Human Tumor Antigens Yesterday, Today, and Tomorrow

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Abstract

The question of whether human tumors express antigens that can be recognized by the immune system has been answered with a resounding YES. Most were identified through spontaneous antitumor humoral and cellular immune responses found in cancer patients and include peptides, glycopeptides, phospho-peptides, viral peptides, and peptides resulting from common mutations in oncogenes and tumor-suppressor genes, or common gene fusion events. Many have been extensively tested as candidates for anticancer vaccines. More recently, attention has been focused on the potentially large number of unique tumor antigens, mutated neoantigens, that are the predicted products of the numerous mutations revealed by exome sequencing of primary tumors. Only a few have been confirmed as targets of spontaneous immunity and immunosurveillance, and even fewer have been tested in preclinical and clinical settings. The field has been divided for a long time on the relative importance of shared versus mutated antigens in tumor surveillance and as candidates for vaccines. This question will eventually need to be answered in a head to head comparison in well-designed clinical trials. One advantage that shared antigens have over mutated antigens is their potential to be used in vaccines for primary cancer prevention. *Cancer Immunol Res; 5(5); 347–54. ©2017 AACR.*

Introduction

As this review was in preparation, the field of tumor antigens lost its two pioneers, Dr. Richmond T. Prehn who died on November 7, 2016, at the age of 93, and Dr. George Klein, who died on December 10, 2016, at the age of 91. Both scientists started their illustrious careers at the time when the immune system was not considered in any way involved in protecting us from tumors, which were assumed to be part of one's self. That thinking changed in 1957 when Dr. Prehn, often referred to as the father of modern tumor immunology, and his coworker and collaborator, Dr. Joan Main, published an article describing the induction of an immune response against carcinogen-induced sarcomas in mice that protected them from a future challenge with the same tumor but not with many other similarly generated sarcomas (1). This work clearly showed that tumors harbored one or more specific antigens capable of eliciting immunity and long-term immune memory. In 1960, Dr. Klein with his life-long collaborator, Dr. Eva Klein, and two other giants of tumor immunology, Drs. Hans Oloff Sjogren and Karl Erik Hellstrom, reported the induction of immunity in primary autochthonous hosts whose tumors had been surgically removed and who were then resistant to a challenge with the same tumor cells (2). These authors further determined that the resistance resided in the lymph nodes and that serum had no effect, suggesting a cellular immune mechanism at play.

These studies stimulated a large tumor antigen discovery effort that to this day has not shown signs of abating. The work has been carried out both as proof-of-principle *in vivo* in mice and *in vitro* with human immune cells and with sera from tumor-bearing patients. One of the first approaches to the identification of human tumor-specific antigens was to immunize mice with human tumor cells and use the new hybridoma technology (3) to immortalize B cells and find the rare clones producing monoclonal antibodies that recognized tumors but not normal cells of the same tissue type. These antibodies were then used for purification of their cognate antigens and for characterization of their expression in tumors versus normal tissues. Although these studies identified many antigens that mouse antibodies recognized preferentially or uniquely on human tumor cells and not on normal cells, much doubt and skepticism remained about these same molecules being recognized as tumor antigens by the human immune system. Most of them were nonmutated self-proteins. This skepticism was eventually defeated when antibodies to some of the same antigens were found in sera of cancer patients. In addition, advances in basic immunology that provided the new understanding of antigen processing and presentation by dendritic cells (DC) to T cells, combined with the ability to grow both T cells and DC *in vitro*, enabled experiments that showed that many of these tumor antigens were recognized by human T cells. Advances in molecular biology and gene-transfer techniques allowed expression of individual tumor genes in target cells and isolation of those recognized by tumor-specific T cells, leading to cloning of genes encoding tumor antigens. Tumor gene expression libraries could be made in bacteria and individual colonies examined for reactivity with sera from cancer patients but not healthy controls.

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Yesterday: The Excitement of Discovery

For the newest generation of tumor immunologists entering this field in ever increasing numbers, it might be hard to imagine what it was like to study immune system–tumor cell interactions without being able to reduce them to antigen-specific interactions. For many years, experiments were performed in mice with highly

foreign model antigens such as ovalbumin (OVA) transfected into tumor cells or viral antigens such as SV-40 expressed by tumor cells. Although some basic principles could be gleaned from these experiments, it was hard to predict how translatable those would be to immunity against self-derived tumor antigens. The field needed its equivalent of the Periodic Table of Elements in order to move the work to a higher level of understanding. An equivalent Table of Tumor Antigens began to be populated in the late eighties and early nineties. Based on fundamental immunological principles that antigen priming takes place in the lymph node, lymph nodes draining tumor site were the first to be interrogated for the presence of T cells with antitumor reactivity. Progress depended on being able to grow human T cells *in vitro* and, in parallel, establishing human tumor cell lines as T-cell targets and as renewable sources of tumor antigens. Because the recognition of tumor antigens by T cells was expected to be HLA-restricted, a large number of tumor cell lines was needed to cover some of the most common HLAs. These requirements were challenging. Nevertheless, with the very first few tumor cell lines established from melanomas, breast, colon, and pancreas cancers and several T-cell lymphomas, the field moved forward.

In 1989, the identification of a putative human tumor antigen recognized by T cells, an epithelial transmembrane glycoprotein in the mucin family, was published (4). It was identified by the ability of a human T-cell line that had been expanded from the draining lymph nodes of a pancreatic tumor to specifically recognize pancreatic tumor cell lines. This antigen had been previously identified by DUPAN-2, a mouse monoclonal antibody to human tumors (5). The same mucin had also been isolated from human milk and named human milk fat globule (HMFG). Interestingly, many mouse monoclonal antibodies specific for HMFG recognized its expression on normal breast epithelia but failed to recognize it on epithelial breast cancer cells (6, 7). Further studies showed that on normal epithelia, this mucin was highly O-glycosylated and expressed at low levels on the apical surfaces of epithelial ducts facing the lumen, whereas on tumors its location was non-polarized, and it was highly overexpressed and severely under-glycosylated. This abnormal expression was later found on all human adenocarcinomas as well as on multiple myelomas and some B-cell lymphomas. Two groups simultaneously cloned the gene for this antigen (8–10) and being that it was the first gene to be cloned of a multi-member mucin family gave it a designation *muc1* and named its protein product MUC1. Transfection of the MUC1 cDNA into MUC1⁻ cells transformed those cells into T-cell targets and allowed identification of specific peptide epitopes and HLA-restriction elements (11–13). It also allowed studies into biochemical and structural modifications (14, 15) that classified MUC1 as a tumor-specific antigen.

In 1991, a gene was cloned from a melanoma cell line that encoded an antigen recognized by HLA-A1–restricted cytotoxic T-cell (CTL) clones specific for a particular melanoma cell line (16). The cell line was cloned, and clones were divided into those killed by the CTL and others not recognized by the CTL. The assumption was that the lack of recognition was due to the loss of the gene encoding the antigen. Cosmid libraries were prepared from DNA of tumor cells that expressed the antigen and transfected into cells negative for the antigen. Transfected clones that were newly recognized by the CTL were considered carriers of the gene encoding the antigen, which was then isolated and sequenced. The encoded antigen, MAGEA1 (melanoma antigen family A, 1),

was expressed in other melanoma cell lines and tumors and also in many human tumors of other histologies, but not in any normal tissues except for testis and trophoblasts. Because neither of these cell types expresses HLA-class I, they could not present MAGEA1 peptides to the immune system, and thus the peptides were recognized as tumor-specific antigens in HLA molecules on tumor cells. MAGEA1 turned out to be the first member of a larger family of molecules with the same pattern of expression, only in tumors and in germ cells, which was called the cancer testis (CT) or cancer-germline antigen family.

Many other melanoma antigens recognized by CTL were identified in quick succession, but unlike the CT antigens, many of these were also expressed on normal melanocytes and were thus given the designation "tissue specific" or "differentiation antigens." Tumors of other histologies were also preferentially recognized by T cells via their own tissue-specific/differentiation antigens. Transgenic mouse models for a number of antigens, with few exceptions, show that inducing immunity against them can lead to tumor rejection without causing autoimmunity against normal tissues expressing those same antigens. Many are expressed at higher levels on tumors than on normal cells, which may be an important mechanism of their recognition as specific tumor targets. Other mechanisms can protect normal tissues from autoimmune damage, but until this is better understood, this category of tumor antigens needs to be approached with more caution when considered as targets for cancer immunotherapy.

Development of genetic approaches, combined with the availability of tumor-specific cell lines and clones, led to the identification of molecules in cancer cells that were a source of T-cell–recognized peptides. A new technology emerged that could identify tumor-specific peptides directly without having to first identify the source protein (17). This involved elution of all peptides from tumor HLA class I molecules, separating them by tandem mass spectrometry and identifying those that are recognized by CTL. Because HLA-A2 is the most frequent HLA class I molecule in Caucasian populations, the initial approach was to elute peptides from purified HLA-A2 molecules from various tumors and present them to CTL clones by HLA-A2⁺ target cells. This technique quickly yielded a melanoma-specific tumor peptide expressed on many melanoma cells lines (18) and many other tumor-specific peptides over the years.

One set of tumor peptides identified using this technology was derived from cyclin B1 (19). CTL, helper T cells, and antibodies specific for nonmutated cyclin B1 can be found in patients with multiple tumor types, both liquid and solid (20, 21), raising a question of why a cell-cycle regulatory molecule, expressed in all dividing cells at the G_2 to M transition in the cell cycle, is seen as a tumor antigen. This became clear looking at the difference of expression of this molecule between normal proliferating cells and tumor cells. In normal cells, cyclin B1 is detected in the nucleus at one particular cell-cycle checkpoint. Once the cells go through that checkpoint, it is exported to the cytoplasm and quickly degraded. This limited expression leads to very few cyclin B1–derived peptides bound to HLA on the normal cell surface. In contrast, large amounts of cyclin B1 in tumors are expressed constitutively in the cytoplasm and degraded at high rates, providing numerous cyclin B1 peptides to bind to, and be presented by, HLA class I to T cells. Dying tumor cells release high concentrations of cyclin B1, which floods regional lymph nodes. Cyclin B1 is then presented to helper T cells and B cells, leading

to the generation of cyclin B1–specific antibodies. Many other cell-cycle regulatory proteins have been reported as tumor antigens, due to similar patterns of abnormal expression in tumors versus normal proliferating cells (21).

Most of the work in the early years of antigen discovery was directed to the identification of CTL targets, under the assumption that CTL would be the major antitumor effector cells. The problem with this assumption was that it was soon determined that CTL needed help from CD4$^+$ helper T cells to proliferate and survive and thus activation of CTL needed to be accompanied by simultaneous activation of CD4$^+$ helper T cells. One way to bypass that requirement in vaccines based on CTL peptides was to include a ready-made heterologous helper epitope not of tumor origin, known to bind HLA class II. Such epitopes were identified and explored, with special emphasis on peptides that would be promiscuous and bind a wide variety of HLA class II antigens (22). Although such heterologous help could improve priming of an antitumor immune response, the helper T-cell memory generated was against an antigen that would not be present on recurring tumor, and thus T-cell help would not be activated to help the CTL. It therefore became important to identify tumor targets of cancer-specific CD4$^+$ T cells. The presence of antitumor antibodies in cancer patients, especially of isotypes that require cognate T-cell help, foreshadowed the existence of tumor-specific helper T cells. The very first human cancer antigens targeted by CD4$^+$ T cells were reported in 1999 (23) and many others quickly fallowed, prompting further studies into the importance of T-cell help in antitumor immunity (24).

Even though a number of tumor-specific peptides recognized by T cells were derived from cell surface molecules, exploring humoral immunity and using cancer patients' antibodies to identify tumor antigens lagged behind. This changed when a new technique was published in 1997 that stimulated many studies in multiple laboratories to identify tumor targets of human antibodies. It was named serological analysis (SER) of autologous tumor antigens by recombinant cDNA expression (EX) cloning—SEREX (25). The technique involved constructing a cDNA library from fresh tumor specimens, cloning into λ phage expression vectors, and transfecting E. coli with the recombinant phages. During the lytic cycle, Escherichia coli (E. coli) clones express the human proteins, which are transferred to nitrocellulose and incubated with the patient's serum. Reactive plaques can be identified and phage inserts sequenced for protein identification. This technique quickly led to the identification of numerous antigens, including quite a few that had already been identified previously as T-cell targets, as well as some new and unexpected ones (26). The shortcoming of this approach was that E. coli could only express unglycosylated epitopes, whereas many antibodies could be expected against tumor glycans or glycopeptides. Many different versions of this technique have since been developed and used to reveal the extensive cancer immunome recognized by spontaneously elicited antibodies in tumor-bearing patients. Improvement in technology allowed detection of posttranslationally modified antigens, the most frequent being differentially glycosylated proteins yielding tumor-specific glycopeptides or glycans, which were then intensively studied as antibody targets on cancer cells (27, 28). Tumor antigen–specific antibodies have been developed as effective therapeutics, either as naked antibodies or conjugated to drugs or radio-

isotopes, to directly kill tumor cells or inhibit their proliferation (29). They have also been used to target T cells to tumor cells either as bispecific antibodies (30) or as chimeric receptors (CAR) on T cells (31).

The all-out effort using the newest cellular and molecular techniques as they were being developed in immunology and other disciplines resulted in the discovery and characterization of several hundred molecules of different types that served as tumor targets for human CD8$^+$ CTL, CD4$^+$ helper T cells, and antibodies. These potential targets have been organized into several categories (32). The cancer-testis antigens category includes some of the oldest and best characterized families of MAGE, GAGE, and BAGE antigens (originally identified on melanomas but also expressed on many other cancers) and NY-ESO-1, also widely expressed on many tumors. The differentiation antigens category is another large group of molecules that includes, among many others, the well-known CEA, gp100, mammoglobin A, melan A/MART-1, PSA, and tyrosinase. The largest group are the overexpressed antigens that include HER-2/neu, hTERT, MUC1, mesothelin, PSA, PSMA, survivin, WT-1, p53, cyclin B1, and many others. Numerous HLA class I– and class II–restricted epitopes have been identified in each of these molecules, and most of them are also recognized by patients' antibodies. Those that are also posttranslationally modified provide tumor-specific epitopes, including tumor glycopeptides (33), phosphopeptides (34, 35), and citrullinated peptides (36). Fewer in number but nevertheless important as confirmed targets of T-cell immunity are the mutated antigens, representing epitopes derived from the protein products of mutated oncogenes such as KRAS, and NRAS, and new epitopes created by known gene translocations and fusions, such as BCR-ABL in chronic myelogenous leukemia, ETV6/AML in acute lymphoblastic leukemia, NPM/ALK in anaplastic large-cell lymphomas, and ALK in neuroblastomas.

Today: Human Tumor Antigens in Therapeutic Cancer Vaccines

Identification of the first few tumor antigens was exciting because it supported a hopeful hypothesis that specifically stimulating the immune system against molecules on tumor cells could awaken and direct its destructive forces to kill the tumor. Unlike in infectious diseases that progress fast and cannot be controlled by post-exposure vaccines, immunologists dared to propose to test such vaccines in tumors that grow slowly and are often removed, providing a "window of opportunity" for a vaccine to elicit a response that might keep tumors from recurring (37). Many antigens were promptly made into vaccines of different formulations, including peptides plus adjuvants vaccines, DNA or RNA vaccines, viral or bacterial vectors carrying sequences encoding antigens, or loaded into DCs. The vaccines were tested in the best available animal models at that time and rushed to the clinic.

These efforts were met with tremendous challenges that did not dim the enthusiasm of cancer immunologists determined to use the best of their science to develop nontoxic, durable, and cost-effective therapy for cancer. One of the biggest hurdles was the requirement that patients receiving a cancer vaccine had to have first failed standard therapy, which in many instances meant multiple toxic treatments after each recurrence of the tumor, until the last recurrence for which no standard therapy was effective. By the time patients were eligible for vaccine therapy, they had

run-away metastatic disease and an immune system severely damaged by previous treatments. It was clear to everyone that a vaccine in this setting was unlikely to have a therapeutic effect. Indeed, the early trials of many such vaccines with many different antigens were done primarily as phase I trials, determining to the extent possible the safety of the vaccines and looking for signals of the vaccine's immunogenicity. The few patients that responded in each trial provided invaluable samples from which data could be derived to inform future trials. Especially valuable were those patients whose response to the vaccine correlated with some clinical improvement.

Although the various antigens and vaccine formulations showed some differences, the consensus was that being able to elicit a multiclonal and a multifunctional T-cell response was going to be important for better vaccine efficacy. DCs had already been established as champion stimulators of T cells, and the new methodology to grow human DCs in culture created an opportunity to deliver tumor antigens via DCs to lymph nodes, where the best immune response would be generated. This breathed a new life into the cancer vaccine field, and hundreds of DC trials were undertaken in many different cancers (38). The expectation that the best immune responses would be achieved with DC-based vaccines was borne out. However, with only a few exceptions, the clinical outcome did not change significantly. One of the important lessons learned was that even the most immunogenic vaccines would have only a limited success because cancer patients are severely immunocompromised by the presence of the tumor. The existence of "exhausted" T cells (39), unable to properly respond to antigen stimulation, was revealed, and increased attention began to be paid to the tumor microenvironment where numerous immunosuppressive cells and soluble factors were discovered, all capable of diminishing vaccine efficacy (40, 41).

Once again in the history of therapeutic cancer vaccines, these results did not discourage or dissuade cancer immunologists. On the contrary, armed with this new knowledge, they have gone back to the drawing board to design vaccines better capable of dealing with the tumor microenvironment. The best example of this effort is the "The Human Vaccine Project," an international not-for-profit public–private partnership with the mission to better understand human antitumor immune responses in the context of the new knowledge about the tumor microenvironment, in order to develop clinically effective cancer vaccines (42). Everything is being re-examined: tumor antigens, antigen prioritization, antigen delivery, requirements for long-term T-cell memory, and the immunocompetence of cancer patients.

The renewed interest in searching for tumor antigens likely to induce clinically significant responses is due to observations that melanoma and lung cancer that patients on checkpoint blockade immunotherapy (43) respond better if their tumors have a large number of mutations identified by exome sequencing (44, 45). The hypothesis that is becoming popular is that the more mutations the tumor has, the more "foreign" antigens are seen on that tumor by T cells, which when released from suppression in the tumor microenvironment by checkpoint inhibition immunotherapy contribute to a good clinical response. The logical outcome, if that hypothesis is shown to be correct, would be to treat each patient with his/her own "personalized" vaccine based on the mutated neoantigens previously confirmed to be immunogenic. These are early days, and there is still very little evidence in human

cancer to support the hypothesis that unique mutated neoantigens would be better vaccine candidates than the hundreds of nonmutated tumor antigens that are widely expressed on tumors and against which T cells and antibodies are readily found in cancer patients. Equally foreign to the immune system are viral antigens expressed in virally caused tumors, such as the human papillomavirus (HPV)–derived antigens expressed by cervical and head and neck cancers. Therapeutic vaccines based on HPV E6 and E7 peptides have had the same marginal immunogenicity and clinical efficacy in patients with cervical cancer as the many self-derived shared antigen vaccines in nonvirally induced cancers. They have shown efficacy only in primary prevention and in premalignant lesions (46). The head-to-head comparison has never been done and should eventually be done in clinical trials. A vaccine trial could be designed in HPV$^+$ cervical or head and neck cancer to compare the immunogenicity and efficacy of HPV peptide vaccines with vaccines based on personal mutated neoepitopes. A third arm would be important to add to such a trial—a vaccine based on one of several shared tumor antigens known to be expressed in these tumors. One could also envision a trial in breast cancer where patients are randomized to receive either a vaccine based on one of the well-known shared breast cancer antigens, such as Her-2neu or MUC1, or a vaccine based on a mutated neoantigen found in individual patient's tumor. If vaccines based on mutated neoantigens show unprecedented efficacy in the clinic, a huge responsibility will fall on cancer immunologists to make these personalized vaccines available to all cancer patients. Cancer vaccines have faced many challenges in the last 25 years, but none will have been as daunting as coming up with a plan for a wide distribution of this potentially curative, but very labor-intensive—and expected to be a very expensive—therapy.

Tomorrow—Human Tumor Antigens for Primary Cancer Prevention

The recent focus on mutated neoantigens and renewed efforts to optimize therapeutic vaccines, so that they could be used in combination with other immunomodulators to enhance their efficacy, has ignored the more suitable role for cancer vaccines—primary cancer prevention (47, 48). The best candidate antigens for designing such vaccines would be the shared tumor antigens and the few mutated antigens derived from highly predictive mutations, such as in *KRAS* (49), or from fusion proteins encoded by predicted gene translocation events, such as *BCR-ABL* in CLL.

There are many outdated reasons why such vaccines have not been more actively pursued. The biggest one is the firmly entrenched idea that shared tumor antigens are all self-antigens under strong immune tolerance and that eliciting immunity against any of them would break that tolerance and cause dangerous autoimmunity. This idea has persisted in spite of very little evidence to support it. Most animal models that have propelled many of these antigens into therapeutic clinical trials have been models of cancer prevention, administration of a vaccine prior to tumor challenge. Autoimmunity has been seen primarily with melanoma antigens. Otherwise, vaccines based on the long list of shared antigens, used either as whole molecules or specific epitopes, have been shown over and over again to elicit tumor-rejection immunity without causing autoimmunity.

The second entrenched idea, in spite of lack of evidence, is that high-affinity T cells and antibodies specific for self-derived tumor antigens would have been deleted as part of self-tolerance, and thus immune responses against them would be weak and ineffective. This had not been explored in humans until it was finally recently done for the shared tumor antigen MUC1. This molecule is abnormally expressed (overexpressed and hypoglycosylated) on all human adenocarcinomas but also on all of their premalignant lesions. After many therapeutic vaccine trials showing marginal immunogenicity and efficacy (50–52), patients at high risk for MUC1$^+$ colon cancer after the removal of MUC1$^+$-advanced premalignant colonic adenomas were vaccinated with the MUC1 peptide plus adjuvant vaccine to test its safety and immunogenicity (53). Of 41 vaccinated individuals, 47% responded to the vaccine with the production of high titer IgG antibodies and a strong immune memory that could be significantly boosted at 1 year. When MUC1-specific antibodies present in a vaccine responder after the 1-year booster were cloned and their individual affinities measured, the entire range of affinities was present, no different than the range of affinities reported for antiviral responses (54). Moreover, all vaccine-induced antibodies were tumor-specific and did not recognize MUC1 on normal epithelial cells and tissues. When the nonresponders (53%) to the vaccine were studied, it was found that they had increased numbers of circulating myeloid-derived suppressor cells, most likely expanded during adenoma growth (53). Thus, what could have been interpreted as self-tolerance against this specific shared tumor antigen was instead the immunosuppressive microenvironment already established in some patients even in the premalignant stage. Most importantly, there were no safety issues, and after almost 10 years after the first individual was vaccinated, no autoimmune problems have been reported. Furthermore, 110 individuals have been immunized with the same vaccine and given 1-year boosters in an ongoing placebo-controlled trial, without any evidence of autoimmunity beyond Grade I (unpublished). Normal MUC1 is expressed in the pancreas, kidney, prostate, breast, lungs, etc. Clearly the well-chosen tumor-specific epitope, even though unmutated and derived from a self-antigen, can elicit high-affinity immunity in healthy individuals without causing autoimmunity.

The safety of the MUC1 vaccine was predicted by earlier work showing that healthy individuals could also have spontaneously induced antibodies to MUC1. Those are generated during febrile viral infections (e.g., mumps) or acute inflammations (e.g., mastitis) affecting epithelial tissues, during which MUC1 transiently changes to the "tumor" form (hypoglycosylated and overexpressed). Immune memory for this antigen appears to be important for immune surveillance of cancer leading to risk reduction (55–59).

Healthy people safely maintain antibodies and T cells to many shared tumor antigens, at levels that would be induced by vaccines. Cyclin B1 is one of them (60, 61). The potential sources are VZV and HCMV infections that raise cytoplasmic levels of this otherwise nuclear protein in infected cells and package it into the virions (62, 63). These virions are perfect delivery vehicles that carry cyclin B1 to DCs, in which it is presented to T cells at the same time as the viral antigens. A report from the Glioma International Case-Control Study with data from 4,533 cases and 4,171 controls showed that a positive history of chicken pox was associated with a 21% lower glioma risk (64). Cyclin B1 is

expressed as a tumor antigen in gliomas, and it is tempting to postulate that this protection is in part mediated by anticyclin B1 immunosurveillance.

More evidence of active immunosurveillance of shared tumor antigens without autoimmunity would further support their use for preventative vaccines. Unfortunately, very few studies are being done on this topic. Yet this knowledge might be important whether the goal is preventative vaccination, therapeutic vaccination, or checkpoint blockade immunotherapy. Patients with pre-existing immunity to shared tumor antigens, which could have been initiated through either nonmalignant events or very early events in tumor development when the immune system was still competent, could be precisely the patients who are most responsive to immunotherapy. A prospective analysis of antigen-specific immunity in the progression of premalignant MGUS (monoclonal gammopathy of undetermined significance) to multiple myeloma was made on 305 patients with MGUS. T-cell immunity against the shared tumor antigen, stem cell molecule SOX2, at study entry was independently correlated with reduced risk of progression to myeloma (65). A vaccine based on SOX2 given during the MGUS state might have boosted anti-SOX2 immunity and eliminated MGUS before it progressed to myeloma.

A large category of shared antigens that could be safely considered for preventative vaccines are the CT antigens. Many tumors express these antigens, and immune responses against them can be easily generated. The field should make it a priority to provide better approaches to prevention in cancers such as BRCA-1–driven breast cancer, in which patients rely upon double mastectomies and ovary removal as their only prevention options. A large number of CT antigens is expressed in all breast cancers, starting with the ductal carcinoma *in situ* (DCIS) and including triple-negative, for which no good therapy has yet been discovered. The CT antigens in breast cancers include most of the MAGE antigens and NY-ESO-1 (66), which are absent from normal epithelial tissues. Perhaps a vaccine that can raise immunity and strengthen immunosurveillance against these antigens, combined with clinical surveillance, could lower the risk and spare these women from having to choose between the agony of cancer diagnosis and the agony of the current prevention approach. Many shared antigens in the other categories would also be appropriate in this setting. Efforts to prevent recurrence in women with DCIS by immunizing with anti–HER-2neu vaccines are ongoing (67, 68), which might set the stage for an even earlier application of this vaccine for primary prevention.

Concluding Remarks

Starting almost two decades ago with only a few putative antigens, at this 60th anniversary of Prehn's 1957 publication, the universe of human tumor antigens has greatly expanded to include a large number of molecules whose role in antitumor immunity and immunosurveillance has been confirmed and their potential to contribute to successful cancer therapy or prevention is now being explored (69). The field is still relatively young and divided into two camps: those that have evidence that shared tumor antigens are important for immunosurveillance of tumors, and thus should be pursued as vaccines to boost immunity for cancer therapy or cancer prevention; and those who are firm believers that only mutated antigens unique to each tumor are capable of stimulating effective antitumor immunity. Each camp
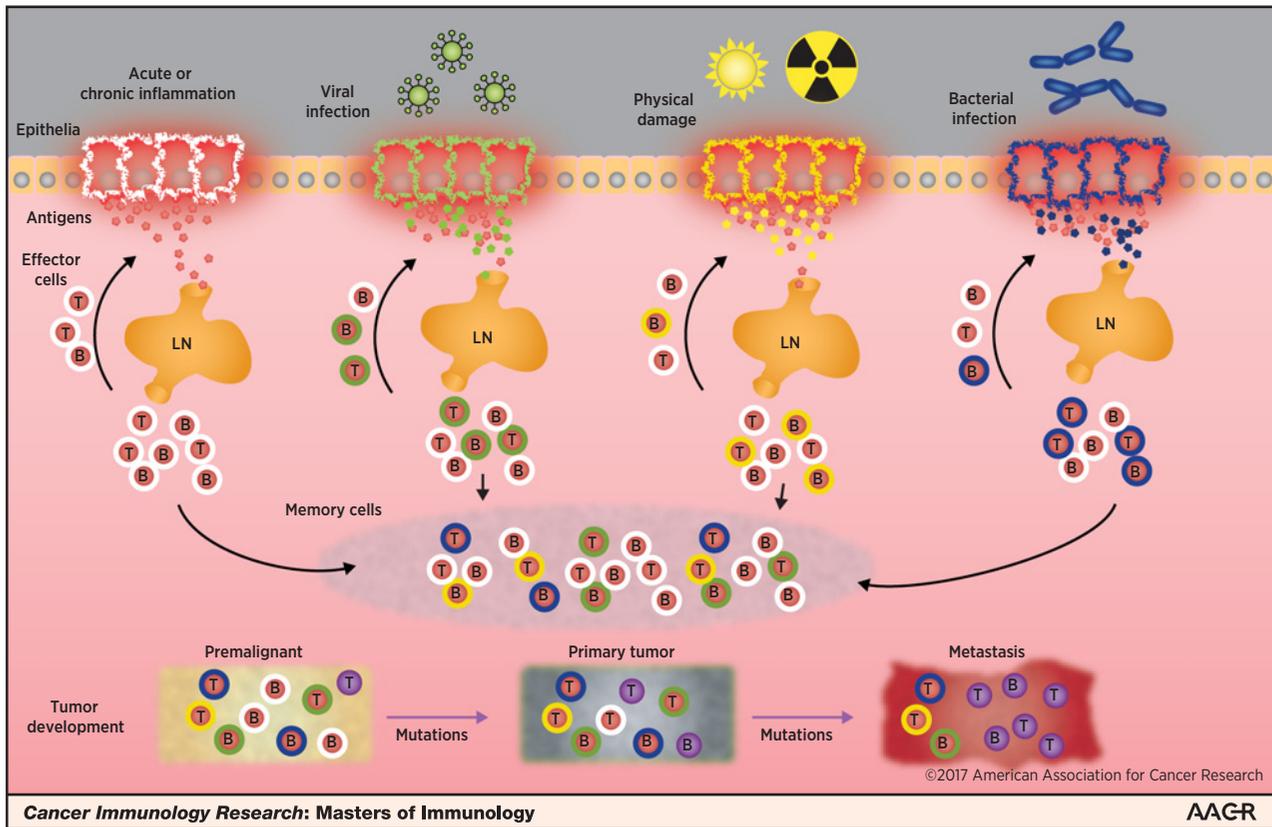
Figure 1.
Proposed origin of shared epithelial tumor antigens and their role in tumor immunosurveillance and promotion of immunity to mutated neoantigens. Epithelial barriers endure injury due to conditions such as chronic inflammation (red with white border) and inflammation accompanying viral infections (red with green border), physical damage (red with yellow border), or bacterial infections (red with blue border). Many normal self-antigens transiently change their expression to abnormal forms (red dots) and drain to the lymph node (LN) together with antigens derived from viruses (green dots), bacteria (blue dots), or mutated antigens (yellow dots) resulting from physical damage. T and B cells are elicited specific for abnormal self-antigens (white halo) as well as to viral antigens (green halo), mutated antigens (yellow halo), and bacterial antigens (blue halo). Some T and B cells, including those specific for abnormal self-antigens, return to the site of injury while others become long-lived memory cells. Tumors express many of these abnormal self-antigens as shared tumor antigens, from premalignant stages through metastasis. Because memory responses are reactivated, shared tumor antigens–specific T and B cells are the first to reach the tumor. Immune pressure exerted by these "first responders" promotes mutations, some of which generate antigens that prime mutated neoantigen-specific T and B cells (purple).

is looking only for evidence supporting its own views. As Fig. 1 hypothesizes, and this hypothesis is experimentally testable, these two categories of antigens might be equally important, and when both are present, more effective immunity can be seen. Immunity and immune memory to shared tumor antigens may develop early in life in response to nonmalignant acute events that transiently change expression of multiple self-antigens that are also transiently presented to the immune system as foreign. This immune memory may be boosted throughout life when such events occur and may serve as the "first responder" at each event, holding back infection, dampening inflammation, and allowing new immune responses to be primed with greater efficiency, such as against viral antigens, if a tissue is infected with a virus. Without these first responders from adaptive immunity, the outcome may be very different, driven primarily by innate immunity and myeloid cells that, unopposed by adaptive immunity, might more quickly establish an immunosuppressive microenvironment. The same may occur during tumor

development. The first responders attracted by the expression of shared tumor antigens may slow down tumor growth, tame the inflammatory microenvironment, and prepare better conditions for the new mutated antigens to prime immune responses. It may indeed be the T cells specific for the unique antigens that effectively destroy the tumor, but their generation depends on the presence of shared antigen-specific T cells. This scenario, if correct, supports the use of shared antigen vaccines either as prevention or as therapy to increase the number of first responders and allow the tumor to do the rest of the work *in vivo*.

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