Cancer Immunology Research

The Second Annual AACR–Cancer Research Institute Lloyd J. Old Award in Cancer Immunology

The second annual American Association for Cancer Research and Cancer Research Institute (AACR–CRI) Lloyd J. Old Award in Cancer Immunology was presented to Dr. Robert D. Schreiber on Wednesday April 9, 2014, at the 105th annual AACR meeting held in San Diego, CA.

Dr. Lloyd J. Old (1933–2011) was the founding scientific and medical director of CRI and an honorary member of the AACR. Dr. Old's singular focus on developing immune-based solutions for cancer, and his collaborative style of leadership for decades, shepherded basic discoveries in tumor immunity from the bench and animal models into clinical research. His efforts in large measure provided the foundation for the current success and excitement in cancer immunology and immunotherapy. It is thus fitting to consider Dr. Old as the father of modern tumor immunology and the original standard-bearer of cancer immunotherapy. The AACR–CRI Lloyd J. Old Award recognizes an active investigator who has conducted outstanding and innovative research in cancer immunology that has had a far-reaching impact on the field. Jill O'Donnell-Tormey, PhD, Chief Executive Officer and Director of Scientific Affairs at CRI, and James P. Allison, PhD, recipient of the inaugural Lloyd J. Old award and a deputy editor of this journal, presented the award to Dr. Schreiber.

Dr. Schreiber is the Alumni Endowed Professor of Pathology and Immunology, Professor of Molecular Microbiology, Director of the Center for Human Immunology and Immunotherapy Programs, and co-leader of the Tumor Immunology Program at the Siteman Cancer Center at the Washington University School of Medicine in Saint Louis (WUSTL), Missouri. Dr. Schreiber is being recognized for his pioneering work that led to our understanding of the roles of the immune system in protecting against tumor outgrowth and in shaping tumor immunogenicity leading to tumor progression.

Dr. Schreiber began his award lecture with a historic summary of the cancer immunosurveillance and cancer immunoediting concepts, which date back to 1909 with the prediction by Nobel laureate Paul Ehrlich that host immunity should protect against cancer by preventing the outgrowth of continuously arising transformed cells (1). In 1951, Sir Peter Medawar, a Nobel laureate and the father of transplantation, performed a series of studies on skin grafting in mammals and defined the components of immunity required for rejecting foreign cells (2). With the development of inbred strains of mice, many investigators were able to study tumor immunity in the following decade (3–6)—including Lloyd Old, namesake of this award. Collectively, this work led to the recognition that tumors express specific antigens that can be recognized by the immune system, which in turn was capable of destroying the transformed cells without affecting the normal tissues from which they were derived. Moreover, tumor immunity could be transferred to isogenic hosts. These observations formed the basis for the cancer immunosurveillance hypothesis proposed by Burnett and Thomas in the late 1950s to early 1960s, positing that host immunity, and specifically T cells, would be able to detect and destroy developing tumor cells almost immediately following their transformation (7, 8). The logical predictions that followed from this hypothesis were that immunodeficient individuals should develop more cancers than those with an intact immune system. However, when these predictions were tested experimentally using nude mice (the only immunodeficient mouse strain that was available at the time), no evidence could be found for an effective cancer immunosurveillance mechanism that affected either spontaneous or carcinogen-induced cancers (9, 10). On the basis of these findings, the cancer immunosurveillance hypothesis was abandoned in the mid-1970s, and the field went on to develop arguments to explain why cancer immunosurveillance could not occur.

However, by the early 1990s, things began to change. By then, it became apparent that nude mice maintained some basal T-cell functions and thus were not ideal models of immunodeficiency. In addition, newer well-defined genetic mouse models of complete immunodeficiency were generated and monoclonal antibodies (mAb) capable of depleting or neutralizing key immune components of innate and adaptive immunity were developed that could induce immunodeficiency in wild-type mice on any genetic background. Moreover, an explosive growth had taken place in the understanding of the composition and function of the immune system such that the roles of immunity in promoting both host defense as well as immunopathologic processes were better understood (11). These events thus set the backdrop for Schreiber's entrance into the field of tumor immunology in the early 1990s.

At that time, Dr. Schreiber was a well-known cytokine biologist particularly recognized for his work on the cytokine interferon gamma (IFNγ). In the course of this work, Schreiber

doi: 10.1158/2326-6066.CIR-14-0094

and colleagues had developed a panel of mAbs against various cytokines and their receptors that were capable of ablating the function of these cytokines *in vivo*. He also had generated several lines of mice with selective defects in cytokine-receptor signaling. Old, on the other hand, had generated a chemically induced mouse sarcoma cell line (Meth A) and had shown that, while it could form progressively growing tumors in syngeneic wild-type mice, it was rejected in a T cell–dependent manner when the mice were stimulated by bacterial lipopolysaccharide (LPS; endotoxin). Although Old had shown that the LPS-induced rejection of Meth A was in part due to elaboration of a cytokine he called TNFα, he was certain that additional cytokines played a role in the rejection process. In 1990, Old (in New York) and Schreiber (in St. Louis) established a collaboration to explore this issue. In short order, the two showed that (i) mAb-dependent blockade of IFNγ inhibited LPS-induced Meth A rejection *in vivo* and ablated the induction of Meth A-specific T cells (ii) that IFNγ responsiveness was required at both the level of the host and the level of the tumor in order for rejection to occur; and (iii) that Meth A tumor cells, rendered insensitive to IFNγ through enforced expression of a truncated, dominant negative form of IFNGR1, a subunit of the IFNγ receptor, were no longer immunogenic (12).

In subsequent years, this collaboration produced a set of key findings that reinvigorated interest in the concept of cancer immunosurveillance. First, Schreiber and colleagues showed that, compared with wild-type mice, gene-targeted mice lacking either the IFNγ receptor or its major signal transduction protein, STAT1, were ten to twenty times less sensitive to primary tumor induction by the chemical carcinogen 3′-methylcholanthrene (MCA; ref. 13). In addition, they showed that mice lacking both the IFNγ receptor and the tumor suppressor p53 developed a wider range of tumor types compared with mice that lacked p53 alone. Second, they showed that immunodeficient $Rag2^{-/-}$ mice, lacking T, B, and NKT cells, were also more sensitive to MCA-induced tumorigenesis compared with immunocompetent wild-type mice (14). Together, these studies provided unequivocal evidence that IFNγ and lymphocytes cooperated to form a host-protective mechanism that prevented outgrowth of transplanted and primary developing tumors *in vivo*. Thus, cancer immunosurveillance indeed occurred, at least for certain types of tumors.

However, this was not the whole story. Schreiber and Old went on to show that MCA-induced tumors from immunodeficient mice were more immunogenic than those from immunocompetent mice (14). Thus, immunity not only protected against tumor outgrowth but also sculpted or shaped the immunogenicity of tumors formed in immunologically intact hosts, rendering them more fit to grow in an immunocompetent host. This work thus demonstrated that tumors are imprinted by the immunologic environment in which they form, and this imprinting determines the ultimate fate of a developing tumor.

These and other observations made by Schreiber and colleagues led to the evolution of the cancer immunosurveillance hypothesis into the cancer immunoediting hypothesis, which emphasizes the dual host-protective and tumor-sculpting/promoting functions of immunity (11, 14, 15). Cancer immunoediting was thus codified as an extrinsic tumor-suppressive mechanism that engages only after cellular transformation has occurred and intrinsic tumor suppressors, such as p53 and the retinoblastoma protein (Rb) have failed (16). In its most complex form, cancer immunoediting is envisaged to occur in three phases: *Elimination*—the phase most similar to cancer immunosurveillance in which innate and adaptive immunity work together to detect and destroy a developing tumor before it becomes clinically apparent; *Equilibrium*—the phase in which occasional tumor cells that might survive elimination are held in a state of immune-mediated dormancy and undergo editing; and *Escape*—the phase in which edited tumor cells with reduced immunogenicity begin to grow progressively, establish an immunosuppressive tumor microenvironment, and emerge as clinically apparent tumors that are the manifestation of the disease we know as cancer, as depicted in Fig. 1, a slide from Dr. Schreiber's lecture (16).

Although strong experimental data had been obtained in Dr. Schreiber's laboratory to support the elimination and escape phases, the proposal of the equilibrium phase was based purely on clinical literature. In 2007, Dr. Schreiber initiated a collaboration with Mark Smyth in Australia that led to the experimental demonstration of the equilibrium phase (17). In these experiments, large cohorts of wild-type mice were treated with low doses of MCA such that only about 20% of the group developed tumors during the 200-day observation period. Mice with progressively growing tumors were removed from the experiment, and the remaining mice were then divided into two groups. Half were treated with control mAb, whereas the other half were rendered highly immunodeficient by treatment with mAbs that depleted $CD4^+$ and $CD8^+$ T cells and blocked the activity of IFNγ. Tumor outgrowth in the two groups was subsequently assessed over the next 100 days. Mice exposed to low-dose MCA and then treated with control mAb did not develop late-forming tumors. In contrast, approximately half the mice treated with low-dose MCA and then immunodepleted displayed tumor outgrowth at the site of the original MCA injection. Additional studies showed that editing of tumor immunogenicity occurred during the equilibrium phase. Finally, equilibrium was shown to be a function only of adaptive immunity, as tumor outgrowth was observed only following depletion of $CD4^+$ and/or $CD8^+$ T cells, IFNγ, or IL12, but not of NK cells, TNFα, type I IFN, or TRAIL (17). These findings clearly distinguished equilibrium from elimination, which required components of both innate and adaptive immunity.

After presenting the historical overview of the development of the cancer immunoediting concept, Dr. Schreiber described the findings from a recently published study from his laboratory showing that tumor-specific mutant antigens are targets of cancer immunoediting (18). This study was one of the first to use next-generation exome sequencing and epitope prediction algorithms to identify tumor-specific mutant proteins expressed in unedited tumor cells from MCA-treated immunodeficient $RAG2^{-/-}$ mice that are responsible for the high immunogenicity of these tumors. They showed that a subset of these mutations form strong neoepitopes for MHC class I–restricted $CD8^+$ T cells, which then eliminate tumor cells expressing these strong antigens, leaving behind less immunogenic tumor-cell variants that do not express strong

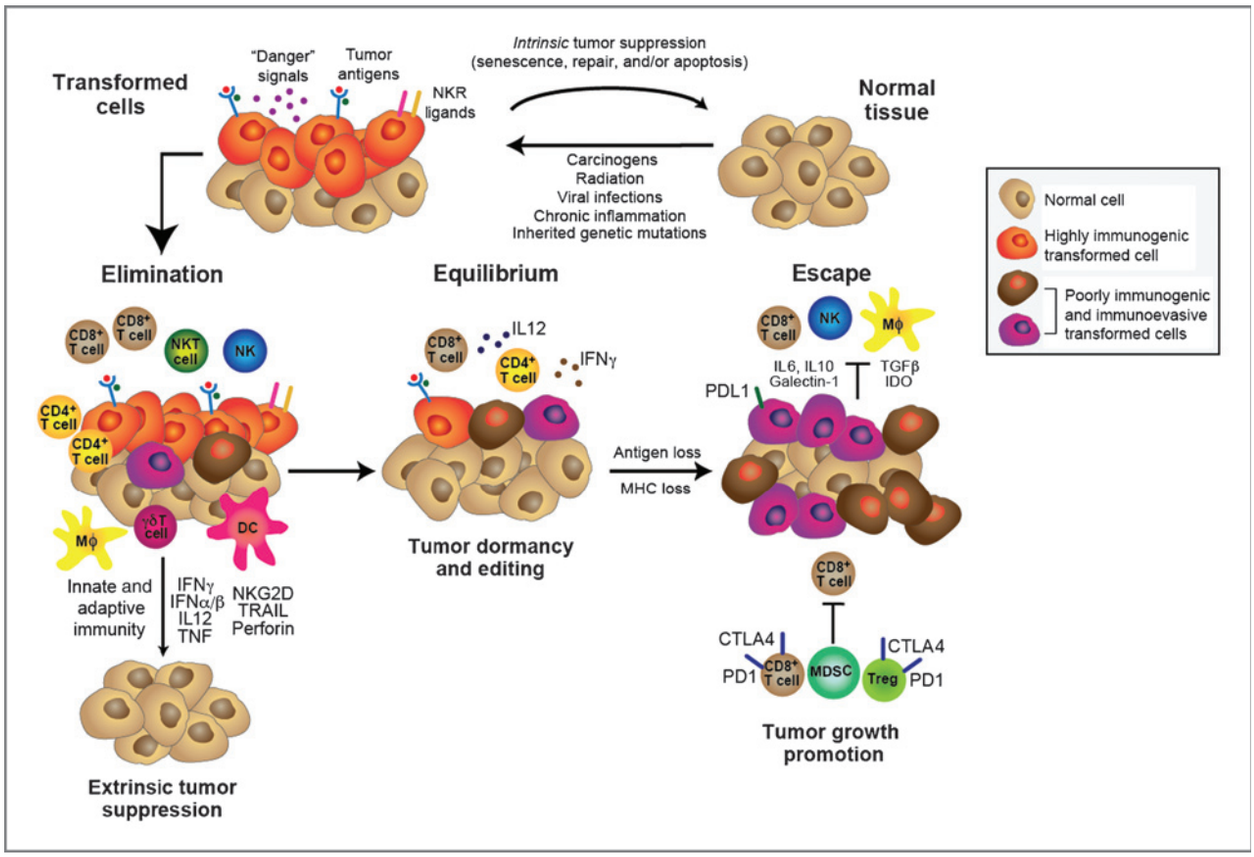

Figure 1. This schematic diagram illustrates the three phases (3 Es) of cancer immunoediting as proposed by Schreiber and colleagues: *Elimination*, *Equilibrium*, and *Escape*. Transformed cells arise from normal tissue as a consequence of either spontaneous or carcinogen-induced mutations. Various intrinsic tumor-suppression mechanisms exist to detect and eliminate these mutant/abnormal cells. When intrinsic tumor suppression fails, the three phases of cancer immunoediting commence. In the elimination phase, transformed cells are detected and killed by innate and adaptive immunity, termed extrinsic tumor suppression. If sporadic transformed cells survive the elimination phase, they can still be held in a state of immune-mediated dormancy in the equilibrium phase. Dormant transformed cells can undergo immunoediting, which leads to loss of immunogenicity, and thus they enter the escape phase, in which they begin to grow progressively, establish an immunosuppressive tumor microenvironment, and give rise to clinically apparent tumors. Adapted from Schreiber et al. (16), with permission from the American Association for the Advancement of Science.

tumor-specific mutant antigens. This work thus identified tumor-specific mutant antigens as targets of cancer immunoediting and demonstrated that immunoediting occurred via a T cell–dependent immunoselection mechanism. These findings also suggested that a genomics/bioinformatics approach might be used to develop personalized cancer immunotherapies. Schreiber pointed out that a similar conclusion was reached independently by another group using a genetic model of sarcoma formation involving *in vivo* induction of a mutant form of KRAS on a p53 knockout genetic background (19). Interestingly, in this latter model, editing was also found to occur via epigenetic silencing of mutant genes.

Dr. Schreiber ended his lecture by discussing ongoing work from his laboratory using his immunogenomic approach to identify the targets and mechanisms underlying immune checkpoint blockade cancer immunotherapy for rejection of progressively growing clinically apparent tumors. He showed that exome analysis and epitope prediction can indeed be used to identify less immunogenic tumor-specific mutant antigens expressed in clinically apparent, immunoedited tumors that are targets of T cells activated by treatment of tumor-bearing mice with either anti-PD-1 and/or anti-CTLA-4 antibodies. He showed that vaccines targeting these tumor-specific mutant antigens can be used to replace checkpoint blockade therapy and thus may be safe and effective cancer treatments when used alone or in combination with shared antigen vaccines and/or other forms of cancer immunotherapies. Dr. Schreiber ended his presentation by suggesting that the time is right to use the immunogenomic approach to treat human patients with cancer.

Dr. Schreiber received his BA in chemistry and PhD in biochemistry from the State University of New York at Buffalo. He completed postdoctoral training at the Scripps Institute in La Jolla, CA, and served on the faculty there before joining WUSTL in 1985 as professor of pathology and accepting the challenging post as director of the graduate program in immunology in 1993. In addition to building a superb graduate program, Dr. Schreiber also focused on elucidating the biochemistry and molecular cell biology of cytokines and defining the role they play in promoting immune responses to cancer. He was the first to demonstrate that IFNγ was the cytokine that

activated mouse macrophage antitumor and antimicrobial activities, and he pioneered the *in vivo* use of monoclonal antibodies to define the physiologic roles of cytokines in promoting host responses to tumors and infectious agents. He was also one of the first to elucidate the structure and function of the IFNγ receptor and went on to establish the physiologic relevance of IFNγ receptor–dependent signaling by generating genetically engineered mice lacking specific components of this pathway. Using IFNγ-unresponsive and immunodeficient gene-targeted mice, Schreiber began his pioneering work on cancer immunoediting, described above, which also began his long and productive collaboration with Dr. Old.

Dr. Schreiber has authored more than 300 peer-reviewed and invited publications and has received many honors and recognitions, including the Milstein Award from the International Society for Interferon and Cytokine Research (1996), the Marie T. Bonazinga Award for Excellence in Leukocyte Biology Research (1998), the CRI William B. Coley Award for Distinguished Research in Basic and Tumor Immunology (2001), the Charles Rodolphe Brupbacher Prize for Cancer Research (2007), the Carl and Gerty Cori Faculty Achievement Award (2008), and a Distinguished Investigator Award (2008) from Washington University. Dr. Schreiber is a Fellow of the American Association for the Advancement of Science (1996) and an elected member of the American Academy of Arts and Sciences (2010) and the U.S. National Academy of Sciences (2013).

– *Connie Gee*
AACR, Philadelphia, PA

Received May 14, 2014; published online July 2, 2014.

References

1. Ehrlich P. [Ueber den jetzigen Stand der Karzinomforschung.] Ned-TijdschrGeneeskd 1909;5(Pt. 1):273–90.
2. Medawar P. The behaviour and fate of skin autografts and skin homografts in rabbits. J Anat 1944;78:176–99.
3. Foley EJ. Antigenic properties of methylcholanthrene-induced tumors in mice of the strain of origin. Cancer Res 1953;13:835–7.
4. Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. J Natl Cancer Inst 1957;18:769–78.
5. Klein G. Tumor antigens. Annu Rev Microbiol 1966;20:223–52.
6. Old LJ, Boyse EA. Immunology of experimental tumors. Ann Rev Med 1964;15:167–86.
7. Burnet FM. Cancer–a biological approach. Br Med J 1957;1: 841–7.
8. Thomas L. On immunosurveillance in human cancer. Yale J Biol Med 1982;55:329–33.
9. Stutman O. Tumor development after 3-methylcholanthrene in immunologically deficient athymic-nude mice. Science 1974;183:534–6.
10. Stutman O. Spontaneous, viral, and chemically induced tumors in the nude mouse. In: Fogh J, Giovanella BC, editors. The nude mouse in experimental and clinical research. New York: Academic Press; 1978. p. 411–35.
11. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002;3:991–8.
12. Dighe AS, Richards E, Old LJ, Schreiber RD. Enhanced *in vivo* growth and resistance to rejection of tumor cells expressing dominant negative IFNg receptors. Immunity 1994;1:447–56.
13. Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ, et al. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci U S A 1998; 95:7556–61.
14. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 2001;410:1107–11.
15. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol 2004;22:329–60.
16. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 2011;331:1565–70.
17. Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature 2007;450:903–7.
18. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 2012;482:400–4.
19. DuPage M, Mazumdar C, Schmidt LM, Cheung AF, Jacks T. Expression of tumour-specific antigens underlies cancer immunoediting. Nature 2012;482:405–9.

Cancer Immunology Research

The Second Annual AACR–Cancer Research Institute Lloyd J. Old Award in Cancer Immunology

Cancer Immunol Res 2014;2:606-609.