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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$^{-/-}$ × STAT1$^{-/-}$ (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)
Jα281$^{-/-}$	Lacks NKT cell subset	↑ MCA-induced sarcomas	(32, 36, 40)
LMP2$^{-/-}$	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)
STAT1$^{-/-}$	IFN-γ-, α/β-insensitive	↑ MCA-induced sarcomas; wider tumor spectrum in STAT1$^{-/-}$ × p53$^{-/-}$	(5, 31)
IFNGR1$^{-/-}$	IFN-γ-insensitive	↑ MCA-induced sarcomas; wider tumor spectrum in IFNGR1$^{-/-}$ × p53$^{-/-}$	(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-12p40$^{-/-}$	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)

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