significance of these findings was underlined by the fact that both authors succeeded in inducing strong rejection reactions against chemically induced tumors of similar genetic origin and histology. Recently, Hewitt *et al.* (8) reported their experience with 27 spontaneous tumors of various histological types, derived from mice of low-tumor strains. On transplantation, the takes of small inocula could not be facilitated by preirradiation of the syngeneic host and could not be prevented or inhibited by repeated immunization with lethally irradiated tumor cells. A certain degree of resistance could be built up against some spontaneous tumors in semisyngeneic F_1 hybrid hosts, but this was dismissed as a transplantation artefact. On the basis of these findings, Hewitt *et al.* delivered a forceful attack against prevailing assumptions in tumor immunology. They suggested that the rejection reactions demonstrated in syngeneic hosts against virally or chemically induced tumors are essentially artefacts of laboratory experimentation.

In our opinion, Hewitt *et al.* rightly stress the importance of the negative rejection experiments with spontaneous tumors. As discussed below, however, we believe that they go too far in disregarding the powerful surveillance against virus-induced tumors and in dismissing the significance of the F_1 resistance effect.

Surveillance against virus-induced tumors

As we pointed out elsewhere (9), virus-induced neoplasms give the most convincing evidence for tumor immunogenicity, rejectability, and surveillance. Neonatal thymectomy, antilymphocytic serum, a variety of immunosuppressive treatments, whole body irradiation, and the thymusless state of *nude* mice can all facilitate the appearance of virus-induced tumors in, e.g., polyoma-, simian-virus-40- or Rous-sarcoma-virus- (RSV) infected animals, sometimes to a dramatic extent. In current

Abbreviations: EBV, Epstein–Barr virus; T cell, thymus-dependent cell.

Table 1. Characteristics for naturally occurring oncogenic viruses and their hosts

Virus	Natural host	Spread	Susceptibility	Effector	Genetics of resistance	Immunosuppression
Polyoma	Mouse	Ubiquitous	Normally complete resistance	T-cell-dependent	Not known	Antilymphocytic serum, x-rays, T-cell deficiency, newborn state may lead to tumor formation
Herpesvirus saimiri	Squirrel monkey	Ubiquitous	Complete resistance (100% susceptibility in previously unexposed marmosets and owl monkeys)	Not known	Not known	Not known
Epstein–Barr virus	Man	Ubiquitous	Complete resistance (except special circumstances)	T-cell-dependent?	Not known	Hypothetical
Feline leukemia virus	Cat	Enzootic	Approximately 5% of leukemia	Antibody? Antibody-dependent lymphocytotoxicity?	Not known	Not known
Marek's disease virus (MDV)	Chicken	Sporadic epizootic	High	T-cell-dependent?	Resistance factor linked to major histocompatibility complex	T-cell deficiency increases risk?

polemics against surveillance, this fact is often dismissed with the argument that it merely reflects antiviral immunity. This is not true. In the polyoma system, for example, a clear distinction could be made between antiviral immunity and the rejection reaction directed against nonviral, but virally induced cell membrane (TSTA) antigens (10). Antiviral immunity was neither necessary nor sufficient to bring about rejection, while immunization with virus-nonproducer, polyoma-induced tumors (syngeneic or allogeneic) effectively protected mice against the isografting of already established polyoma tumors. In Marek's disease, vaccination against the related but apathogenic turkey herpesvirus (HVT) could protect against tumor development, but not against virus shedding (11, 12). The mammalian Rous sarcoma virus system is entirely nonpermissive and antiviral immunity cannot play any role in the relatively strong protection observed (13), to mention only a few examples.

Table 1 summarizes some relevant facts for a few, naturally occurring oncogenic viruses. In three systems, polyoma in mice, herpesvirus saimiri in squirrel monkeys, and Epstein–Barr virus (EBV) in man, the virus-infected but immunologically intact adult of the natural host species enjoys virtually complete protection against the oncogenic effect of these powerful transforming agents. All three viruses are ubiquitous and at least EBV must have lived with its natural human and primate hosts for a very long time. Close relatives of EBV are ubiquitous in a variety of Old World (but not New World) nonhuman primates (14). The evolution of powerful protective mechanisms against the oncogenic effect of these viruses must have been a necessity for the survival of the species. The fact that the oncogenic effect of EBV can only be demonstrated in New World monkeys (14) is in line with this conclusion. It may be also noted that EBV and its simian cousins appear to be largely if not completely harmless in their natural resistant host species. The virus appears to live in harmony with most individuals and remains latent during a lifetime; apparently no mechanisms needed to evolve to protect the host from the viral infection as such, as contrasted to the oncogenic effect.

Protection of mice against the oncogenic products of the powerfully transforming polyoma, a naturally occurring mouse virus, is largely, if not entirely mediated by thymus-dependent (T) cells (15). There is also suggestive evidence that T-cell-mediated responses may restrain the proliferation of EBV-transformed bone-marrow-dependent (B) blasts in acute infectious mononucleosis (16) and may "lose out" in competition with progressively growing, EBV-carrying B-lymphoma cells in the Burkitt tumor (17). There is no corresponding information for the herpesvirus saimiri system, although it is interesting to note that the naturally resistant squirrel monkey host responds to virally induced antigens more promptly than the tumor-susceptible marmoset and owl monkey (18).

Feline leukemia virus reflects a somewhat different situation. It is much less ubiquitous than the viruses discussed above; the exact frequency of virus-carrying street cats is not known, but it is estimated to range between 0.1 and 1% (19). The risk of leukemic development in the virus-infected cat is around 5%. There is suggestive evidence that non-T-cell mechanisms may play a protective role against this T-cell disease (19, 20). Antibody to a virally determined but nonviral membrane antigen (FOCMA) appears to play an important part, alone or in concert with cellular effectors.

The "intermediate" resistance of cats to the leukemogenic effect of feline leukemia virus, inferior to the complete resistance in the above systems but nevertheless protective for the majority of the infected animals, may be attributed to an "intermediate" selective impact of the virus, with less ubiquity and perhaps even a less ancient history.

At the far end of the susceptibility scale we find Marek's disease virus (MDV). This virus is not a normal contaminant of healthy chicken breeds. Some countries (e.g., Sweden) have

been free of the disease altogether. Upon infection, Marek's disease virus causes a virulent epizootic disease with full expression of the oncogenic effect and high mortality. The protective effect of turkey herpesvirus vaccination (not merely an antiviral effect, as already mentioned) shows that the birds are responsive to virally induced, tumor-associated antigens. Relatively resistant fowl strains exist, and, in certain crosses, resistance was found to be under the control of a single dominant gene (11, 12, 21–23). Recently (22, 23), a linkage was found between a resistance gene and the major histocompatibility complex. It appears likely that *Ir* genes contribute to genetic resistance.

In conclusion, the admittedly fragmentary story on the relationship between known oncogenic viruses and their natural host species suggests the following:

(*i*) Resistance against the development of virus-induced tumors is mainly if not entirely due to immune responses against virally determined cell antigens, not against viral multiplication *per se*.

(*ii*) Resistance is often mediated through T-cell-dependent mechanisms. This may include T-killer cells and/or indirect, T-cell dependent (e.g., antibody-mediated) effects. The relative role of T-cell-independent mechanisms is not clear but there are strong indications that they may play a certain role, at least in some systems (2, 3, 15, 19, 20).

(*iii*) Ubiquitous viruses appear to have preselected their host species for immunologically mediated resistance against their preneoplastic or frankly neoplastic ("transformed") cell products. Enzootic viruses may have been less efficient and resistance may be only partial. Tumor epizootics may be induced by viruses that infect susceptible and previously largely unexposed hosts.

(*iv*) It is a particularly important corollary of these considerations that virus-induced, tumor-associated membrane changes *can* be regularly recognized as antigenic, independently of virus production (i.e., even in completely nonpermissive systems). It must be realized, however, that such recognition is not automatic, but is strongly influenced by host genetics.

The lessons of tumor progression

The laboratory worker favors virus models with high transforming and/or tumorigenic efficiency. He often uses laboratory variants selected for transforming activity. *In vivo* oncogenesis by such viruses (not to speak of *in vitro* transformation) does not even remotely resemble the natural history of cancer. The admirable review of Foulds, now almost 20 years old (24), gives an entirely different picture. Multiple stepwise changes, collectively designated under the general term "tumor progression" are responsible for the development of most, if not all, naturally occurring tumors. It may be questioned whether a one-step change is *ever* sufficient for tumor development. In a discussion on the tumorigenic action of some of the most directly transforming small DNA viruses, Dulbecco made a similar point recently (25). In the RNA virus field, we may consider the extreme case of laboratory inbreeding with selection for leukemia susceptibility, as it occurred in the AKR strain. At least four different genetic systems that favor leukemia development are known to have been fixed in AKR: the integrated Gross-viral genome (26), the amplifying *Fv-1*^{n} system (27), the H-2 linked *Rgv-1*^{s} allele (28) that is probably analogous to the unresponsive form of an *Ir* gene, and genetic susceptibility at the target cell level, favoring neoplastic transformation (29). In spite of this cumulation of susceptibility factors, leukemia develops only after a latency of several months. When it appears, the cells usually carry one (or more rarely several) extra chromosomes (30). It is known that the thymus of AKR mice is highly abnormal long before leukemia development and contains a substantial number of "preleukemic" cells. Conceivably, the virus induces a preleukemic condition only and further cytogenetic evolution is required to achieve full malignancy.

Table 2. Unit characteristics

Classical, *in vivo*	Modern, *in vitro*
Growth rate	Degree of contact inhibition
Invasiveness	Saturation density
Metastasizability	Serum dependence
Ascitic form of growth	Anchorage dependence
Hormone dependence	Clonability—relationship to *in vivo* tumorigenicity?

The EBV-associated Burkitt lymphoma is another case in point. Between 80 and 90% of all tumors and derived, established lines were found to carry a highly specific 14q+ chromosome marker, with an extra band at the distal end of the long arm of one chromosome 14 (31–33). The remaining 10–20% tumors had other chromosomal anomalies; none of them were purely diploid. In contrast, EBV-transformed cell lines derived from normal EBV-positive donors, or infectious mononucleosis patients, or other nonmalignant sources were often diploid. In no case were they found to contain the 14q+ marker. Even if normal peripheral lymphocytes of Burkitt lymphoma patients with 14q+ positive tumors were transformed by EBV *in vitro*, the derived, established lines lacked the marker and were purely diploid (33).

The cellular genome exerts an important influence on the apparently more direct *in vitro* transformation induced by the small DNA viruses, polyoma or simian virus 40, in sensitive monolayer cultures (25). This can be exemplified by (*i*) the occurrence of virus-carrying phenotypic revertants, with maintained, integrated viral DNA and T-antigen but a changed chromosomal constitution (34); (*ii*) the demonstration that a temperature-sensitive mutation in a *cellular* function can control the transformed phenotype without any change in the viral genome (35); and (*iii*) the suppression of the virally induced malignant phenotype by hybridization with normal cells, in spite of continued viral antigen expression (36).

The likelihood that tumor-associated chromosomal changes play an important role in the neoplastic process is also suggested by their nonrandom nature (37, 38). Specific and reproducible chromosomal changes are associated with certain forms of tumor development, different for different etiological agents.

Tumor progression was defined by Foulds (24) as the gradual evolution of a tumor towards increased autonomy by a series of stepwise changes in multiple unit characteristics. He particularly stressed the independent progression of various unit characteristics, i.e., their ability to reassort in many different combinations, and concluded that each form of autonomous neoplasia may evolve along a variety of alternative pathways.

Table 2 lists some of the relevant unit characteristics of tumor progression. It is interesting to contrast the *in vivo* properties of Foulds against the "more modern" *in vitro* parameters, based on cell behavior in artificial culture systems. It has been a general experience that the *in vitro* "correlates" of neoplastic behavior do not faithfully reflect tumorigenicity *in vivo*, although they may represent parts of the whole. Each part ap-

pears to be interchangeable and none of them is absolutely necessary. This is in line with the rules of Foulds for *in vivo* progression.

Somatic cell hybridization between in vitro transformed and/or tumorigenic cells and normal cells showed, paradoxically, dominance of the *in vitro* transformed phenotype but suppression of tumorigenicity, as long as the hybrids carried most of the chromosomes of both parental cells (39). This apparent contradiction may be resolved if the various transformation characteristics in vitro represent parts of the whole, i.e., tumorigenicity. If so, negative ("repressive") control of only one partial characteristic is already sufficient to provide the impression that *in vivo* tumorigenicity as a whole is also under negative control.

The logical connection between tumor progression and the nonrejectability of spontaneous tumors

As discussed above, current experimentation in tumor immunology is often based on the notion that most, if not all, tumors are potentially rejectable in the autologous host and tumor growth is therefore a failure of the rejection response. Experimental evidence on spontaneous tumors does not support this concept, however. One might regard this as the "central fallacy" of tumor immunology.

The poor rejectability of spontaneous tumors may have two main reasons:

(*i*) During tumor progression, immune restrictions are only one among the many categories of homeostatic growth-controlling forces. Tumor progression represents the gradual and essentially clonal evolution of independence from these restrictions, step by step, property by property. This process is likely to involve the selection of cells with decreased immunogenicity and/or resistance to immune effectors.

(*ii*) It may be questioned why the host species has not been selected in the opposite direction, towards improved immune recognition and rejection. This has actually occurred in the ubiquitous oncogenic virus systems, as discussed. However, the common spontaneous tumors occur at a relatively advanced age, as a rule, at a time when most affected individuals have passed their main reproductive age. No immune or other mechanisms that would protect aged individuals against tumors (or any other nosological impact characteristic for that age) would be fixed by selection.

Genetic control of tumor antigen recognition

We have argued above that host selection for *Ir*-gene-mediated recognition of tumor cell membrane antigens was highly successful in virus-induced tumor systems. It is implicit in this reasoning that genetic variability in *Ir* genes could serve as a potential basis for recognition (rejection) of other tumor-associated membrane changes. It has been frequently proposed that neoplastic behavior reflects a cell membrane disease (40), and it is quite likely that most if not all tumor cells are characterized by membrane changes that could serve as rejection targets, for an immune system with the appropriate repertoire. However, the fact that spontaneous tumors have "selected themselves" for nonrejectability on the genetic background of their original host tends to cancel this possibility. While the syngeneic host cannot mount an efficient rejection reaction, the F_1 hybrid host that has a double *Ir* gene repertoire may still do so. This may be the explanation of the "hybrid resistance" effect, described by Snell and Stevens (41) as the decreased take incidence of small tumor inocula in various F_1 hybrids, as compared to syngeneic hosts. In recent years this was interpreted by Oth and Burg (42) and by Sanford *et al.* (43, 44), as reflecting a relatively increased ability of F_1 hybrid hosts to respond against tumor-specific antigens. Backcross and F_2 tests, together with linkage analysis, may pinpoint the gene(s) responsible for hybrid resistance, and permit the study of the effector mechanisms involved. In a system analyzed at our laboratory (2, 45–48), resistance against a Moloney-virus-induced T-cell lymphoma was mediated by a natural killer (NK) cell that was under polygenic control, with a strong H-2 linked factor. Corresponding analyses concerned with F_1 resistance against spontaneous tumors are greatly needed.

It may be objected that the rejection responses reflected by F_1 hybrid resistance are largely haphazard and simply reflect the coincidental matching of a given tumor membrane change and a corresponding *Ir*-gene-mediated rejection. This is possible and may be akin to the somewhat erratic recognition (rejection) of chemically induced tumors. Unlike the virus-induced tumors, the products of chemical carcinogenesis evoke a variable response, ranging from relatively strong rejection to virtually no demonstrable rejection; the latter resembles the situation with spontaneous tumors (49). The most readily rejectable tumors arise after short latency periods. It may be surmised that, in cases where chemical carcinogenesis proceeds very rapidly, there is insufficient time for the somatic evolution of nonrejectability by tumor progression. Also, in view of the wide antigenic variability of chemically induced tumors, rejection remains haphazard and there is virtually no evidence for immune protection against the oncogenic process itself. Thus, the antigenic diversity of the chemically induced tumors appears to prevent a concerted action of the *Ir*-gene equipment of a given host genotype.

Antigenic modification to overcome host unresponsiveness

The nonrejectability of spontaneous tumors does not necessarily justify a pessimistic view about the possibilities of utilizing immunological approaches in tumor therapy. However, it is important to base them on a realistic reevaluation of the host–tumor relationships. The misconception that the tumor patient is always or often the victim of an immunological breakdown can be replaced by the idea that the microevolution of the tumor clone has also involved an escape from immune rejection. The problem of the host of a spontaneous tumor resembles the situation of a genetically unresponsive animal to a nonrecognized antigen. Unresponsiveness may be overcome by appropriate antigen modification. For tumor cells, three ways are open for experimentation: (*i*) chemical modification of tumor cell membranes, particularly the coupling of haptens or other strong antigens; (*ii*) virally induced "xenogenization"; and (*iii*) the biological coupling of the tumor cell membranes to strongly allo- or xenoantigenic partners by somatic cell hybridization. Encouraging model experiments have been published in all three areas but systematic application to the problem of spontaneous tumors has not yet been attempted.

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