Immunity, Vol. 11, 263–270, September, 1999, Copyright ©1999 by Cell Press

The Makings of a Tumor Rejection Antigen

Review

Eli Gilboa*
Center for Genetic and Cellular Therapies
Department of Surgery
Duke University Medical Center
Durham, North Carolina 27710

The demonstration that naturally induced tumors in rodents were largely nonimmunogenic and disappointing results from clinical studies were responsible for the notion that tumors are not sufficiently distinct from normal tissue to activate the immune system and led to the inevitable conclusion that immunological intervention in cancer is futile (Hewitt et al., 1976). In a seminal work, van Pel and Boon have shown that a protective immune response can be generated against a "nonimmunogenic" murine tumor, providing the first experimental evidence that lack of immunogenicity could be due to the tumor's inability to activate the immune system rather then the absence of tumor antigens (van Pel and Boon, 1982). This observation, subsequently confirmed and extended to other rodent nonimmunogenic tumor models, has shown that by proper manipulation—otherwise called vaccination—the tumor antigens present in nonimmunogenic tumors can be "exposed" to the immune system to generate an immune response capable of eradicating the tumor. If this conclusion can be extrapolated to human cancer—and I see no reason why it cannot—all forms of cancer should be susceptible to immunological intervention; namely, all forms of cancer contain tumor antigens that can be targeted for immunotherapy.

The recognition that tumors could after all be sufficiently "foreign" to be recognized by the immune system has reinvigorated the efforts to identify and isolate tumor antigens (Boon and van der Bruggen, 1996; Rosenberg, 1999). This review will focus on what makes a tumor antigen a good or not-so-good target for immunotherapy.

Which Tumor Antigens Function as (Better) Tumor Rejection Antigens

Tumor antigens can be classified according to the type of immune response they elicit: humoral, cellular, $CD4^+$ (T helper), or $CD8^+$ cytotoxic T lymphocyte (CTL) responses. As will be discussed below, the fact that a tumor antigen elicits a tumor-specific immune response does not necessarily mean that the immune response will cause the rejection of the tumor in vivo. Thus, from a vaccination standpoint, the question is which tumor antigen can or is better at inducing a clinically beneficial response. We refer to such antigens as "tumor rejection antigens." Tumor-rejection antigen is therefore an operational term describing how well an immune response elicited against a tumor antigen will impact on tumor growth. Tumor antigens can be poor, intermediate, or strong tumor rejection antigens, describing quantitatively the impact of the immune response on tumor growth. The extent to which an antigen is a tumor rejection antigen is also a function of the immunization protocol. A weak tumor rejection antigen can record as a strong tumor rejection antigen by using an effective vaccination protocol, such as protocols that favor the induction of CTL responses and the Th-1 subset of $CD4^+$ T cell responses (see below). It is therefore not surprising if different studies reach divergent conclusions as to whether a tumor antigen is or is not a tumor rejection antigen (Ramarathinam et al., 1995; Rosato et al., 1997; Brandle et al., 1998). The take-home message is that the potency of a tumor rejection antigen is a relative value that can be assessed only by comparing tumor antigens using the same vaccination protocol.

Historically, it was thought that tumor immunity is best mediated by antibodies. Hence, extensive efforts were devoted to the development of serological approaches for identifying antigens expressed on the surface of tumor cells, which are recognized by antibodies from cancer patients (Old, 1981). Disappointing results from early clinical vaccination trials, animal studies indicating the importance of the cellular arm in the antitumor immune response, and new insights into immunological mechanisms have refocused the attention on the cellular response arm of the immune response as the mediator of tumor immunity. The seminal work of Boon and his colleagues, first introducing the methodology of isolating tumor antigens recognized by CTL (De Plaen et al., 1988) and then isolating the first human antigen recognized by CTL from melanoma patients (van der Bruggen et al., 1991), represents yet another important milestone in the annals of contemporary cancer immunotherapy.

There are three good reasons why tumor antigens recognized by CTL would make effective tumor rejection antigens. (1) The major histocompatibility complex (MHC) class I processing pathway ensures that CTL are able to recognize subtle changes in the repertoire of antigens expressed by most (MHC class I–expressing) somatic cells (Townsend and Bodmer, 1989). (2) Murine studies using antibody depletion or adoptive transfer of T cell subsets have shown that the $CD8^+$ CTL arm of the immune response, alone or sometime in combination with $CD4^+$ T cells, constitutes the primary antitumor effector arm of the adaptive immune response. (3) Perhaps the most compelling evidence stems from the frequent correlation seen between tumor progression and loss of histocompatibility leukocyte antigen (HLA) class I expression in cancer patients (Garrido et al., 1997; Hicklin et al., 1999), strongly suggesting that progressing tumors in cancer patients must have elaborate means of escaping an apparently effective MHC class I–restricted immune response. It is, however, becoming clear that the $CD4^+$ T cell response also plays an essential role in tumor rejection. The primary role of $CD4^+$ T cells, specifically, the Th-1 subset, is to enhance the induction and/or extend the persistence of $CD8^+$ CTL in vivo (Frasca et al., 1998; Zajac et al., 1998; Toes et al., 1999). $CD4^+$ T cells have also been ascribed a direct effector

*E-mail: e.gilboa@cgct.duke.edu.

function (Hu et al., 1993; Levitsky et al., 1994; Overwijk et al., 1999), but their contribution relative to other effector arms has not yet been fully elucidated. Clearly, tumor antigens capable of eliciting $CD4^+$ T cell responses will also function as important tumor rejection antigens, and their incorporation into effective tumor vaccination protocols is absolutely essential.

And what about humoral tumor antigens? Humoral responses do not appear to play an important role in protective tumor immunity. Tumor-reactive antibodies occur with high frequency in cancer patients but do not correlate with disease state (Disis and Cheever 1996). Vaccination of mice with tumors can elicit tumor-specific humoral responses that are not protective (Qin et al., 1998). Thus, humoral tumor antigens would make poor tumor rejection antigens and under some circumstances could even prevent the establishment of a T cell–based antitumor response (Rowley and Stach, 1993). Yet, monoclonal antibody therapy can exert a significant therapeutic benefit in murine models and in cancer patients, suggesting that humoral tumor antigens could function as tumor rejection antigens (Hara et al., 1995; Herlyn and Birebent, 1999). Interestingly, in a recent study, the protective effects of DNA vaccination against an idiotype expressed on a murine B cell lymphoma could be largely attributed to humoral but not cellular immunity (Syrengelas and Levy, 1999). Thus, at present the prospects of using humoral tumor antigens in cancer vaccine formulations is "down but not out." New and vastly improved methods for isolating tumor antigens recognized by the humoral response have reinvigorated the efforts to develop antibody-based treatments to cancer (Sahin et al., 1997).

Tumor Rejection Antigens Recognized by CTL: Shared or Patient Specific

Whether effective tumor rejection antigens are unique patient-specific antigens or correspond to normal gene products shared among many patients is of enormous practical value. Identification and isolation of tumor rejection antigens from each patient is currently not an option. If nonmutated shared antigens can function as tumor rejection antigens, common "off-the-shelf" reagents could be used to treat many cancer patients.

A Tumor Antigen Encoding a CTL Epitope Is Not by Default a Tumor Rejection Antigen

While the notion that tumor antigens encoding CTL epitopes will make good tumor rejection antigens is compelling, a tumor antigen encoding a CTL epitope is not necessarily a potent tumor rejection antigen. Three examples will illustrate this point.

P1A is a murine prototype of a nonmutated shared tumor antigen recognized by tumor-specific CTL (Van den Eynde et al., 1991). P1A was isolated from the P185 mastocytoma cell line by virtue of the fact that tumor cells which lost P1A antigen (as well as other tumor antigens) escaped tumor rejection in vivo (Uyttenhove et al., 1983). P1A is expressed in several unrelated tumors including Meth A sarcoma and J558 plasmacytoma (Ramarathinam et al., 1995) and immunization of mice with each tumor elicits P1A-specific CTL. Nevertheless, cross-protection among the P1A-expressing tumors is weak and could be demonstrated only when the P1A-expressing tumor cells were also engineered to express B7-1 (Ramarathinam et al., 1995; Brandle et al., 1998). It would appear, therefore, that P1A is at best a weak tumor rejection antigen.

MART-1/Melan A is a human melanocyte–specific antigen that is recognized by CTL from melanoma patients. Tumor infiltrating lymphocytes (TIL) from nine out of ten patients exhibited CTL activity against MART-1, but only four TILs exhibited activity against another tumor-associated antigen, gp100 (Kawakami et al., 1994). Yet, upon adoptive transfer of TIL to patients, tumor regression was correlated with TIL, which recognized gp100 and not MART-1 (Kawakami et al., 1995). Furthermore, vaccination with the dominant MART-1 peptide administered in adjuvant generated significant increases in MART-1-specific CTL, yet no clinical responses were seen (Jaeger et al., 1996; Cormier et al., 1997). Notwithstanding the preliminary nature of such phase I clinical studies, MART-1/Melan A is a dominant antigen recognized by CTL in melanoma patients, yet it does not exhibit properties of a tumor rejection antigen.

In a recent study, vaccination of melanoma patients with an anchor residue–modified gp100 derived peptide stimulated strong CTL responses in most patients, yet no clinical responses were seen. Interestingly, patients who also received IL-2 exhibited reduced CTL activities but experienced a dramatic increase in clinical responses. Clearly, there was no simple correlation between the measured CTL responses and clinical responses in this particular case (Rosenberg et al., 1998).

What Makes a Tumor Antigen a Good Tumor Rejection Antigen?

At the heart of the matter is the growing appreciation that the biological impact of a CTL response is not only a function of the experimentally determined magnitude of the immune response, but it is also, and perhaps even more so, a function of the avidity of the CTL to their targets. Several studies have shown that whereas low-avidity CTL can be readily detected by standard immunological assays, only high-avidity CTL exert biological function in vivo in viral (Speiser et al., 1992; Alexander-Miller et al., 1996a; Gallimore et al., 1998) or tumor (Zeh 3rd et al., 1999) models. Thus, perhaps the single most important parameter that determines the potency of a tumor rejection antigen is the avidity of the cognate T cells that can be activated and marshaled against the progressing tumor.

Two assumptions are necessary to complete this argument. The first assumption is that tolerance to self-antigen is incomplete. Antigens presented by thymic antigen-presenting cells (APC) cause the clonal deletion of autoreactive T cells (central tolerance), whereas the response to peripherally expressed antigens with no access to the thymus varies from physical elimination or functional anergy to complete "ignorance" (peripheral tolerance). If self-antigens induce tolerance by causing the functional or physical elimination of *all* autoreactive T cells, self-antigens could not be tumor antigens, let alone tumor rejection antigens. This, however, does not appear to be the case. As a rule, tolerogenic responses are limited to the inactivation of high-avidity but not low-avidity T cells. The threshold is determined by the properties of the APC and epitope density. Tolerogenic responses to self are calibrated to eliminate only the high-avidity T cells capable of reacting to physiologically

relevant amounts of antigen presented by somatic cells. Thus, low-avidity autoreactive T cells, which are not capable of recognizing self-antigens under normal conditions, persist in the circulation. The presence of such low-avidity anti-self T cells has been amply documented in animal studies (Poindexter et al., 1992; Cibotti et al., 1994; Oehen et al., 1994; von Herrath et al., 1994; Poplonski et al., 1996; Morgan et al., 1998). It is therefore conceivable that a spectrum of autoreactive T cells are present in the mature T cell population, ranging from high-avidity T cells corresponding to antigens that have been completely "ignored" to very low-avidity T cells directed against antigens that have triggered effective tolerance. The second assumption, which constitutes the underlying rationale for using nonmutated self-antigens as tumor rejection antigens, is that through vaccination low-avidity anti-self T cells can be activated and marshaled to eradicate tumors. Conceivably, activation of such low-avidity T cells will be less efficient and more difficult to achieve than activation of high-avidity T cells, and hence the corresponding antigens will score as weak tumor rejection antigens. The key is the effectiveness of the vaccination protocol required to activate the low-avidity T cells in order to compensate for their relative weakness in recognizing their tumor targets. That low-avidity T cells corresponding to a self-transgene can be activated to impact on tumor growth has been demonstrated in the elegant studies by Morgan et al. (1998).

The potency of a tumor rejection antigen is also dictated by the frequency of cognate T cells in the mature T cell population and, as suggested from viral models, the TCR diversity of the responding population (Busch et al., 1998; Cooper et al., 1999). Conceivably, a high frequency of responding T cells can offset their low avidity, and their activation in the course of vaccination would elicit an effective antitumor response. Naturally, both frequency and diversity of the responding T cells will be reduced by tolerance.

The overall implications are that mutated-self, tumor-specific antigens will make effective tumor rejection antigens, whereas shared tumor antigens corresponding to nonmutated tissue-spe cific gene products will make weak tumor rejection antigens. Fortunately, as will be discussed below, this is not as simple as that and there is a gray area that can be exploited for cancer vaccination.

Which of the Known Human Tumor Antigens Will Make Good Tumor Rejection Antigens?

Guided by the principle that the potency of tumor rejection antigens is foremost a function of cognate T cell avidity, the current list of human tumor antigens was divided into four groups shown in Figure 1.

Group I. Group I antigens represent tumor antigens that have arisen as a result of somatic mutations in normal gene products. The mutations in this group of antigens are incidental to the oncogenic process reflecting the genetic instability of tumor cells. Tumor antigens in this group will be therefore patient specific, not expected to trigger tolerance, and should make potent tumor rejection antigens.

One caveat is that tumors could tolerize the immune system against antigens expressed on the tumor cells. In one study, tumor antigen–specific $CD4^+$ T cell anergy was established in tumor-bearing mice (Staveley-O'Carroll et al., 1998), yet in other studies no impairment of tumor antigen–specific ($CD8^+$) T cell responses was observed (Wick et al., 1997; Prevost-Blondel et al., 1998; Ochsenbein et al., 1999). We have not seen evidence that in vitro stimulation of CTL against a "self" tumor-associated antigen, carcinoembryonic antigen (CEA), is significantly blunted in cancer patients as compared to healthy individuals (Nair et al., 1999). Thus, whether or to what extent progressing tumors impact negatively on tumor-specific immunity is yet unresolved, but it clearly merits more investigation.

Unique tumor antigens recognized by CTL have been isolated from tumor-bearing mice and from cancer patients. Mut-1 was the first murine tumor antigen recognized by CTL isolated from the spontaneously induced Lewis lung carcinoma line (Mandelboim et al., 1994). The Mut-1 epitope was generated by a point mutation in the connexin 37 protein generating a novel H-$2K^b$-restricted epitope. Immunization of mice with Mut-1 peptide elicit CTL that recognize the tumor of origin and can lead to the regression of established metastases in tumor-bearing mice (Mandelboim et al., 1995). Tumor antigens resulting from point mutations in normal gene products recognized by CTL from cancer patients have been also identified, although they represent a small fraction of the human tumor antigens isolated so far (Boon and van der Bruggen, 1996; Rosenberg, 1999). Nevertheless, as will be discussed below, experimental bias in the methodology of isolating human tumor antigens may be responsible for underrepresentation of this group of antigens.

Group II. Group II antigens correspond to tumor-specific antigens (antigens expressed in tumor cells but not in normal tissue), which nevertheless could be shared among cancer patients. This group of antigens is further subdivided into two subgroups. One subgroup consists of antigens that have arisen as a result of mutations related to the oncogenic process and hence are conceivably shared among some of the patients. For example, mutated or translocated oncogens could provide new epitopes and serve as tumor rejection antigens. Indeed, vaccination of mice with peptides spanning mutations in p53 or *ras* elicit CTL, but evidence that vaccination with mutated or translocated oncogens will provide effective tumor immunity is lacking (Disis and Cheever, 1996). This is not surprising, since the likelihood that a mutation will generate a potent epitope was estimated to be less than 1/300 per haplotype (Yewdell and Bennink, 1999). The second subgroup consists of viral antigens present in cancers of viral etiology such as Epstein Barr virus (EBV)-induced lymphoma and human papilloma virus (HPV)-associated cervical cancer. Overall, antigens belonging this subgroup are not expected to trigger tolerance and should make excellent tumor rejection antigens.

Group III. Group III antigens are shared tumor antigens that correspond to normal gene products with highly restricted tissue distribution. Antigens belonging to this group are not expressed in most somatic tissues, usually with the exception of the testis, and are reactivated in a number of tumors of various histological types, including melanoma. Prototypes of this group are the melanoma MAGE, GAGE, and BAGE families (Boon and

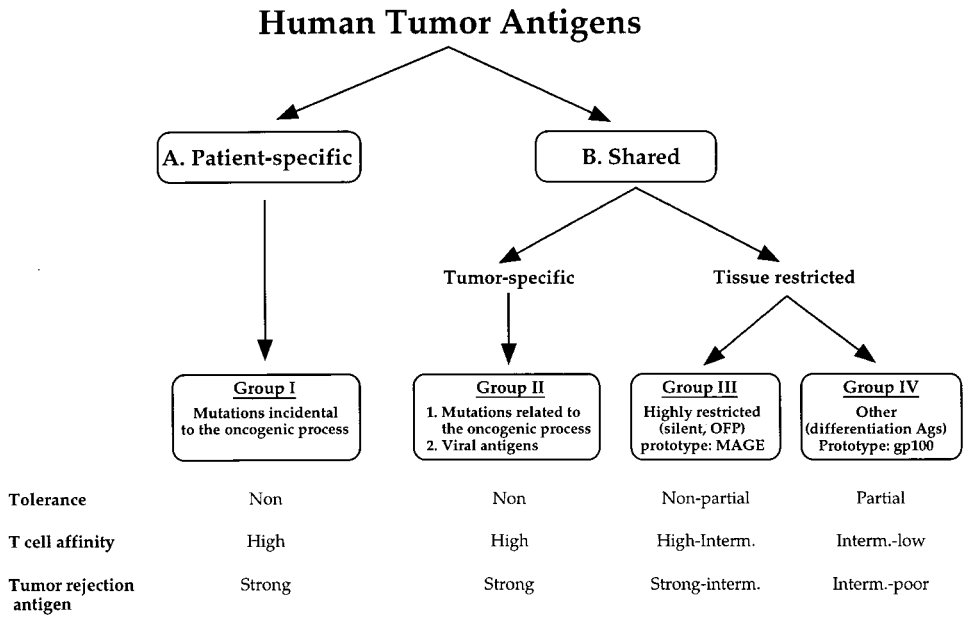

Figure 1. Classification of Human Tumor Antigens Recognized by CTL from Cancer Patients

van der Bruggen, 1996; Rosenberg, 1999). The prediction is that antigens belonging to this group are by and large ignored by the immune system and hence will make good tumor rejection antigens.

Group IV. Group IV antigens consist of shared tumor antigens that correspond to normal tissue-specific gene products, also called "differentiation antigens." Such antigens have been isolated from melanoma patients and include MART-1/Melan A, gp100, and tyrosinase. If—and this is debatable—antigens belonging to this group have triggered some level of tolerance, they will make poor tumor rejection antigens. However, it should be noted that the distinction between groups III and IV is somewhat arbitrary and is more a matter of degree. Antigens corresponding to either group are likely to represent a spectrum of antigens ranging from antigens that have not triggered tolerance to antigens that have triggered tolerance to varying degrees.

In the light of what was discussed above, the prediction is that tumor-specific antigens, whether patient specific or shared (groups I and II, respectively), and nonmutated self-antigens with highly restricted tissue distribution ignored by the immune system (group III) will make potent tumor rejection antigens, whereas antigens corresponding to tissue-specific gene products (group IV) will have triggered tolerance to various degrees and will be less effective tumor rejection antigens. Experimental observations by and large support this view though the evidence is far from conclusive.

Tumor transplantation studies in mice have provided compelling evidence that antigens belonging to group I are responsible for protective immunity in tumor-bearing animals. This conclusion is based on the demonstration that mice vaccinated with irradiated tumor cells became resistant to a subsequent tumor challenge, and that resistance was exquisitely tumor specific (Prehn and Main, 1957; Klein et al., 1960; Old et al., 1962). Many of the tumors used in these studies were carcinogen induced, and hence the unique tumor-specific rejection antigens most likely consisted of mutated forms of normal genes resulting from carcinogen exposure. A low level of cross-protection could be occasionally seen, suggesting the presence of shared, albeit weak, tumor rejection antigens (Coggin, 1989). The tumor transplantation studies are also supported by CTL analysis in the tumor-bearing mice. Dudley and Roopenian have shown that whereas unique antigens represent the dominant antigens recognized by CTL in tumor-bearing mice, tumors also express immunosubdominant antigens, which stimulate a low level of CTL (Dudley and Roopenian, 1996).

The rodent tumor transplantation studies are often criticized on account of their artificial nature. Unlike most human tumor or spontaneously arising rodent tumors, experimental tumors derived by exposure to large doses of carcinogen are immunogenic. (But exactly what is an immunogenic versus nonimmunogenic tumor and how is it determined, especially for human tumors, is imprecise at best.) In addition, the circumstances of inducing immunity in tumor-free mice subsequently challenged with tumor may be quite different from the conditions prevailing in patients with cancer. However, lack of cross-protection, taken as evidence for the dominance of unique tumor rejection antigens, has been also seen in spontaneously induced nonimmunogenic tumors monitoring induction of immunity in tumor-bearing animals (Gilboa and Lyerly, 1994). Overall, while the concerns are valid, by no means do they disprove the notion that tumor rejection antigens which dominate an effective antitumor response are unique. As will be discussed below, careful examination of the burgeoning field of human tumor antigens has done little to change this conclusion.

At first blush, the data from human studies does suggest otherwise; the majority of human antigens isolated to date correspond to nonmutated shared antigens

belonging to groups III and IV. Only a minority of antigens in the list correspond to mutated forms of normal gene products (Boon and van der Bruggen, 1996; Rosenberg, 1999). However, closer examination suggests that this could be a "mirage." Tumor antigens recognized by CTL are isolated by fractionating the antigenic content of tumor cells and determining which fraction is recognized by tumor-specific CTL. The key, or catch if you wish, is what constitutes "tumor-specific CTL." Tumor-specific CTL cannot be detected or generated from most patients. In a few cases, notably melanoma, tumor-specific CTL can be generated following extensive in vitro culture of T cells in the presence of tumor cells. The tumor cells serve as APC to stimulate tumor-specific CTL present at low frequency in melanoma patients. The question is whether CTL generated via repeated in vitro stimulations recognize strong tumor rejection antigens or whether the CTL generated in this fashion are skewed to recognize weaker tumor rejection antigens. The answer may be the latter. There is evidence that upon in vitro restimulation, high-avidity CTL corresponding to immunodominant epitopes are more prone to undergo apoptotic death in a process known as activation-induced cell death (AICD) (Alexander-Miller et al., 1996b; Bush and Pamer 1998). Thus, unless special care is taken, repeated stimulation of CTL from cancer patients could lead to the preferential expansion of low-avidity CTL corresponding to weaker tumor antigens. It is perhaps not surprising that many of the known tumor antigens are (melanocyte) differentiation antigens belonging to group IV.

Another complicating factor stems from the fact that the CTL used to identify tumor antigens are generated from cancer patients—from individuals who failed to reject their cancer. The concern is that CTL present in patients with a long history of cancer are functionally compromised (Whiteside 1998) and were rescued from "irrelevance" by the ex vivo culture conditions. The tumor-specific T cells remaining in the cancer patient may represent low-avidity T cells that were not eliminated by AICD (Alexander-Miller et al., 1996b).

One way to increase the odds of isolating relevant tumor antigens would be to use CTL from patients experiencing a spontaneous remission. This would be difficult because such events are very rare. Nevertheless, Zorn and Hercend succeeded in isolating and characterizing two antigens recognized by T cells infiltrating a spontaneously regressing melanoma lesion (Zorn and Hercend, 1999a, 1999b). Their findings are revealing. The antigen recognized by the dominant CTL clone corresponds to a neoantigen resulting from a point mutation in a myosin class I gene. Thus, this antigen belongs to group I of mutated-self antigens shown in Figure 1. A second antigen represented by a less prevalent T cell clone was identified as MAGE-6 (group III, Figure 1). While no firm conclusions can be drawn from two examples, it does bolster the argument that tumor-specific antigens and nonmutated "silent" antigens would make better tumor rejection antigens.

It is also informative to examine the antigenic profile from patients with highly favorable clinical courses. LB33 and MZ2 are two such melanoma patients that were alive 10 years following initial diagnosis. Both patients were treated with autologous irradiated melanoma cells and exhibited very high levels of melanoma-specific CTL in the blood. Assuming that the unusually long remission state of the patients was due to the measured CTL response—a highly plausible assumption yet lacking definitive proof—the antigens recognized by the CTL would constitute effective tumor rejection antigens. In patient LB33, four antigens characterized were neoantigens generated by point mutations in normal gene products. In patient MZ2, seven of eight antigens were encoded by nonmutated group III antigens of the MAGE, BAGE, and GAGE family, and the eighth antigen corresponded to the group IV differentiation antigen tyrosinase (Coulie et al., 1999).

Clonal and frequency analysis of CTL from four melanoma patients have revealed that the majority of CTL were directed against tumor-specific, albeit shared, group III antigens (Anichini et al., 1996). In another study, dendritic cells transfected with tumor-derived peptides stimulated tumor-specific CTL, which did not recognize any of the previously characterized melanoma antigens expressed in that particular tumor (Imro et al., 1999). The implication from these studies are that the majority of the melanoma-specific CTL may be targeted to new yet unidentified antigens, the majority of which are not melanocyte differentiation antigens.

Cumulatively, these findings bring into question the current strategies used for the identification of tumor rejection antigens and is consistent with the notion that tumor-specific antigens, arising from somatic mutations or introduced as viral antigens (groups I and II), or self-antigens with highly restricted tissue distribution (group III) will make more effective tumor rejection antigens compared to antigens that correspond to tissue-specific gene products (group IV).

Isolation of human tumor rejection antigens could be improved in several ways. One approach is to modify the ex vivo culture conditions in ways that will preserve and enrich for high-avidity CTL. This could be achieved by employing measures which minimize Fas-mediated apoptosis (van Parijs and Abbas, 1996) and CTLA-4-mediated attenuation of proliferation (Thompson and Allison, 1997), or measures which engage the 4–1BB receptor (Hurtado et al., 1997), etc. Another approach to increase the representation of tumor-specific T cells in the responder population would be to develop improved in vitro stimulation protocols capable of generating primary tumor-specific CTL responses from naive precursors, as opposed to the current strategies, which have been used to expand a preexisting memory CTL response from cancer (melanoma) patients.

Induction of Autoimmunity by Vaccinating with Self-Tumor Antigens

This important topic will be dealt with briefly here. Autoimmunity with pathological consequences following vaccination with self-tumor antigens should be expected and will likely be encountered with the advent of increasingly effective vaccination protocols. Two lines of not unrelated observations suggest that autoimmunity may not be a serious problem. First, potent tumor immunity can be generated in mice by vaccination with specific self-antigens or with unfractionated tumor-derived material, with little or no evidence of autoimmunity. A low level of autoimmunity has been occasionally seen but appears to be the exception rather than the rule (Hara et al., 1995; Naftziger et al., 1996; Fong et al.,

1997; Overwijk et al., 1999). Second, a host of studies have shown that an activated effector arm consisting of antigen-specific $CD8^+$ CTL (Mayordomo et al., 1996; Roth et al., 1996; Melero et al., 1997; Vierboom et al., 1997; Morgan et al., 1998) or $CD4^+$ T cells (Hu et al., 1993) could selectively eradicate tumors without apparently attacking or damaging normal tissue expressing the same antigen. The implication is that even the effector arm of the immune response in its activated form can distinguish between normal and tumor tissue, i.e., tumor tissue is more susceptible to immune destruction than normal tissue.

Implications for Vaccination

Vaccination with patient-specific mutated-self group I antigens is the approach of choice, but it would require isolation and characterization of such antigens from each patient, clearly not an option. Theoretical considerations and circumstantial evidence presented here argue that vaccination with shared self-antigens will be less effective. As a rule, group III antigens should be more effective than group IV antigens, and those antigens that are completely ignored by the immune system should make potent tumor rejection antigens. Whether this is indeed the case must wait clinical testing.

The limited efficacy of vaccination with self tumor antigens can be offset in two ways: use of potent vaccination protocols capable of activating and expanding the remaining low-avidity T cells and vaccination with a mixture of tumor antigens. It is safe to assume that the current vaccination protocols do not meet expectations. Vaccination with genetically modified irradiated tumor cells, tumor-derived heat shock proteins, DNA-encoded antigens, or dendritic cells represent some of the promising approaches currently explored. With vaccination using a mixture of tumor antigens, it is conceivable that the combined immune response directed against several antigens expressed by tumor cells would have an additive if not synergistic effect. Consistent with this notion, Johnston et al. have shown that a polyclonal CTL response directed against multiple tumor antigens correlated with an improved antitumor effect as compared to a CTL response directed against a single antigen (Johnston et al., 1996).

Tumor (Rejection) Antigen Discovery

The essential prerequisites for isolating tumor antigens from cancer patients are foremost the availability of sufficient tumor tissue for antigen isolation and ex vivo T cell stimulation, a method for generating a representative tumor-specific T cell response, and a target cell to measure the successful generation of a tumor-specific T cell response. The problem is that adequate amounts of tumor tissue of sufficient purity cannot be obtained from many cancer patients. In addition, a preexisting antitumor T cell response is not detectable in most cancer patients, and current ex vivo T cell expansion protocols are not sufficiently powerful in stimulating a primary polyclonal antitumor response in vitro. The reason why antigen discovery has been limited primarily to melanoma is that unlike many other types of cancers, melanoma can be established in culture and thus provides the necessary amount of tumor tissue for antigen isolation. In addition, melanoma patients exhibit a weak melanoma-specific CTL response, which current techniques are capable of expanding ex vivo.

To expand the scope of vaccination with tumor antigens, general protocols for tumor antigen discovery applicable to other forms of cancers have to be developed. One such approach could involve the use of dendritic cells transfected with tumor mRNA. mRNA-transfected dendritic cells are potent stimulators of T cell immunity in vitro and in vivo (Boczkowski et al., 1996; Ashley et al., 1997; Nair et al., 1998). Dendritic cells transfected with tumor-derived mRNA may be therefore capable of stimulating primary tumor-specific CTL responses in vitro to provide a more representative CTL response from many cancer patients. In addition, mRNA-transfected dendritic cells can function as targets in CTL cytotoxicity assays (Nair et al., 1998), offering a practical substitute for tumor cells for such assays. Finally, mRNA should provide an inexhaustible source of tumor antigen, since the mRNA content of cells could be amplified from microscopic amounts of tumor tissue.

Vaccination with Undefined Tumor Antigens

If isolation of unique group I–type antigens from each cancer patients is not practical, and the use of shared nonmutated antigens must await the development of appropriate antigen isolation methodologies, what can one do in the meantime? One attractive option is to vaccinate with autologous tumor-derived antigenic mixtures. The tumor-derived antigenic mixtures will include the complete antigenic repertoire of the tumor, including the potent group I patient-specific antigens, yet will obviate the need to identify the relevant tumor antigens in each patient. In animal tumor models, vaccination with genetically modified irradiated tumor cells (Gilboa and Lyerly, 1994), tumor-derived heat shock proteins (Tamura et al., 1997), or with dendritic cells loaded with tumor-derived peptides or proteins (Gilboa et al., 1998) is very potent. A common limitation of these strategies is that sufficient tumor tissue for antigen preparation cannot be obtained or generated from many cancer patients. In such instances, use of mRNA amplified from small amounts of available tumor tissue could provide unlimited amounts of antigen for vacccination protocols.

References

Alexander-Miller, M.A., Leggatt, G.R., and Berzofsky, J.A. (1996a). Selective expansion of high or low-avidity cytotoxic T lymphocytes and efficacy for adoptive immunotherapy. Proc. Natl. Acad. Sci. USA *93*, 4102–4107.

Alexander-Miller, M.A., Leggatt, G.R., Sarin, A., and Berzofsky, J.A. (1996b). Role of antigen, CD8, and cytotoxic T lymphocyte (CTL) avidity in high dose antigen induction of apoptosis of effector CTL. J. Exp. Med. *184*, 485–492.

Anichini, A., Mortarini, R., Maccalli, C., Squarcina, P., Fleischhauer, K., Mascheroni, L., and, Parmiani. G. (1996). Cytotoxic T cells directed to tumor antigens not expressed on normal melanocytes dominate HLA-A2.1-restricted immune repertoire to melanoma. J. Immunol. *156*, 208–217.

Ashley, D.M., Faiola, B., Nair, S., Hale, L.P., Bigner, D.D., and Gilboa, E. (1997). Bone marrow-generated dendritic cell pulsed with tumor extracts or tumor RNA induce anti-tumor immunity against central nervous system tumors. J. Exp. Med. *186*, 1177–1182.

Boczkowski, D., Nair, S.K, Snyder, D., and Gilboa, E. (1996). Dendritic

cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. J. Exp. Med. *184*, 465–472.

Boon, T., and van der Bruggen, P. (1996). Human tumor antigens recognized by T lymphocytes. J. Exp. Med. *183*, 725–729.

Brandle, D., Bilsborough, J., Rülicke, T., Uyttenhove, C., Boon, T., and Van den Eynde, B.J. (1998). The shared tumor-specific antigen encoded by mouse gene P1A is a target not only for cytolytic T lymphocytes but also for tumor rejection. Eur. J. Immunol. *28*, 4010–4019.

Busch, D.H., and Pamer, E.G. (1998). MHC class I/peptide stability, implications for immunodominance, in vitro proliferation, and diversity of responding CTL. J. Immunol. *160*, 4441–4448.

Cibotti, R., Cabaniols, J.P., Pannetier, C., Delarbre, C., Vergnon, I., Kanellopoulos, J.M., and Kourilsky, P. (1994). Public and private V_β T cell receptor repertoires against hen egg white lysozyme (HEL) in non-transgenic versus HEL transgenic mice. J. Exp. Med. *180*, 861–872.

Coggin, J.H. (1989). Cross-reacting tumor associated transplantation antigen on primary 3-methylcholantrene-induced BALB/c sarcomas. Mol. Biother. *4*, 223–228.

Cooper, S., Erickson, A.L., Adams, E.J., Kansopon, J., Weiner, A.J., Chien, D.Y., Houghton, M., Parham, P., and Walker, C.M. (1999). Analysis of a successful immune response against hepatitis C virus. Immunity *10*, 439–449.

Cormier, J.N., Salgaller, M.L., Prevette, T., Barracchini, K.C., Rivoltini, L., Restifo, N.P., Rosenberg, S.A., and Marincola, F.M.. (1997). Enhancement of cellular immunity in melanoma patients immunized with a peptide from MART-1/melan A. Cancer J. *3*, 37–44.

Coulie, P.G., Ikeda, H., Baurain, J.-F., and Chiari, R. (1999). Antitumor immunity at work in a melanoma patient. Adv. Cancer Res. *76*, 213–242

De Plaen, E., Lurquin, C., Van Pel, A., Mariame, B., Szikora, J.P., Wölfel, T., Sibille, C., Chomez, P., and Boon, T. (1988). Immunogenic (tum^-) variants of mouse tumor P815. Cloning of the gene of tum^- antigen P91A and identification of the tum^- mutation. Proc. Natl. Acad. Sci. USA *85*, 2274–2278.

Disis, M.L., and Cheever, M.A. (1996). Oncogenic proteins as tumor antigens. Curr. Opin. Immunol. *8*, 637–642.

Dudley, M.E., and Roopenian, D.C. (1996). Loss of a unique tumor antigen by cytotoxic T lymphocyte immunoselection from a 3-methylcholanthrene-induced mouse sarcoma reveals secondary unique and shared antigens. J. Exp. Med. *184*, 441–447.

Fong, L., Ruegg, C.L., Brockstedt, D., Engleman, E.G., and, Laus, R. (1997). Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization. J. Immunol. *159*, 3113–3117.

Frasca, L., Piazza, C., and Piccolella, E. (1998). $CD4^+$ T cells orchestrate both amplification and deletion of $CD8^+$ T cells. Crit. Rev. Immunol. *18*, 569–594.

Gallimore, A., Dumrese, T., Hengartner, H., Zinkernagel, R.M., and Rammensee, H.G. (1998). Protective immunity does not correlate with the hierarchy of virus-specific cytotoxic T cell responses to naturally processed peptides. J. Exp. Med. *187*, 1647–1657.

Garrido, F., Ruiz-Cabello, F., Cabrera, T., Perez-Villar, J.J., Lopez-Botet, M., Duggan-Keen, M., and Stern, P.L. (1997). Implications for immunosurveillance of altered HLA class I phenotypes in human tumors. Immunol. Today *18*, 89–95.

Gilboa, E., and Lyerly, H.K. (1994). Specific active immunotherapy of cancer using genetically modified tumor vaccines. In Biologic Therapy of Cancer Updates, S.A. Rosenberg, ed. (Philadelphia: J.B. Lippincott Company), pp. 1–16.

Gilboa, E., Nair, S.K., and Lyerly, H.K. (1998). Immunotherapy of cancer with dendritic-cell based vaccines. Cancer Immunol. Immunother. *46*, 82–87

Hara, I., Takechi, Y., and Houghton, A.N. (1995). Implicating a role for immune recognition of self in tumor rejection, passive immunization against the Brown locus protein. J. Exp. Med. *182*, 1609–1614.

Herlyn, D., and Birebent, B. (1999). Advances in cancer vaccine development. Ann. Med. *31*, 66–78.

Hewitt, H., Blake, E., and Walder, A. (1976). A critique of the evidence for active host defense against cancer, based on person studies of 27 murine tumors of spontaneous origin. Cancer *33*, 241–259.

Hicklin, D.J., Marincola, F.M., and Ferrone, S. (1999). HLA class I antigen downregulation in human cancers. Mol. Med. Today, in press.

Hu, J., Kindsvogel, W., Busby, S., Bailey, M.C., Shi, Y.-Y., and Greenberg, P.D. (1993). An evaluation of the potential to use tumor-associated antigens at targets for antitumor T cell therapy using transgenic mice expressing a retroviral tumor antigen in normal lymphoid tissues. J. Exp. Med. *177*, 1681–1690.

Hurtado, J.C., Kim, Y.-J., and Kwon, B.S. (1997). Signals through 4–1BB are costimulatory to previously activated splenic T cells and inhibit activation-induced cell death. J. Immunol. *158*, 2600–2609.

Imro, M.A., Manici, S., Russo, V., Consogno, G., Bellone, M., Rugarli, C., Traversari, C., and Protti, M.P. (1999). Major histocompatibility class I restricted cytotoxic T cells specific for natural melanoma peptides recognize unidentified shared melanoma antigen(s). Cancer Res. *59*, 2287–2291.

Jaeger, E., Bernhard, H., Romero, P., Ringhoffer, M., Arand, M., Karbach, J., Ilsemann, C., Hagedorn, M., and Knuth, A. (1996). Generation of cytotoxic T-cell responses with synthetic melanoma-associated peptides in vivo, implications for tumor vaccines with melanoma-associated antigens. Int. J. Cancer *66*, 162–169.

Johnston, J.V., Malacko, A.R., Mizuno, M.T., McGowan, P., Hellström, I., Hellström, K.E., Marquardt, H., and Chen, L. (1996). B7-CD28 costimulation unveils the hierarchy of tumor epitopes recognized by major histocompatibility complex class I-restricted $CD8^+$ cytolytic T lymphocytes. J. Exp. Med. *183*, 791–800.

Kawakami, Y., Eliyahu, S., Sakaguchi, K., Robbins, P.F., Rivoltini, L., Yannelli, J.R., Appella, E., and Rosenberg, S.A. (1994). Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. J. Exp. Med. *180*, 347–352.

Kawakami, Y., Eliyahu, S., Jennings, C., Sakaguchi, K., Kang, X., Southwood, S., Robbins, P.F., Sette, A., Appella, E., and Rosenberg, S.A. (1995). Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with *in vivo* tumor regression. J. Immunol. *154*, 3961–3968.

Klein, G., Sjogren, H.O., and Klein, E. (1960). Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. Cancer Res. *20*, 1561–1572.

Levitsky, H.I., Lazenby, A., Hayashi, R.J., and Pardoll, D.M. (1994). *In vivo* priming of two distinct antitumor effector populations. The role of MHC class I expression. J. Exp. Med. *179*, 1215–1224.

Mandelboim, O., Berke, G., Fridkin, M., Feldman, M., Eisenstein, M., and Eisenbach, L. (1994). CTL induction by a tumor-associated antigen octapeptide derived from a murine lung carcinoma. Nature *369*, 67–71.

Mandelboim, O., Vadai, E., Fridkin, M., Katz-Hillel, A., Feldman, M., Berke, G., and Eisenbach, L. (1995). Regression of established murine carcinoma metastases following vaccination with tumor-associated antigen peptides. Nat. Med. *11*, 1179–1183.

Mayordomo, J.I., Loftus, D.J., Sakamoto, H., De Cesare, C.M., Appasamy, P.M., Lotze, M.T., Storkus, W.J., Appella, E., and DeLeo, A.B. (1996). Therapy of murine tumors with p53 wild-type and mutant sequence peptide-based vaccines. J. Exp. Med. *183*, 1357–1365.

Melero, I., Singhal, M.C., McGowan, P., Haugen, H.S., Blake, J., Hellstrom, K.E., Yang, G., Clegg, C.H., and Chen, L. (1997). Immunological ignorance of an E7-encoded cytolytic T-lymphocyte epitope in transgenic mice expressing the E7 and E6 oncogenes of human papillomavirus type 16. J. Virol. *71*, 3998–4004.

Morgan, D.J., Kreuwel, H.T.C., Fleck, S., Levitsky, H.I., Pardoll, D.M., and Sherman, L.A. (1998). Activation of low avidity CTL specific for a self epitope results in tumor rejection but not autoimmunity. J. Immunol. *160*, 643–651.

Naftziger, C., Takechi, Y., Kohda, H., Hara, I., Vijayasaradhi, S., and Houghton, A.N. (1996). Immune response to a differentiation antigen induced by altered antigen, a study of tumor rejection and autoimmunity. Proc. Natl. Acad. Sci. USA *93*, 14809–14814.

Nair, S.K., Boczkowski, D., Morse, M., Cumming, R.I., Lyerly, H.K.,

and Gilboa, E. (1998). Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. Nat. Biotech. *16*, 364–369.

Nair, S.K., Hull, S., Coleman, D., Gilboa, E., Lyerly, H.K., and Morse, M. A. (1999). Induction of carcinoembryonic antigen (CEA)-specific CTL responses in vitro using autologous dendritic cells loaded with CEA peptide or CEA RNA in patients with metastatic malignancies expressing CEA. Int. J. Cancer, in press.

Ochsenbein, A.F., Klenerman, P., Karrer, U., Ludewig, B., Pericin, M., Hengartner, H., and Zinkernagel, R.M. (1999). Immune surveillance against a solid tumor fails because of immunological ignorance. Proc. Natl. Acad. Sci. USA *96*, 2233–2238.

Oehen, S.U., Ohashi, P.S., Burki, K., Hengartner, H., Zinkernagel, R.M., and Aichele, P. (1994). Escape of thymocytes and mature T cells from clonal deletion due to limiting tolerogen expression levels. Cell. Immunol. *158*, 342–352.

Old, L.J. (1981). Cancer immunology, the search for specificity—G.H.A. Lowes memorial lecture. Cancer Res. *41*, 361–375.

Old, L.J., Boyse, E.A, Clarke, D.A., and Carswell, E.A. (1962). Antigenic properties of chemically induced tumors. Ann. N. Y. Acad. Sci. *101*, 80–106.

Overwijk, W.M., Lee, D.S., Surman, D.R., Irvine, K.R., Touloukian, C.E., Chan, C.-C., Carroll, M.W., Moss, B., Rosenberg, S.A., and Restifo, N.P. (1999). Vaccination with a recombinant vaccinia virus encoding a "self" antigen induces autoimmune vitiligo and tumor cell destruction in mice. Requirement for $CD4^+$ T lymphocytes. Proc. Natl. Acad. Sci. USA *96*, 2982–2987.

Poindexter, N.J., Landon, C., Whiteley, P.J., and Kapp, J.A. (1992). Comparison of the T cell receptors on insulin-specific hybridomas from insulin transgenic and nontransgenic mice. J. Immunol. *149*, 38–44.

Poplonski, L., Vukusic, B., Pawling, J., Clapoff, S., Roder, J., Hozumi, N., and Wither, J. (1996). Tolerance is overcome in beef insulin-transgenic mice by activation of low-avidity autoreactive T cells. Eur. J. Immunol. *26*, 601–609.

Prehn, R.T., and Main, J.M. (1957). Immunity to methylcholanthrene-induced sarcomas. J. Natl. Cancer Inst. *18*, 769–778.

Prevost-Blondel, A., Zimmermann, C., Stemmer, C., Kulmburg, P., Rosenthal, F.M., and Pircher, H. (1998). Tumor-infiltrating lymphocytes exhibiting high ex vivo cytolytic activity fail to prevent murine melanoma tumor growth in vivo. J. Immunol. *161*, 2187–2194.

Qin, Z., Richter, G., Schüler, T., Ibe, S., Cao, X., and Blankenstein, T. (1998). B cells inhibit induction of T cell-dependent tumor immunity. Nat. Med. *4*, 627–630.

Ramarathinam, L., Sarma, S., Maric, M., Zhao, M., Yang, G., Chen, L., and Liu, Y. (1995). Multiple lineages of tumors express a common tumor antigen, P1A, but they are not cross-protected. J. Immunol. *155*, 5323–5329.

Rosato, A., Zambon, A., Milan, G., Ciminale, V., D'Agostino, D.M., Macino, B., Zanovello, P., and Collavo, D. (1997). CTL response and protection against P815 tumor challenge in mice immunized with DNA expressing the tumor-specific antigen P815A. Hum. Gene Ther. *8*, 1451–1458.

Rosenberg, S.A. (1999). A new era for cancer immunotherapy based on the genes that encode cancer antigens. Immunity *10*, 281–287.

Rosenberg, S.A., Yang, J.C., Schwartzentruber, D.J., Hwu, P., Marincola, F. M., Topolian, S.L., Restifo, N.P., Dudley, M.E., Schwartz, S.L., Spiess, P.J., et al. (1998). Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat. Med. *4*, 321–327

Roth, J., Dittmer, D., Rea, D., Tartaglia, J., Paoletti, E., and Levine, A.J. (1996). p53 as a target for cancer vaccines. Recombinant canarypox virus vectors expressing p53 protect mice against lethal tumor cell challenge. Proc. Natl. Acad. Sci. USA *93*, 4781–4786.

Rowley, D.A., and Stach, R.M.. (1993). A first or dominant immunization. I. Suppression of simultaneous cytolytic T cell responses to unrelated alloantigens. J. Exp. Med. *178*, 835–840.

Sahin, U., Türeci, Ö., and Pfreundschuh, M. (1997). Serological identification of human tumor antigens. Curr. Opin. Immunol. *9*, 709–716.

Speiser, D.E., Kyburz, D., Stübi, U., Hengartner, H., and Zinkernagel, R.M. (1992). Discrepancy between in vitro measurable and in vivo virus neutralizing cytotoxic T cell reactivities. J. Immunol. *149*, 972–980.

Staveley-O'Carroll, K., Sotomayor, E., Montgomery, J., Borrello, I., Hwang, L., Fein, S., Pardoll, D., and Levitsky, H. (1998). Induction of antigen-specific T cell anergy, an early event in the course of tumor progression. Proc. Natl. Acad. Sci. USA *95*, 1178–1183.

Syrengelas, A.D., and Levy, R. (1999). DNA vaccination against the idiotype of a murine B cell lymphoma: mechanism of tumor protection. J. Immunol. *162*, 4790–4795.

Tamura, Y., Peng, P., Liu, K., Daou, M., and Srivastava, P.K. (1997). Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science *278*, 117–120.

Thompson, C.B., and Allison, J.P. (1997). The emerging role of CTLA-4 as an immune attenuator. Immunity *7*, 445–450.

Toes, R.E.M., Ossendorp, F., Offringa, R., and Melief, C.J.M. (1999). $CD4^+$ T cells and their role in antitumor immune responses. J. Exp. Med. *189*, 753–756.

Townsend, A., and Bodmer, H. (1989). Antigen recognition by class I-restricted T lymphocytes. Annu. Rev. Immunol. *7*, 601–624.

Uyttenhove, C., Maryanski, J., and Boon, T. (1983). Escape of mouse mastocytoma P815 after nearly complete rejection is due to antigen-loss variants rather than immunosuppression. J. Exp. Med. *157*, 1040–1052.

Van den Eynde, B., Lethé, B., Van Pel, A., De Plaen, E., and Boon, T. (1991). The gene coding for a major tumor rejection antigen of tumor P815 is identical to the normal gene of syngeneic DBA/2 mice. J. Exp. Med. *173*, 1373–1384.

van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., Knuth, A., and Boon, T. (1991). A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science *254*, 1643–1647.

van Parijs, L., and Abbas, A.K. (1996). Role of Fas-mediated cell death in the regulation of immune responses. Curr. Opin. Immunol. *8*, 355–361.

van Pel, A., and Boon, T. (1982). Protection against a nonimmunogenic mouse leukemia by an immunogenic variant obtained by mutagenesis. Proc. Natl. Acad. Sci. USA *79*, 4718–4722.

Vierboom, M.P.M., Nijman, H.W., Offringa, R., van der Voort, E.I.H., van Hall, T., van den Broek, L., Fleuren, G.J., Kenemans, P., Kast, W.M., and Melief, J.M (1997). Tumor eradication by wild-type p53-specific cytotoxic T lymphocytes. J. Exp. Med. *186*, 695–704.

von Herrath, M.G., Dockter, J., and Oldstone, M.B.A. (1994). How virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. Immunity *1*, 231–242.

Whiteside, T.L. (1998). Immune cells in the tumor microenvironment. Mechanisms responsible for functional and signaling defects. Adv. Exp. Med. Biol. *451*, 167–171.

Wick, M., Dubey, P., Koeppen, H., Siegel, C.T., Fields, P.E., Chen, L., Bluestone, J.A., and Schreiber, H. (1997). Antigenic cancer cells grow progressively in immune hosts without evidence for T cell exhaustion or systemic anergy. J. Exp. Med. *186*, 229–238.

Yewdell, J.W., and Bennink, J.R. (1999). Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Annu. Rev. Immunol. *17*, 51–88.

Zajac, A.J., Murali-Krishna, K., Blattman, J.N., and Ahmed, R. (1998). Therapeutic vaccination against chronic viral infection, The importance of cooperation between $CD4^+$ and $CD8^+$ T cells. Curr. Opin. Immunol. *10*, 444–449.

Zeh, III, H.J., Perry-Lalley, D., Dudley, M.E., Rosenberg, S.A., and Yang, J.C. (1999). High avidity CTLs for two self-antigens demonstrate superior in vitro and in vivo antitumor efficacy. J. Immunol. *162*, 989–994.

Zorn, E., and Hercend, T. (1999a). A MAGE-6-encoded peptide is recognized by expanded lymphocytes infiltrating a spontaneously regressing human primary melanoma lesion. Eur. J. Immunol. *29*, 602–607.

Zorn, E., and Hercend, T. (1999b). A natural cytotoxic T cell response in a spontaneously regressing human melanoma targets a neoantigen resulting from a somatic point mutation. Eur. J. Immunol. *29*, 592–601.