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THE THREE ES OF CANCER IMMUNOEDITING

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■ **Abstract** After a century of controversy, the notion that the immune system regulates cancer development is experiencing a new resurgence. An overwhelming amount of data from animal models—together with compelling data from human patients—indicate that a functional cancer immunosurveillance process indeed exists that acts as an extrinsic tumor suppressor. However, it has also become clear that the immune system can facilitate tumor progression, at least in part, by sculpting the immunogenic phenotype of tumors as they develop. The recognition that immunity plays a dual role in the complex interactions between tumors and the host prompted a refinement of the cancer immunosurveillance hypothesis into one termed "cancer immunoediting." In this review, we summarize the history of the cancer immunosurveillance controversy and discuss its resolution and evolution into the three Es of cancer immunoediting—elimination, equilibrium, and escape.

INTRODUCTION

The concept that the immune system can recognize and eliminate primary developing tumors in the absence of external therapeutic intervention has existed for nearly 100 years. However, the validity of this concept has, in the past, been difficult to establish. When first proposed in 1909 (1), the hypothesis could not be experimentally tested because so little was known at the time about the molecular and cellular basis of immunity. Later on, as the field of immunology developed and the concept acquired its name—cancer immunosurveillance (2, 3)—experimental testing became possible but failed to provide evidence for the process, using mice with spontaneous mutations that rendered them immunocompromised but not completely immunodeficient (4). Only recently, with the development of gene targeting and transgenic mouse technologies and the capacity to produce highly specific blocking monoclonal antibodies (mAb) to particular immune components, has the cancer immunosurveillance hypothesis become testable in unequivocal, molecularly defined murine models of immunodeficiency. Over the past ten years, the use of these

improved in vivo cancer models has provided strong and convincing data that have rekindled interest in the cancer immunosurveillance hypothesis. Most recently, this conundrum has been further clarified by the demonstration that the immune system not only can protect the host against tumor development but also, by selecting for tumors of lower immunogenicity, has the capacity to promote tumor growth. These dual effects of the immune system on developing tumors prompted us to refine the cancer immunosurveillance hypothesis into one we termed cancer immunoediting (5, 6). We envisage that this process is comprised of three phases that are collectively denoted the three Es of cancer immunoediting: elimination, equilibrium, and escape. In this review, we first present data supporting the existence of the elimination phase (i.e., cancer immunosurveillance) as it occurs in mice and humans and propose a model for the molecular and cellular events that underlie this process. Second, we provide evidence for a tumor-sculpting role of immunity and discuss the relationship between this function and the equilibrium and escape phases of cancer immunoediting. Third, we outline the implications of this concept for the understanding and treatment of human cancer.

CANCER IMMUNOSURVEILLANCE IN MICE

Historical Perspective

The validity of the cancer immunosurveillance hypothesis has emerged only recently from a long history of heated debate (reviewed in 6). The notion that the immune system could protect the host from neoplastic disease was initially proposed by Ehrlich (1) and formally introduced as the cancer immunosurveillance hypothesis nearly 50 years later by Burnet and Thomas (2, 3, 7–9). Based on an emerging understanding of the cellular basis of transplantation and tumor immunity (10–15), Burnet and Thomas predicted that lymphocytes were responsible for eliminating continuously arising, nascent transformed cells. However, when this prediction was put to the experimental test using nude mice, which were the most congenitally immunodeficient mice available at the time (16, 17), no convincing evidence for such a process was obtained. Specifically, CBA/H strain nude mice neither developed increased incidences of carcinogen [methylcholanthrene (MCA)]-induced or spontaneous tumors nor did they show shortened periods of tumor latency compared with wild-type controls (4, 18–22).

However, in retrospect, there are several important caveats to these experiments that could not have been appreciated at the time. First, the nude mouse is now recognized to be an imperfect model of immunodeficiency. These mice produce low but detectable numbers of functional populations of $\alpha\beta$ T cells (23–25) and therefore can manifest at least some degree of adaptive immunity. Second, the existence of natural killer (NK) cells (which are present and function normally in nude mice) was not well established at the time (26) and thus very little was known about their origins, actions, or roles in promoting innate immunity. In addition, the profound influence of innate immunity on adaptive immunity was

not recognized (27). Thus, the residual adaptive immune system in the presence of a fully functional innate immune system may provide the nude mouse with at least some cancer immunosurveillance capacity. Third, the CBA/H strain mice used in Stutman's MCA carcinogenesis experiments express the highly active isoform of the aryl hydroxylase enzyme that is required to metabolize MCA into its carcinogenic form (28, 29). Therefore, it is conceivable that MCA-induced cellular transformation in CBA/H strain mice occurred so efficiently that it masked any protective effect that immunity could provide. Nevertheless, since these caveats can only be appreciated in hindsight, the Stutman experiments were considered to be so convincing that by the end of the 1970s, the death knell had sounded for the cancer immunosurveillance hypothesis.

THE RENAISSANCE OF CANCER IMMUNOSURVEILLANCE

IFN-γ, Perforin, and Lymphocytes in Tumor Immunity

In the 1990s, two sets of studies incited renewed interest in cancer immunosurveillance. First, endogenously produced interferon-γ (IFN-γ) was shown to protect the host against the growth of transplanted tumors and the formation of primary chemically induced and spontaneous tumors (30–33). The injection of neutralizing monoclonal antibodies specific for IFN-γ into mice bearing transplanted, established Meth A tumors blocked LPS-induced tumor rejection (30). In addition, transplanted fibrosarcomas grew faster and more efficiently in mice treated with IFN-γ-specific mAb. These observations were then extended to models of primary tumor formation. IFN-γ-insensitive 129/SvEv mice lacking either the IFNGR1 ligand-binding subunit of the IFN-γ receptor or STAT1, the transcription factor responsible for mediating much of IFN-γ's biologic effects on cells (34), were found to be 10–20 times more sensitive than wild-type mice to tumor induction by methylcholanthrene (31). Specifically, these mice developed more tumors, more rapidly, and at lower MCA doses than did wild-type controls. These results were subsequently confirmed by independent experiments using C57BL/6 strain mice lacking the gene encoding IFN-γ itself (32). Similarly, in models of genetically driven tumorigenesis, mice lacking the p53 tumor suppressor gene and either IFNGR1 or STAT1 formed a wider spectrum of tumors compared with IFN-γ-sensitive mice lacking only p53 (31). In addition, compared to their IFN-γ-sufficient counterparts, IFN-$\gamma^{-/-}$ C57BL/6 mice showed an increased incidence of disseminated lymphomas, and IFN-$\gamma^{-/-}$ BALB/c mice displayed an increased incidence of spontaneous lung adenocarcinomas (33).

Second, mice lacking perforin (pfp$^{-/-}$) were found to be more susceptible to MCA-induced and spontaneous tumor formation compared with their wild-type counterparts (32, 33, 35–37). Perforin is a component of the cytolytic granules of cytotoxic T cells and NK cells that plays an important role in mediating lymphocyte-dependent killing (38). Following challenge with MCA, pfp$^{-/-}$ mice developed significantly more tumors compared with wild-type mice treated in the

same manner (32, 35, 36). Untreated pfp$^{-/-}$ mice also showed a high incidence of spontaneous disseminated lymphomas, which was accelerated on a p53$^{-/-}$ background (37). BALB/c mice lacking perforin also displayed a low incidence of spontaneous lung adenocarcinomas, which was not observed in wild-type mice (33). Taken together, these observations demonstrated that tumor development in mice was controlled by components of the immune system and stimulated a considerable amount of work aimed at better defining this process (Table 1).

The definitive work demonstrating the existence of an IFN-γ- and lymphocyte-dependent cancer immunosurveillance process was based on experiments employing gene-targeted mice that lack the recombinase activating gene (RAG)-2 (5). Mice lacking RAG-2 (or its obligate partner RAG-1) cannot rearrange lymphocyte antigen receptors and thus lack T, B, and NKT cells (39). Since RAG-2 expression is limited to cells of the immune system, the use of RAG-2$^{-/-}$ mice provided an appropriate model to study the effects of host immunodeficiency on tumor development because, unlike other genetic models of immunodeficiency (such as SCID mice), the absence of RAG-2 would not result in impaired DNA repair in nonlymphoid cells undergoing transformation. Following challenge with MCA, 129/SvEv RAG-2$^{-/-}$ mice developed sarcomas more rapidly and with greater frequency than genetically matched wild-type controls (5) (Figure 1*A*). After 160 days, 30/52 RAG-2$^{-/-}$ mice formed tumors, compared with 11/57 wild-type mice. Similar findings were obtained in MCA tumorigenesis experiments that used RAG-1$^{-/-}$ C57BL/6 mice (40). Moreover, *Helicobacter*-free RAG-2$^{-/-}$ 129/SvEv mice aged in a specific pathogen-free mouse facility and maintained on broad-spectrum antibiotics formed far more spontaneous epithelial tumors than did wild-type mice housed in the same room (5; A.T. Bruce & R.D. Schreiber, unpublished observations) (Figure 1*B*). Specifically, 26/26 RAG-2$^{-/-}$ mice ranging in age from 13–24 months developed spontaneous neoplasia, predominantly of the intestine; 8 of these mice had premalignant intestinal adenomas, 17 had intestinal adenocarcinomas, and 1 had both an intestinal adenoma and a lung adenocarcinoma. In contrast, only 5/20 wild-type mice aged 13–24 months developed spontaneous neoplasia, which was predominantly benign. Three wild-type mice developed adenomas of the Harderian gland, lung, and intestine, respectively; one developed a Harderian gland adenocarcinoma; and one developed an endometrial stromal carcinoma. Thus, lymphocytes protect mice against the formation of both chemically induced and spontaneous tumors.

The overlap between the IFN-γ- and lymphocyte-dependent tumor suppressor pathways was explored by comparing tumor formation in 129/SvEv mice lacking either IFN-γ responsiveness (IFNGR1$^{-/-}$ or STAT1$^{-/-}$ mice), lymphocytes (RAG-2$^{-/-}$ mice), or both [RAG-2$^{-/-}$ X STAT1$^{-/-}$ (RkSk) mice] (5). Each of the four lines of gene-targeted mice formed three times more chemically induced tumors than syngeneic wild-type mice when injected with a single 100 μg dose of MCA (Figure 1*A*). Since no significant differences were detected between any of the gene-targeted mice, the conclusion was reached that the IFN-γ/STAT1 and lymphocyte-dependent extrinsic tumor suppressor mechanisms were heavily

TABLE 1 Enhanced susceptibility of immunodeficient mice to chemically induced and spontaneous tumors

Technology	Immune status	Tumor susceptibility relative to wild type	References
RAG-$2^{-/-}$	Lacks T, B, NKT cells	↑ MCA-induced sarcomas; ↑ spontaneous intestinal neoplasia	(5)
RAG-$2^{-/-}$ × STAT$1^{-/-}$ (RkSk)	Lacks T, B, NKT cells; IFNγ-, α/β-insensitive	↑ MCA-induced sarcomas; ↑ spontaneous intestinal and mammary neoplasia	(5)
RAG-$1^{-/-}$	Lacks T, B, NKT cells	↑ MCA-induced sarcomas	(40)
BALB/c SCID	Lacks T, B, NKT cells	↑ MCA-induced sarcomas	(40)
TCR$\beta^{-/-}$	Lacks $\alpha\beta$ T cells	↑ MCA-induced sarcomas	(58)
TCR$\delta^{-/-}$	Lacks $\gamma\delta$ T cells	↑ MCA-induced sarcomas; ↑ DMBA/TPA-induced skin tumors	(58)
J$\alpha$$281^{-/-}$	Lacks NKT cell subset	↑ MCA-induced sarcomas	(32, 36, 40)
LMP$2^{-/-}$	Lacks LMP2 subunit	↑ Spontaneous uterine neoplasms	(169)
Anti-asialo-GM1	Lacks NK cells, mono-cytes/macrophages	↑ MCA-induced sarcomas	(40)
Anti-NK1.1	Lacks NK, NKT cells	↑ MCA-induced sarcomas	(36, 40)
Anti-Thy1	Lacks T cells	↑ MCA-induced sarcomas	(36)
STAT$1^{-/-}$	IFN-γ-, α/β-insensitive	↑ MCA-induced sarcomas; wider tumor spectrum in STAT$1^{-/-}$ × p$53^{-/-}$	(5, 31)
IFNGR$1^{-/-}$	IFN-γ-insensitive	↑ MCA-induced sarcomas; wider tumor spectrum in IFNGR$1^{-/-}$ × p$53^{-/-}$	(5, 31)
IFN-$\gamma^{-/-}$	Lacks IFN-γ	↑ MCA-induced sarcomas; B6: ↑ spontaneous disseminated lymphomas; BALB/c: ↑ spontaneous lung adenocarcinomas	(32, 33)
GM-CSF/IFN-$\gamma^{-/-}$	Lacks GM-CSF, IFN-γ	↑ Spontaneous lymphomas; ↑ nonlymphoid solid cancers	(55)
Pfp$^{-/-}$ × IFN-$\gamma^{-/-}$	Lacks Perforin, IFN-γ	↑ MCA-induced sarcomas; ↑ spontaneous disseminated lymphomas	(32, 33)
Pfp$^{-/-}$	Lacks Perforin	↑ MCA-induced sarcomas; ↑ spontaneous disseminated lymphomas	(32, 33, 35–37)
TRAIL$^{-/-}$	Lacks TRAIL	↑ MCA-induced sarcomas	(61)
Anti-TRAIL	Blockade of TRAIL function	↑ MCA-induced sarcomas; ↑ spontaneous sarcomas, disseminated lymphomas	(60)
IL-12p$40^{-/-}$	Lacks IL-12	↑ MCA-induced sarcomas	(36)
Wt + IL-12	Exogenous IL-12	↓ MCA-induced sarcomas	(62)
Wt + α-GalCer	Exogenous NKT cell activation	↓ MCA-induced sarcomas	(63)

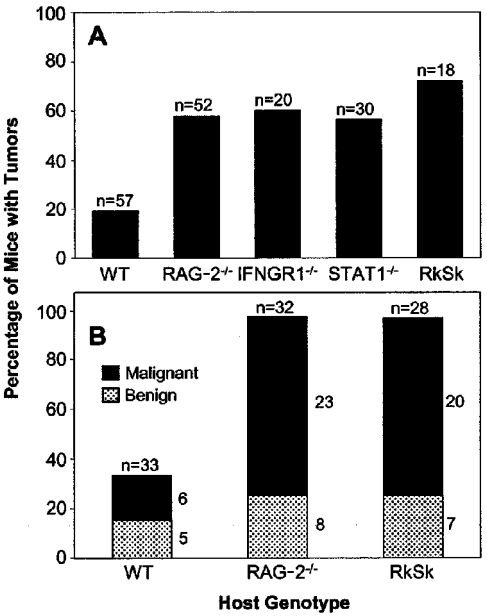

Figure 1 Increased incidence of chemically induced and spontaneous tumors in immunodeficient mice. (*A*) Age- and sex-matched mice were inoculated with 100 μg MCA and monitored for tumor development for 160 days. (*B*) Mice housed in a specific pathogen-free facility were monitored for spontaneous tumor development between 13–24 months. Adapted from Shankaran et al. (5).

overlapping. However, RkSk mice developed spontaneous breast tumors that were not observed in wild-type or RAG-$2^{-/-}$ mice, therefore demonstrating that the overlap between the two pathways was incomplete (Figure 1*B*). Similar findings were made in carcinogenesis experiments employing mice that lacked either perforin, IFN-γ, or both, where a small increase was observed in tumor induction in the doubly deficient mice compared with mice lacking only one of the two components (32).

Identification of the Components of the Immmunosurveillance Network

TUMOR CELLS AS KEY TARGETS OF IFN-γ The finding that endogenously produced IFN-γ played a critical role in protecting mice against tumor development stimulated a search for the physiologically important cellular targets of this cytokine. Two approaches demonstrated that the tumor cell itself is an important IFN-γ target in tumor rejection. In the first, the effects of ablating IFN-γ sensitivity on the immunogenicity of IFN-γ-sensitive tumor cells was assessed using models of tumor cell transplantation (30). Meth A tumor cells, when

engineered to be unresponsive to IFN-γ by overexpression of a dominant-negative IFNGR1 mutant (mgR.ΔIC) (41), grew more aggressively than mock-transfected controls when transplanted into naïve syngeneic wild-type hosts and were resistant to LPS-induced tumor rejection (30, 41). Unlike their IFN-γ-sensitive counterparts, IFN-γ-insensitive Meth A.mgR.ΔIC cells failed to prime naïve recipients for development of Meth A immunity and were poorly recognized when injected into mice with pre-established immunity to the parental wild-type tumor cell line. Similar results were obtained with a second fibrosarcoma derived from a C57BL/6 mouse (MCA-207). The second approach employed an opposite strategy where the effects on in vivo tumor growth were assessed following restoration of IFN-γ sensitivity to tumor cells generated in IFN-γ-insensitive IFNGR1$^{-/-}$ mice (31). When transplanted into wild-type mice, IFNGR1-deficient RAD.gR.28 tumor cells were highly tumorigenic and formed progressively growing tumors even when injected at very low cell number (10–100 cells/mouse). In contrast, when RAD.gR.28 cells were rendered responsive to IFN-γ by complementation with wild-type IFNGR1, the resulting tumor cell line (RAD.gR.28.mgR) was highly immunogenic and failed to form progressively growing tumors in wild-type recipients even when injected at high cell number (5×10^6 cells/mouse). Demonstration that RAD.gR.28.mgR rejection occurred via an IFN-γ-dependent immunologic mechanism was evidenced by the observations that (*a*) rejection of RAD.gR.28.mgR cells in wild-type mice was inhibited by administration of IFN-γ mAb (31), (*b*) rejection was inhibited if wild-type mice were depleted of either CD4$^+$ or CD8$^+$ T cells (A.T. Bruce & R.D. Schreiber, unpublished observations), and (*c*) RAD.gR.28.mgR cells formed progressively growing tumors when injected into RAG-2$^{-/-}$ mice (31). Thus, the effects of using IFN-γ-insensitive tumor cells are the same as blocking IFN-γ availability in the intact mouse: Immune rejection of the tumor is inhibited. Together, these results formed the basis for the conclusion that the tumor cell is a physiologically relevant target of IFN-γ in the tumor rejection process.

Subsequent studies have pointed to several effects of IFN-γ on tumor cells that could promote tumor elimination. IFN-γ's capacity to enhance tumor cell immunogenicity by upregulating components of the MHC class I antigen processing and presentation pathway has been shown to be sufficient for tumor rejection. IFN-γ-insensitive RAD.gR.28 tumor cells engineered for enforced expression of either TAP-1 (5) or H-2D^b (A.T. Bruce & R.D. Schreiber, unpublished observations) were rejected when transplanted into naïve syngeneic recipients in an immunologic manner that was indistinguishable from that of IFN-γ-responsive RAD.gR.28.mgR cells. In contrast, RAD.gR.28 cells engineered for expression of H-2K^b were not rejected (5). The finding that enforced expression of H-2D^b, but not H-2K^b, caused rejection of RAD.gR.28 corresponds to the H-2D^b MHC restriction displayed by protective CD8$^+$ T cells that arise naturally in mice immunized with RAD.gR.28.mgR (A.T. Bruce & R.D. Schreiber, unpublished observations). Thus, the capacity of IFN-γ to regulate tumor cell immunogenicity via enhancement of MHC class I pathway function is a physiologically relevant action that promotes tumor rejection.

Other known IFN-γ-dependent effects on developing tumors may also contribute to the rejection process; however, their physiologic relevance to the process has not yet been established. IFN-γ has profound antiproliferative and/or proapoptotic effects on certain tumor cells. In the former case, IFN-γ can induce expression of cell cycle inhibitors such as $p21^{WAF1/CIP1}$ or $p27^{KIP1}$ that bind to and inhibit the cyclin-dependent kinase CDK-2 (42) or CDK-4 (43), respectively. In the latter case, IFN-γ can induce expression of gene products such as caspase-1 (44, 45), Fas, and Fas ligand (46) that, under the proper conditions, can promote tumor cell apoptosis. IFN-γ can also stimulate tumor cells to produce the chemokines CXCL-9 (Mig) and CXCL-10 (IP-10), which, in addition to having potent chemoattractant activity for CXCR3-expressing leukocyte populations, also function as powerful inhibitors of angiogenesis (47–52). Although all the aforementioned processes likely contribute in some way to the antitumor response, the relative importance and interrelationships between the immunologic and nonimmunologic actions of IFN-γ on developing tumors in promoting tumor rejection requires further analysis.

HOST CELLS AS POTENTIAL ADDITIONAL TARGETS OF IFN-γ Evidence has also been obtained supporting a role for IFN-γ and/or STAT1 at the level of the host immune system in the tumor rejection process. IFN-γ-unresponsive mice lacking STAT1 failed to reject highly immunogenic P198 tumor cells that were completely eliminated in wild-type mice (53). Similar findings have also been made using the highly immunogenic RAD.gR.28.mgR fibrosarcoma cell line that was rejected in wild-type mice but grew progressively in $STAT1^{-/-}$ mice (V. Shankaran & R.D. Schreiber, unpublished observations). In addition, T cells derived from $STAT1^{-/-}$ mice immunized with poorly immunogenic P1.HTR tumor cells in the presence of IL-12 failed to express cytolytic activity against the tumor. In contrast, T cells derived from similarly immunized wild-type mice developed potent cytocidal capacity. Mice lacking STAT6, which tend to polarize their $CD4^+$ T cell compartment more easily into Th1 cells, spontaneously rejected poorly immunogenic P1.HTR tumor cells that grew progressively in wild-type mice (54). Thus, these studies suggest that IFN-γ's well-recognized STAT1-dependent promotion of $CD4^+$ T cell polarization into Th1 cells facilitates development of the appropriate type of cellular immune response needed for tumor rejection.

Another study revealed a more indirect immunological action of IFN-γ at the level of the host in preventing tumor development (55). Both GM-CSF/IFN-$\gamma^{-/-}$ doubly deficient and GM-CSF/IL-3/IFN-$\gamma^{-/-}$ triply deficient mice were found to be highly susceptible to bacterial infection, displayed acute and chronic inflammation in a variety of different organs, and developed high incidences of spontaneous lymphoma and nonlymphoid solid cancers. The incidences of infection, inflammation, and neoplasia were much reduced in mice lacking GM-CSF alone, IL-3 and GM-CSF only, or IFN-γ alone. Tumor development in the IL-3/GM-CSF/IFN-γ triply gene-targeted mice was prevented or delayed by maintaining the mice on broad-spectrum antibiotics from birth. These results suggest a role for IFN-γ, in combination with GM-CSF, in controlling chronic infections that can lead to

a chronic inflammatory state that ultimately may result in cancer development. Clearly, the relationship between bacterial/microbial immunosurveillance and cancer immunosurveillance warrants further analysis but must await the development of in vivo models that can unequivocally differentiate between the two processes.

Finally, other studies have suggested that host cells of nonimmunologic origin may also be important targets of IFN-γ in the antitumor response (56, 57). These studies report that IFN-γ can induce angiostatic effects in tumors by targeting nontransformed host cells that are in close proximity to the tumor. It is possible that the underlying mechanism of this effect is similar to the one that has already been discussed in the context of the tumor cells themselves—the IFN-γ-dependent induction in host stromal cells of the angiostatic chemokines IP-10 and Mig.

THE CELLULAR EFFECTORS OF CANCER IMMUNOSURVEILLANCE Other studies have begun to shed light on the specific lymphocyte subsets that are involved in cancer immunosurveillance. Together, these studies have shown that components of both the adaptive and innate immune systems participate in the process. Girardi et al. (58) examined the relative contributions of different T-cell subsets in blocking primary tumor formation in mice lacking $\alpha\beta$ T cells (TCR$\beta^{-/-}$) and/or $\gamma\delta$ T cells (TCR$\delta^{-/-}$). MCA treatment of either type of TCR$^{-/-}$ mouse led to an increased incidence of fibrosarcomas and spindle cell carcinomas compared with wild-type controls, thereby showing that both $\alpha\beta$ and $\gamma\delta$ T-cell subsets play critical and nonredundant host-protective roles in this particular model of tumor development. However, in an initiation/promotion model of DMBA- and TPA-induced skin tumorigenesis, TCR$\delta^{-/-}$ mice showed an increased susceptibility to tumor formation and a higher incidence of papilloma-to-carcinoma progression than wild-type mice, whereas TCR$\beta^{-/-}$ mice did not. This result suggests that immunosurveillance may be a multivariable process requiring the actions of different immune effectors in a manner dependent on the tumor's cell type of origin, mechanism of transformation, anatomic localization, and mechanism of immunologic recognition.

NK and NKT cells represent cellular populations of the innate immune compartment that were shown to protect the host from tumor formation. C57BL/6 mice depleted of both NK and NKT cells using the NK1.1 mAb were two to three times more susceptible to MCA-induced tumor formation than wild-type controls (40). In the same study, C57BL/6 mice depleted of NK cells following anti-asialo-GM1 treatment were two to three times more prone to developing MCA-induced tumors than control counterparts. Although anti-asialo-GM1 can also deplete activated macrophages, this study nevertheless supports the involvement of cells of innate immunity in blocking primary tumor development. A role for NKT cells in this process was implicated when Jα281$^{-/-}$ mice, which lack a large population of Vα14Jα281-expressing invariant NKT cells, were found to develop MCA-induced sarcomas at a higher incidence than their wild-type counterparts (36).

Additional evidence pointing to cells of innate immunity as critical effectors of cancer immunosurveillance comes from studies of the TNF-related

apoptosis-inducing ligand (TRAIL). A member of the TNF superfamily that induces apoptosis through engagement of the TRAIL-R2 (DR5) receptor in mice, TRAIL is expressed constitutively on a subset of liver NK cells and is induced by either IFN-γ or IFN-α/β in monocytes, NK cells, and dendritic cells (59). When injected with low doses of MCA, C57BL/6 strain mice treated with neutralizing antibodies to TRAIL (60) or lacking the TRAIL gene (61) developed fibrosarcomas at a higher incidence than wild-type controls. Moreover, C57BL/6 strain $p53^{+/-}$ mice treated with the same neutralizing TRAIL antibody exhibited a higher incidence of spontaneous sarcoma and disseminated lymphoma formation over a two-year span than control IgG-treated mice (60). Further study will be required to identify the specific innate cell subsets that manifest the TRAIL-dependent antitumor effects.

Finally, evidence also exists showing that enhancing immune system activity leads to reduced primary tumor formation in models of MCA tumorigenesis. Mice treated with either IL-12 (62) or the prototypic NKT cell activator α-galactosylceramide (α-GalCer) (63) throughout the MCA carcinogenesis process had a reduced incidence of tumors after longer latency periods than control mice.

In summary, using a variety of well-characterized gene-targeted mice, specific immune system activators, and blocking monoclonal antibodies highly specific for distinct immunologic components, a large body of work has now accumulated to support the statement that the immune system indeed functions to protect the murine host against development of both chemically induced and spontaneous tumors (Table 1).

CANCER IMMUNOSURVEILLANCE IN HUMANS

Given that there is significant evidence supporting the existence of a cancer immunosurveillance process in mice, does a similar process exist in humans? Analysis of individuals with congenital or acquired immunodeficiencies or patients undergoing immunosuppressive therapy has documented a highly elevated incidence of virally induced malignancies such as Kaposi's sarcoma, non-Hodgkin's lymphoma, and cancers of the anal and urogenital tracts compared with immunocompetent individuals (64–66). However, the study of the incidence of cancers of nonviral origins that may take many years to develop is confounded by the variety of viral and bacterial infections to which these immunodeficient/immunosuppressed patients are susceptible and by the more rapid appearance of virally induced tumors. Nevertheless, one can draw upon three lines of evidence to suggest that cancer immunosurveillance indeed occurs in humans: (*a*) immunosuppressed transplant recipients display higher incidences of nonviral cancers than age-matched immunocompetent control populations; (*b*) cancer patients can develop spontaneous adaptive and innate immune responses to the tumors that they bear, and (*c*) the presence of lymphocytes within the tumor can be a positive prognostic indicator of patient survival.

Transplant Recipients Display Increased Incidences of Malignancies

Increased relative risk ratios have indeed been observed in immunosuppressed transplant recipients for a broad subset of tumors that have no apparent viral origin. Assessment of 5692 renal transplant patients from 1964–1982 in Finland, Denmark, Norway, and Sweden showed increased standardized cancer incidence ratios for colon, lung, bladder, kidney, ureter, and endocrine tumors compared to the general population (67). For example, the relative risks for colon cancer were 3.2 for men and 3.9 for women. In addition, analysis of 925 patients who received cadaveric renal transplants from 1965 to 1998 in Australia and New Zealand exhibited increased risk ratios for the development of a variety of cancers, including those of the colon, pancreas, lung, and endocrine tumors as well as malignant melanomas (68). When tumor incidence was examined in 608 cardiac transplant patients at the University of Pittsburgh between 1980 and 1993, the prevalence of lung tumors was 25-fold higher than in the general population (69). Furthermore, Penn researchers documented several examples of the increased incidence of tumors of nonviral etiology in immunosuppressed transplant patients through analysis of the Cincinnati Transplant Tumor Registry (CTTR). A review of data accumulated by this database from 1968 to 1995 found a twofold increase in risk in transplant patients for developing melanoma over that of the general population (70). Moreover, whereas only 0.3% to 0.4% of melanomas occur in the general pediatric population, the occurrence in pediatric transplant patients followed in the CTTR was 4% (70). These data complemented other studies that showed approximately fourfold increases in the incidence of de novo malignant melanoma after organ transplantation (71, 72). Finally, analysis of the CTTR showed that transplant patients were three times more likely to develop non-Kaposi's sarcomas (73). Thus, individuals with normal immune systems who undergo immunosuppression display an increased probability of developing a variety of cancers that have not been linked to a viral etiology. This observation may indicate that immunosuppressive intervention predisposed the transplant patients either to de novo tumor formation or allowed the outgrowth of occult tumors whose growth was contained by a functioning immune system. Either way, these results suggest a protective action of immunity in preventing human tumors.

Spontaneous Tumor Recognition by Adaptive and Innate Immunity

ADAPTIVE IMMUNE RESPONSES Substantial amounts of data support the concept that cancer patients can spontaneously develop specific adaptive immune responses to tumor antigens. Because the transplantation techniques used to demonstrate the presence of tumor-specific antigens in the mouse could not be employed in humans, in vitro approaches to identify immune responses to human tumor antigens needed to be developed. A systematic survey of the humoral and cellular immune

responses of patients to their own tumors was initiated in the 1970s using an approach termed autologous typing (74). Tumor cell lines were established from a large series of patients with melanoma or other tumor types that could be propagated in vitro, and these cells were used as targets for analysis of the humoral or cellular antitumor immune responses of the autologous patient. Fibroblasts and other autologous normal cell types served as control targets to assess the specificity of the antitumor response. Using this system, a small subset of patients was identified who had specific antibody to cell-surface antigens (75, 76) or who had T cells that recognized the autologous tumor (77). The characterization of the molecular targets recognized by autologous typing was made possible by application of the gene cloning and expression systems developed by Boon and colleagues to identify tumor antigens recognized by $CD8^+$ T cells (78, 79) and by Pfreundschuh and colleagues for antibody-defined tumor antigens (80). More recently, it has been possible to identify MHC class II restricted tumor antigens recognized by $CD4^+$ T cells (81).

A large array of immunogenic human tumor antigens has now been identified (82–84). These can be segregated into the following four classes: *Differentiation Antigens*, e.g., melanocyte differentiation antigens, Melan-A/MART-1, tyrosinase, gp-100; *Mutational Antigens*, e.g., abnormal forms of p53; *Overexpressed/Amplified Antigens*, e.g., HER-2/neu; *Viral Antigens*, e.g., EBV and HPV; and *Cancer-Testis* (CT) *Antigens*. Using the currently available methodologies, the search for immunogenic human tumor antigens continues. The ultimate objective of this work is to define the human cancer immunome—the complete repertoire of human tumor antigens eliciting an immune response in humans—and a human cancer immunome database containing over 1000 human tumor antigens has been established (https://www2.licr.org/CancerImmunomeDB/).

Because of their unique characteristics, CT antigens are of particular interest (85). In adult normal tissues, their expression is limited to germ cells in the testis, whereas in cancer, a variable proportion of a wide range of different tumor types expresses CT antigens. The first members of the CT family of antigens (MAGE, BAGE, GAGE) were cloned by Boon and his colleagues using $CD8^+$ T cells from a patient having strong $CD8^+$ T cell reactivity to autologous melanoma cells (79). The serological expression cloning technique (SEREX) developed by Pfreundschuh and colleagues (80) to detect the humoral response to human cancer has greatly expanded the list of CT antigens as well as other categories of tumor antigens, and there are now more than 20 CT antigens or antigen families recognized in human cancer (85).

The analysis of the immune response to NY-ESO-1, a SEREX-defined CT antigen, is one of the best-documented examples of an integrated, naturally occurring spontaneous immune response to a nonviral human cancer. NY-ESO-1 was identified using SEREX analysis of an esophageal squamous cell carcinoma (86). Analysis of NY-ESO-1 at the mRNA and protein levels showed that NY-ESO-1 expression is limited to testis, fetal ovary, and placenta, but is detected in a variety of tumors including melanoma, bladder cancer, lung cancer, and

synovial sarcomas (87). Antibody to NY-ESO-1 has been found only in patients with NY-ESO-1-expressing tumors; no antibody has been detected in patients with NY-ESO-1-negative tumors or in normal individuals (88). NY-ESO-1 antibody is rare in patients with early stage cancer, but can be found in up to 50% of patients with advanced NY-ESO-1^+ tumors. The presence of antibody appears to be antigen driven, as removal of the tumor by surgery or following chemotherapy is frequently followed by disappearance of antibody (89). $CD8^+$ and $CD4^+$ T cell responses to NY-ESO-1 have been detected in patients with NY-ESO-1^+ tumors, and a large number of MHC class I– and II–restricted NY-ESO-1 epitopes have been defined (90–92). These cellular responses are almost invariably associated with a strong NY-ESO-1 humoral immune response, documenting the integrated spontaneous immune response to this tumor antigen. Although there are indications that patients with spontaneous NY-ESO-1 immunity have a more favorable prognosis, proof that such an association exists is difficult to establish because of the variable clinical course of cancer and the influence of chemotherapy and other therapeutic interventions. The development of immunogenic NY-ESO-1 vaccines and randomized clinical trials will undoubtedly be necessary before definitive evidence linking NY-ESO-1 immunity to patient benefit can be substantiated.

A second, well-characterized example of spontaneous immune responses to developing tumors in humans comes from the analysis of paraneoplastic neurologic disorders/degenerations (PNDs). PNDs are rare autoimmune neurologic diseases that are thought to be caused by "remote effects of cancer on the nervous system" (93), i.e., they are not caused by either direct primary or metastatic tumor invasion into nervous tissue but rather may be caused by cross reactivity of host antitumor responses with cells of the nervous system. Clinically, PNDs may affect any part of the nervous system and are most commonly associated with tumors of the breast, lung, and ovary (93). In the 1980s, an immunologic link between neuronal degeneration and the presence of cancer was established by the discovery that the serum and cerebrospinal fluid of PND patients harbored high titers of antibodies that reacted with neuronal antigens present in both the affected neuronal population and the associated cancer [antigens are discussed in depth in (94, 95)]. Furthermore, CTLs have been identified in the peripheral blood (96) and cerebrospinal fluid (97) of PND patients that can react with peptides from one of these antigens. However, CTL reactivity and cytotoxicity against intact neuronal cells and antigen-expressing tumor cells has yet to be demonstrated. Data from several clinical studies suggest that the presence of neuronal-reactive autoantibodies is associated with improved prognosis in cancer patients (98–100). Specifically, these studies have noted a positive correlation between the presence of antibody and the extent of disease, response to anticancer therapy, and survival.

INNATE IMMUNE RESPONSES Recent studies indicate that the innate arm of the human immune system may also discriminate between tumor cells and normal cells and thus has the potential of participating in cancer immunosurveillance. These studies have centered largely on the human MHC class I chain-related proteins

A and B (MICA/B) that are differentially expressed on tumor cells and function as ligands for two receptors expressed on cells of the innate immune system: NKG2D and the T cell receptor on Vδ1 $\gamma\delta$ T cells. MICA/B are highly polymorphic nonclassical MHC cell surface glycoproteins that do not associate with β2 microglobulin, nor do they require TAP for expression (101, 102). When a panel of normal tissues was screened by immunohistochemistry for expression of these proteins, MICA expression was found only on gastrointestinal epithelium of the stomach and large and small intestines. However, MICA/B gene expression could be induced in certain nontransformed cell lines by heat shock or viral infection (101, 103). In contrast, constitutive MICA/B expression has been documented in a high percentage of primary carcinomas of the lung, breast, kidney, ovary, prostate and colon (104), melanomas (105), and hepatocellular carcinomas (106).

MICA/B are recognized by an activating receptor on NK cells that is also expressed on most human $\gamma\delta$ T cells and CD8$^+$ $\alpha\beta$ T cells (107). This receptor is comprised of two subunits: a ligand-binding NKG2D subunit and either a DAP-10 or DAP12 signaling subunit (108). This receptor also reacts with other ligands such as those of the ULBP family that were independently identified as cell surface markers present on transformed cells and cells undergoing stress (109, 110). Tumor cells expressing MICA/B are killed by effector cells with functional NKG2D receptors, and lysis can be inhibited by pretreating the effector cell with blocking NKG2D mAb (107). However, recognition of MICA/B has also been ascribed to the direct binding of the $\gamma\delta$ TCR on Vδ1 $\gamma\delta$ T cells, as the lysis of MICA-expressing target cells by Vδ1 $\gamma\delta$ T cells can be inhibited by a Vδ1 $\gamma\delta$ TCR mAb (111), and soluble MICA tetramers can bind specifically to transfected cells expressing various Vδ1 $\gamma\delta$ TCRs but not NKG2D (112). Thus, $\gamma\delta$ T cells possess two mechanisms to recognize the MIC markers on tumors: one involving a direct interaction with the $\gamma\delta$ TCR and the other mediated by a more globally expressed NKG2D activating receptor.

Two data sets link MICA/B recognition to immunosurveillance. First, Groh et al. (111) demonstrated that MIC-expressing cells were recognized and killed by the Vδ1 $\gamma\delta$ T-cell subset, and observed a strong in vivo correlation ($p < 0.0001$) between MICA/B expression on tumors and tumor infiltration by Vδ1 $\gamma\delta$ T cells (104). Second, recent data demonstrated a correlation between downregulation of NKG2D on tumor-infiltrating lymphocytes (TILs) and the expression of MICA/B in the tumor (113). Compared with NKG2D expression in lymphocytes from patients with MIC$^-$ tumors, NKG2D expression was reduced on tumor-infiltrating CD8$^+$ $\alpha\beta$ T cells, $\gamma\delta$ T cells, and NK cells and also on peripheral blood mononuclear cells (PBMCs) from individuals with MIC$^+$ tumors. Further analysis revealed a correlation between the presence of soluble MIC proteins in the circulation of 7/14 cancer patients and a downregulated expression of NKG2D on lymphocytes. This downregulation could be recapitulated in vitro. Results of a separate study suggested that shedding of MIC proteins from tumor cell surfaces was the result of the actions of an unknown matrix metalloproteinase (114). These observations thus establish a common mechanism of tumor recognition—and potential

elimination—by both the innate and adaptive immune systems. The finding that soluble MIC proteins may attenuate the expression/function of NKG2D on host immune cells provides one explanation for how a growing tumor could escape cancer immunosurveillance. Recent work has established the generalizable importance of NKG2D-dependent tumor recognition in murine tumor models as well. In these studies, the overexpression of NKG2D ligands H60 and Rae-1 family members in tumors capable of growing progressively led to their rejection in an NK cell-dependent manner (115–117).

In summary, a large amount of data has begun to accumulate indicating that human cancer patients indeed develop immune responses to the tumors that they bear. Although these responses may not always be able to prevent cancer development, they may nevertheless function to restrain tumor growth.

The Presence of Tumor-Infiltrating Lymphocytes Correlates with Patient Survival

The third line of evidence that a cancer immunosurveillance process exists in humans comes from a growing body of evidence showing that the presence of tumor-infiltrating lymphocytes (TILs) in a cancer patient's tumor presages an improved clinical outcome for that individual. Some of the groundbreaking studies that established a strong correlation between patient survival and the presence of TILs involved collectively nearly 900 patients with primary or metastatic melanoma (118–120). The paradigm that was established in these studies has been upheld by several recent studies involving patients with other types of cancer. In a recent analysis, Zhang et al. (121) reported a relationship between the presence of $CD3^+$ TILs and favorable clinical outcomes in patients with advanced ovarian adenocarcinoma. In this study, 186 frozen specimens of stage III or IV ovarian cancers from patients undergoing debulking surgery were assessed by immunostaining for the presence of TILs. Of the 174 tumors that could be evaluated, 102 contained TILs, whereas 72 did not. Patients with TIL-containing tumors had five-year overall survival rates of 38% compared with 4.5% for patients whose tumors lacked TILs. In a subset of 74 of these patients who experienced complete responses to surgical debulking and chemotherapy, the five-year overall survival rate was 73.9% for those with TIL-containing tumors versus 11.9% for patients with tumors that lacked TILs. Furthermore, in a multivariate analysis, it was shown that the presence or absence of TILs and the extent of residual tumor were the only independent prognostic factors of progression-free and overall survival in these patients; other variables such as the type of chemotherapy, histologic type of the tumor, tumor grade, or patient age were not predictive of both rates.

Other studies examined the prognostic significance of individual T-cell subsets that infiltrate tumors. Naito et al. (122) found that the extent of $CD8^+$ T cell infiltration specifically into cancer cell nests correlated with the survival of patients with colorectal cancer; 56 patients with no infiltration had five-year survival rates of 50%, whereas the 23 patients showing pronounced $CD8^+$ T cell infiltration into

cancer cell nests had five-year survival rates of 100%. Moreover, multivariate analysis revealed that the presence $CD8^+$ T cells in cancer cell nests was an independent prognostic factor with an impact on patient survival similar to conventional Dukes' tumor-staging classifications. Analogous findings were made by Schumacher et al. (123) who tracked the clinical course of 70 patients with esophageal squamous cell carcinomas or adenocarcinomas. When histological analysis of the tumor was compared to clinical outcome, the presence of intratumoral lymphocytes correlated with both increased time to disease recurrence and also increased time to death over a five-year period after diagnosis. As in the aforementioned study, a multivariate analysis of the data showed that the presence of intratumoral $CD8^+$ T cells was an independent prognostic factor for survival.

Still other studies have shown similar positive correlations between NK cell infiltration and patient survival for gastric carcinoma (124), squamous cell lung carcinoma (125), and colorectal cancer (126). Thus, significant evidence links the presence of TILs to increased survival of cancer patients. Since tumors may attract distinct TIL subsets depending on their tissue of origin (127), it will be important in the future to clarify which particular immune cells are prognostic for each distinct type of cancer.

Thus, after a century of controversy, substantial amounts of direct experimental data from mice coupled with correlative data from humans show that innate and adaptive immunity function together to protect the host against neoplastic disease and thereby converge on the original conviction of Burnet and Thomas: immunosurveillance exists.

IMMUNOLOGIC SCULPTING DURING TUMOR DEVELOPMENT

Despite strong evidence supporting the existence of a functional cancer immunosurveillance process, immunocompetent individuals still develop cancer. This clinical reality may be explained by the existence of an immune process that facilitates the outgrowth of tumors with reduced immunogenicity that have a better chance of surviving in an immunocompetent host. Recent work from several laboratories now supports this hypothesis.

Our laboratory used tumor transplantation approaches to assess the immunogenic characteristics of a large number of primary MCA-induced sarcomas generated in the presence or absence of a functional immune system (5). Tumor cells from either wild-type or RAG-$2^{-/-}$ mice grew progressively with similar kinetics when transplanted into RAG-$2^{-/-}$ recipients, indicating that there were no inherent growth differences between tumors generated in the presence or absence of an intact immune system (Figure 2*A*,*B*). Moreover, tumor cells derived from wild-type mice grew progressively when transplanted into naïve immunocompetent 129/SvEv hosts (Figure 2*C*). In contrast, 8/20 of the tumors originally generated in RAG-$2^{-/-}$ mice were rejected when transplanted into immunocompetent hosts,

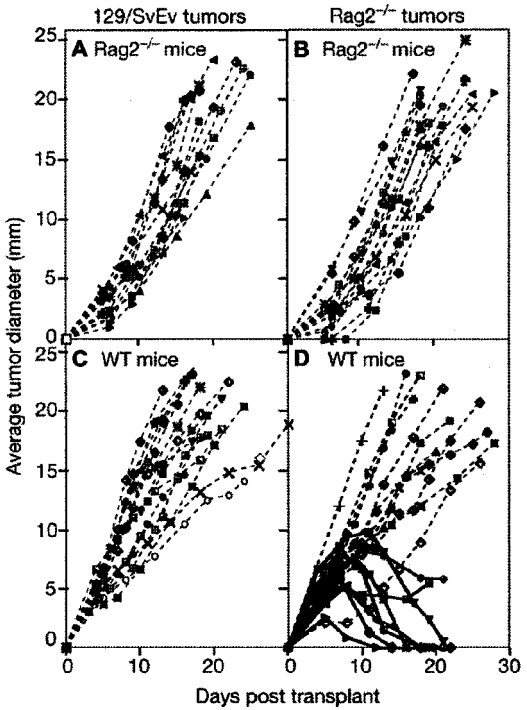

Figure 2 Increased immunogenicity of tumours derived from MCA-treated RAG-$2^{-/-}$ mice. Immunodeficient RAG-$2^{-/-}$ hosts were injected with a dose of 10^5 tumor cells derived from wild-type 129/SvEv mice (*A*) or RAG-$2^{-/-}$ mice (*B*). Tumor growth is plotted as mean tumor diameter of 3–5 mice inoculated with each tumor. Groups of 5–8 immunocompetent 129/SvEv × RAG-$2^{-/-}$ F1 mice were injected on day 0 with doses of 10^6 tumor cells derived from 17 individual 129/SvEv mice (*C*) or 20 individual RAG-$2^{-/-}$ mice (*D*) and tumor growth was monitored as above. In (*D*), the dashed lines denote tumors that grew progressively, whereas solid lines represent tumors that were rejected. Data from Shankaran et al. (5).

even when injected at high cell number (Figure 2*D*). Thus, tumors formed in the absence of an intact immune system are, as a group, more immunogenic than tumors that arise in immunocompetent hosts.

Experiments performed in other laboratories have led to similar conclusions. MCA sarcomas derived from nude (128) or SCID mice (129) were rejected more frequently than similar tumors derived from wild-type mice when transplanted into wild-type hosts. In addition, two MCA-induced sarcomas derived from TCR Jα281$^{-/-}$ mice grew more slowly when transplanted into wild-type hosts than did sarcomas originally isolated from wild-type mice (36). In contrast, these tumors grew in a comparable manner when transplanted into Jα281$^{-/-}$ recipients. It was

also shown that lymphomas derived from $pfp^{-/-}$ mice grew avidly when transplanted into $pfp^{-/-}$ mice but most were rejected when transplanted into wild-type mice (33).

Finally, results from one recent study suggest that immunocompetent mice select tumors that are less sensitive to TRAIL-mediated cytotoxicity (60). MCA-induced fibrosarcomas produced in C57BL/6 strain wild-type or $p53^{+/-}$ mice that were treated with either control IgG, a neutralizing monoclonal TRAIL-specific antibody, or an antibody specific for asialo-GM1 throughout tumor development were tested for susceptibility to TRAIL-mediated killing in vitro. Although only 1/6 tumors derived from control IgG-treated wild-type mice and 1/8 tumors derived from control IgG-treated $p53^{+/-}$ mice were lysed by TRAIL, 5/6 tumors generated in antiasialo-GM1-treated wild-type mice and 5/8 tumors derived from anti-TRAIL-treated $p53^{+/-}$ mice displayed susceptibility to TRAIL killing.

Taken together, these results show that tumors are imprinted by the immunologic environment in which they form. By eliminating tumor cells of high intrinsic immunogenicity, this imprinting process may select for tumor cell variants of reduced immunogenicity and therefore favor the generation of tumors that are either poorly recognized by the immune system or that have acquired mechanisms that suppress immune effector functions. In this manner, the immunologic sculpting of developing tumor cells provides them with mechanisms to resist the extrinsic tumor-suppressor actions of the immune system. While the shaping of tumor immunogenicity most likely occurs continuously during tumor development, the major effects of this process probably occur early when the tumor is perhaps histologically—but not clinically—detectable. It follows, then, that the immunogenicity of most clinically apparent tumors has already been attenuated to some degree by the sculpting hand of immunity.

CANCER IMMUNOEDITING: REFINING CANCER IMMUNOSURVEILLANCE

Based on the studies summarized in this review, the term "cancer immunosurveillance" no longer suffices to accurately describe the complex interactions that occur between a developing tumor and the immune system of the host. As originally conceived, cancer immunosurveillance was thought to be a host-protective function carried out by the adaptive immune system only at the earliest stages of cellular transformation. In contrast, we now recognize that both the innate and adaptive immune compartments participate in the process and serve not only to protect the host from tumor development but also to sculpt, or edit, the immunogenicity of tumors that may eventually form. Therefore, we have proposed the use of the broader term "cancer immunoediting" to more appropriately emphasize the dual roles of immunity in not only preventing but also shaping neoplastic disease (5, 6). Cancer immunoediting thus represents a refinement of the original cancer immunosurveillance hypothesis but is more comprehensive in its scope. As such,

we envisage that the cancer immunoediting process is comprised of three phases that we have termed the "three Es of cancer immunoediting:" elimination, equilibrium, and escape. In the following sections, we discuss each of these three phases in more detail (Figure 3). Specifically, we attempt to integrate our enhanced and evolving understanding of immune system-tumor interactions with ongoing work in classical tumor biology. Our intention is not to be dogmatic but rather to present a testable model that will stimulate further work in defining the molecular and cellular basis of each of the three phases of cancer immunoediting.

Elimination

The elimination phase represents the original concept of cancer immunosurveillance (Figure 3*A*; Figure 4). If this phase successfully eradicates the developing tumor, it represents the complete immunoediting process without progression to the subsequent phases. The immune components that participate in the elimination phase are now being identified but their precise roles need to be further clarified. As an extrinsic tumor suppressor, we envisage that the immune system manifests its effects only after transformed cells have circumvented their intrinsic tumor-suppressor mechanisms (130). Immunologic rejection of a developing tumor, as in host defense to microbial pathogens, likely requires an integrated response involving both the innate and adaptive arms of the immune system (27). Initiation of the antitumor immune response (Figure 4*A*) occurs when cells of the innate immune system become alerted to the presence of a growing tumor, at least in part owing to the local tissue disruption that occurs as a result of the stromal remodeling processes integral to the basic physiology of solid tumor development. This stromal remodeling could result from two of the six "hallmarks of cancer" (131): angiogenesis (132, 133) and tissue-invasive growth (134). The stromal remodeling induced during these processes could produce proinflammatory molecules that, together with chemokines that may be produced by the tumor cells themselves (135), summon cells of the innate immune system to this new source of local "danger" (136, 137). Once recruited to the developing tumor mass, NKT cells, $\gamma\delta$ T cells, NK cells, and/or macrophages may recognize molecules, such as the ligands for NKG2D previously discussed, that have been induced on tumor cells either by the incipient inflammation or the cellular transformation process itself. In addition, $\gamma\delta$ T cells and NKT cells may recognize developing tumors via TCR interaction with either NKG2D ligands or glycolipid-CD1 complexes expressed on tumor cells, respectively (138). Regardless of the precise mechanism of recognition, these events lead to a common outcome that is critical for progression of the antitumor response—the production of IFN-γ.

In the second step (Figure 4*B*), the effects of innate immune recognition of the tumor are amplified. The initial amount of IFN-γ released at the tumor site induces the local production of chemokines that recruit more cells of the innate immune system to the tumor. Products generated during remodeling of the extracellular matrix may induce tumor-infiltrating macrophages to produce low amounts of

IL-12 (139) that stimulate tumor-infiltrating NK cells to produce low amounts of IFN-γ, which in turn activate macrophages in the tumor to produce more IL-12, leading to increased IFN-γ production by NK cells. In addition to this positive feedback system (140), the binding of NK cell–activating receptors to their cognate ligands on tumor cells stimulates even more NK cell IFN-γ production (115) that can now activate a number of IFN-γ-dependent processes—including antiproliferative (43), proapoptotic (141), and angiostatic (47, 52, 56) effects—that result in the killing of a proportion of the tumor. In addition, macrophages activated by IFN-γ that express tumoricidal products such as reactive oxygen and reactive nitrogen intermediates (142–144) and NK cells activated either by IFN-γ or via engagement of their activating receptors can kill tumor cells via TRAIL- (145, 146) or perforin-dependent (147) mechanisms, respectively. As a result of these processes, a source of tumor antigens from dead tumor cells becomes available and the adaptive immune system is recruited into the process.

In the third step (Figure 4*C*), tumor antigens liberated by the effects of innate immunity on the tumor drive the development of tumor-specific adaptive immune responses. Immature dendritic cells (DCs) that have been recruited to the tumor site become activated either by exposure to the cytokine milieu created during the ongoing attack on the tumor by innate immunity or by interacting with tumor-infiltrating NK cells (148). The activated DCs can acquire tumor antigens directly by ingestion of tumor cell debris or potentially through indirect mechanisms involving transfer of tumor cell–derived heat shock protein/tumor antigen complexes to DCs (149, 150). Activated, antigen-bearing mature DCs then migrate to the draining lymph node (151), where they induce the activation of naïve tumor-specific Th1 $CD4^+$ T cells. Th1 cells facilitate the development of tumor-specific $CD8^+$ CTL induced via cross-presentation of antigenic tumor peptides on DC MHC class I molecules (152–155).

In the fourth step (Figure 4*D*), the development of tumor-specific adaptive immunity provides the host with a capacity to completely eliminate the developing tumor. Tumor-specific $CD4^+$ and $CD8^+$ T cells home to the tumor site, where they participate in the killing of antigen-positive tumor cells. $CD4^+$ T cells produce IL-2 that, together with host cell production of IL-15, helps to maintain the function and viability of the tumor-specific $CD8^+$ T cells. Tumor-specific $CD8^+$ T cells will efficiently recognize their tumor targets [owing to the enhanced immunogenicity of tumor cells that have been exposed to the IFN-γ produced in steps 1 and 2 (5)] and will induce tumor cell death by both direct and indirect mechanisms. It is likely that these $CD8^+$ T cells directly kill many of the tumor cells in vivo. However, these cells will also produce large amounts of IFN-γ following interaction with their tumor targets and thus should also induce tumor cell cytostasis and killing by the IFN-γ-dependent mechanisms of cell cycle inhibition, apoptosis, angiostasis, and induction of macrophage tumoricidal activity. These two scenarios are not mutually exclusive and most likely occur concomitantly; however, their relative contributions may vary among different tumors. Thus, the elimination phase of cancer immunoediting is a continuous process that must be repeated each time antigenically

distinct neoplastic cells arise. For this reason, it is particularly noteworthy that cancer is more prevalent in aged populations where immune system function, and therefore cancer immunosurveillance, begins to decline.

Equilibrium

In the equilibrium phase (Figure 3*B*), the host immune system and any tumor cell variant that has survived the elimination phase enter into a dynamic equilibrium, wherein lymphocytes and IFN-γ exert potent and relentless selection pressure on the tumor cells that is enough to contain, but not fully extinguish, a tumor bed containing many genetically unstable and mutating tumor cells. We envision this period to be a crucible of Darwinian selection: Although many of the original tumor cell escape variants are destroyed, new variants arise carrying different mutations that provide them with increased resistance to immune attack. The end result of the equilibrium process is a new population of tumor clones with reduced immunogenicity, hewn from a heterogeneous parental population by the sculpting forces of the immune system.

Equilibrium is probably the longest of the three phases and may occur over a period of many years in humans. Indeed, it has been estimated that for many solid human tumors there can be a 20-year interval between initial carcinogen exposure and clinical detection of the tumor (156). During this period, the heterogeneity and genetic instability of cancer cells that survive the elimination phase are possibly the principal forces that enable tumor cells to eventually resist the host's immunological siege. It has been proposed that the "mutator phenotype" of tumor cells (157) may result from the three types of genetic instability observed in cancer: nucleotide-excision repair instability (NIN), microsatellite instability (MIN), and chromosomal instability (CIN) (158). Of the three, CIN is thought to be the predominant mechanism responsible for destabilizing genomic integrity, and the observation that cancer cell genomes display gains or losses of whole chromosomes (i.e., aneuploidy) associated with an estimated loss of 25%–50% of their alleles reflects the degree of genomic upheaval associated with the CIN phenotype (158). Clearly, genomic instability has the potential to spawn tumor variants of reduced immunogenicity, and some of these will display an enhanced capacity to grow in an unfettered immune selecting environment. A complete mechanistic understanding of the equilibrium phase will require the development of new tumor models to better define the cell-intrinsic mechanisms that generate new tumor phenotypes and to identify the tumor-sculpting immune "editors."

One clinical scenario that may illustrate the equilibrium phase in humans is the transmission of cancer from transplant donors to recipients. In these cases, transplanted organs are grossly normal and cancer-free at the time of harvest. While some donors are subsequently found to harbor disease in other anatomic sites, other transplant donors either have no clinical history of cancer or have been in durable remission from cancer prior to transplantation. Recently, Mackie et al. (159) reported the occurrence of metastatic melanoma 1–2 years post-transplant

in two allograft recipients who had each received kidneys from the same donor. Upon subsequent analysis, it was found that the donor had been treated for primary melanoma 16 years before her kidneys were donated but was considered tumor-free at the time of her death. Two other case reports described the appearance of donor-derived melanoma in two renal transplant patients and one liver transplant recipient less than one year after these organs were transplanted from a donor with no known history of malignancy (160, 161). These observations, together with others that appear in the clinical literature (70, 162), suggest that the pharmacologic suppression of the immune systems of these transplant recipients facilitated the rapid and progressive outgrowth of occult tumors that had previously been maintained in the equilibrium phase by the donor's competent immune system.

Escape

In the escape phase (Figure 3*C*), tumor cell variants selected in the equilibrium phase now can grow in an immunologically intact environment. This breach of the host's immune defenses most likely occurs when genetic and epigenetic changes in the tumor cell confer resistance to immune detection and/or elimination, allowing the tumors to expand and become clinically detectable. Because both the adaptive and innate compartments of the immune system function in the cancer immunosurveillance network, tumors most likely would have to circumvent either one or both arms of immunity in order to achieve progressive growth. Individual tumor cells may employ multiple immunoevasive strategies to elude the powerful integrated innate and adaptive antitumor immune responses to their immunogenic progenitors. Thus, it is likely that several distinct immunologically driven tumor sculpting events must occur before the final immunogenic phenotype of a malignant cell is ultimately established.

Much work has recently focused on defining the molecular bases of tumor escape. It is now recognized that tumors can either directly or indirectly impede the development of antitumor immune responses either through the elaboration of immunosuppressive cytokines (such as TGF-β and IL-10) or via mechanisms involving T cells with immunosuppressive activities (i.e., regulatory T cells). Because the mechanisms that target the immune system to achieve tumor escape have been the subject of recent review articles (163, 164), they are not discussed further.

Tumor escape can also result from changes that occur directly at the level of the tumor. These can include alterations that affect tumor recognition by immune effector cells [such as loss of antigen expression, loss of MHC components (165), shedding of NKG2D ligands (113), and development of IFN-γ insensitivity (31)] or provide tumors with mechanisms to escape immune destruction [such as defects in death-receptor signaling pathways (60) or expression of antiapoptotic signals such as those induced by constitutively active STAT3 (166)]. Two of these mechanisms, dysregulation of MHC class I processing and presentation and development of IFN-γ insensitivity in tumor cells, would allow tumors to escape from the events discussed in the elimination phase of the cancer immunoediting process and have,

in fact, been identified in tumor cells. Analysis of large banks of human tumor specimens has shown that between 40%–90% of human tumors display total or selective allelic losses of HLA class I proteins (165, 167). Moreover, other components of this pathway, including TAP1 and the immunoproteasome subunits LMP2 and 7, are also frequently deficient in human tumors (168). The physiologic relevance of LMP2 deficiency in cancer is evidenced by the observation that $LMP2^{-/-}$ mice are more prone to the development of uterine neoplasms than their wild-type counterparts (169). IFN-γ receptor signaling dysfunction represents another potential mechanism of tumor immune escape. In one study, 4/17 (25%) human lung adenocarcinoma cell lines were found to be completely unresponsive to IFN-γ (31). The unresponsive state in these tumors was found to be caused by either the absence or abnormal function of distinct components of the IFN-γ receptor signaling pathway. In addition, unpublished work has shown that 15%–30% of primary MCA-induced fibrosarcomas derived from 129/SvEv mice display IFN-γ insensitivity (G.P. Dunn & R.D. Schreiber, unpublished observations). Clearly, identifying additional escape mechanisms will yield critical insights into how tumor cell immunogenicities are edited by the immune system.

CONCLUSIONS AND IMPLICATIONS

In this review, we have summarized some of the salient data supporting the existence and physiologic relevance of a cancer immunoediting process. The recent development of sophisticated tumor models using genetically altered mice and function-blocking monoclonal antibodies has made possible the critical experiments that not only resolved the long-standing controversy surrounding the cancer immunosurveillance hypothesis of Burnet and Thomas but also led to its refinement into the cancer immunoediting hypothesis (5, 6). The continued clarification of the three Es of cancer immunoediting has important implications for cancer immunotherapy in humans. By gaining an improved understanding of the cellular and molecular processes that lead to immunologic tumor rejection in the elimination phase, it will be possible to identify which immune forces need to be augmented to facilitate natural protection against tumors of different tissue origins. By studying the equilibrium phase, it will be possible to understand the genetic processes that lead to development of tumors with reduced immunogenicities and identify the molecular targets of the cancer immunoediting process in order to gain insight into how tumor sculpting can be prevented by stabilizing tumor cell genomes. Finally, by elucidating how tumors escape immune detection and elimination, it will be possible to develop methods to determine the extent to which a tumor has been edited and devise molecular strategies to reverse these cloaking mechanisms and thus unmask tumor immunogenicity.

In recent years, there has been a paradigm shift in how cancer is viewed. Rather than emphasizing the differences in the greater than 100 types of cancer, researchers have begun to consider the similarities between these seemingly disparate

malignancies (170). Hanahan &Weinberg have codified this view by proposing that cancer cells must acquire six enabling characteristics in order to form progressively growing tumors (131). Specifically, they must be able to grow autonomously, develop insensitivity to negative growth regulation, evade intrinsic apoptotic signals, display unlimited replicative potential, develop the capacity for angiogenesis, and develop competence for invasive growth and metastasis. In the current review, we have provided strong evidence that supports the existence of the seventh "hallmark of cancer:" the capacity of a malignant cell to evade the extrinsic tumor suppressor functions of the immune system. Moreover, we have discussed the possibility that this seventh hallmark is a result of a cancer immunoediting process, wherein the malignant cell's immunogenic phenotype—forged by its interaction with the host immune system—may determine its fitness for continued survival and growth in an immunocompetent environment. We hope a generalized recognition of this new hallmark of cancer will stimulate new efforts to elucidate the pivotal events of cancer immunoediting so that the long history of thinking on the immune system and cancer will have as its *dénouement* the enhanced understanding and treatment of neoplastic disease.

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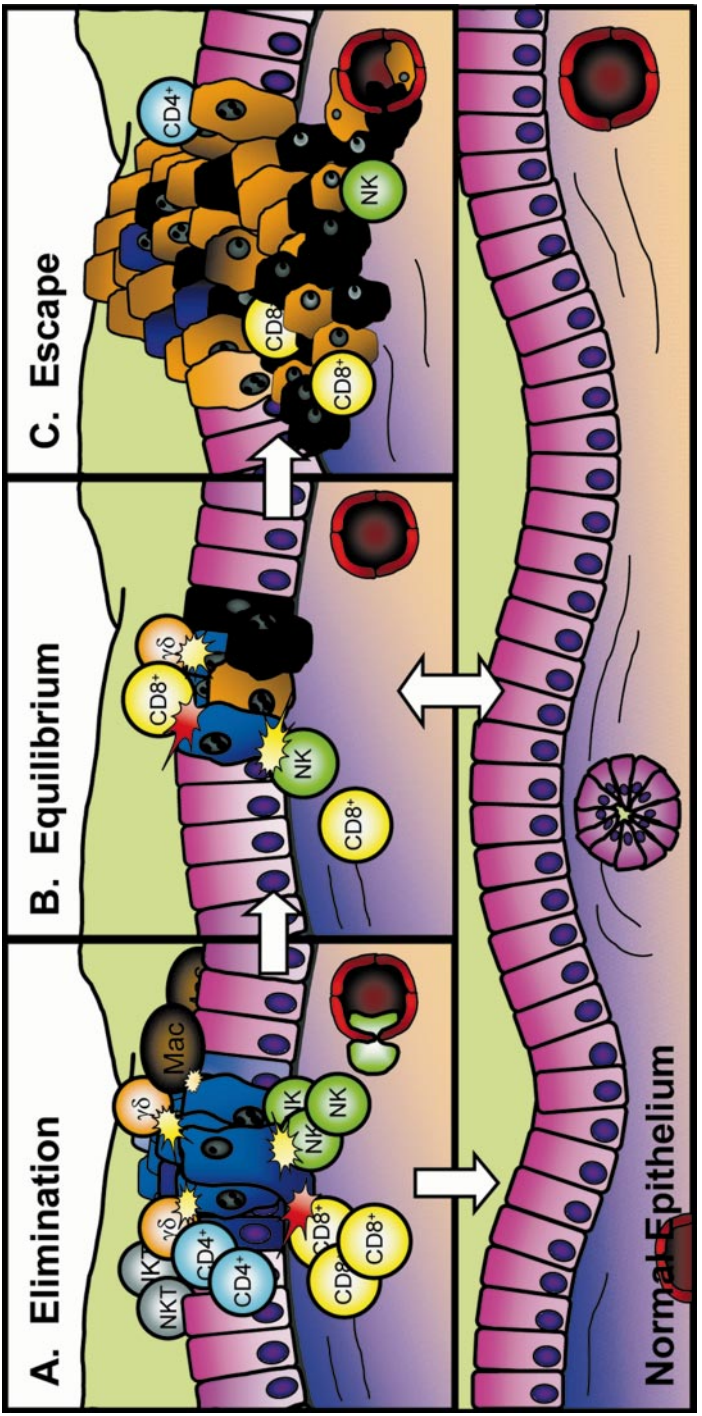

Figure 3 The three Es of cancer immunoediting: elimination, equilibrium, and escape. As indicated by the arrows, the immune system may eliminate the tumor in either the elimination or equilibrium phases, returning the tissue to normal.

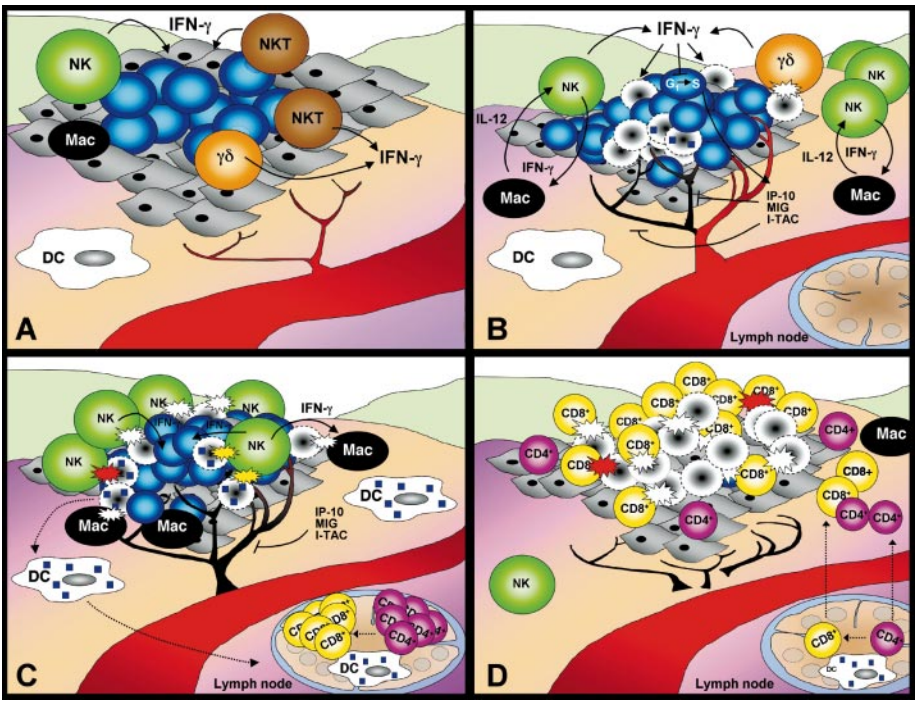

Figure 4 A proposed model for the elimination phase of the cancer immunoediting process. The events underlying this process are described in the text. Tumor cells are in blue; nontransformed cells in gray; lymphocytes, dendritic cells (DC), and macrophages (Mac) are marked and colored appropriately. Dead tumor cells are identified as white to gray gradient circles surrounded by a dashed black line, and tumor antigens are in blue squares. Panel (*A*) represents the initiation of the response, wherein cells of innate immunity recognize the nascent tumor. In panel (*B*), the initial amount of IFN-γ produced starts a cascade of innate immune reactions that result in some tumor cell death by both immunologic and nonimmunologic mechanisms. In panel (*C*), events of innate immunity charge the adaptive response; tumor cells killed due to the increased cytocidal activities of NK cells and activated macrophages are ingested by DCs, which migrate to the draining lymph node and present antigen to naïve $CD4^+$ and $CD8^+$ T cells. In panel (*D*), tumor-specific $CD4^+$ and $CD8^+$ T cells home to the tumor along a chemokine gradient where they recognize and destroy tumor cells expressing distinctive tumor antigens.