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Melanoma Cancer Vaccines and Anti-Tumor T Cell Responses

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Abstract Melanoma is a disease which has been shown to be responsive to immune intervention. This has been suggested by reports of spontaneous responses of metastatic disease with strong immune infiltrates, and supported by recent data correlating clinical response after IFNα treatment with development of generalized autoimmunity. Since the identification of melanoma-associated tumor antigens, many groups have performed clinical trials to take advantage of this discovery with melanoma-specific cancer vaccines. These trials, in which multiple antigen delivery strategies have been tested in hundreds of patients, have demonstrated that these vaccines are safe, immunogenic, and yield a low frequency of objective clinical responses. The ability to perform careful immunological monitoring has allowed important insights into the nature of the anti-tumor immunity generated by these vaccinations. While many trials have found that the absolute frequency of T cells specific for a vaccine-encoded antigen are a marker of immunization, it does not correlate with objective clinical response. Induction of broad immunity to multiple tumor antigens, taking advantage of cross-reactive T cells and activation of persistent T cells may be more important. Harnessing additional modes of amplifying immune responses (lymphodepletion, cytokine support, inhibition of negative immune self-regulation) are now being tested and should improve clinical responses from 5% to 10% complete response seen currently. J. Cell. Biochem. 102: 301–310, 2007.

Key words: peptides/epitopes; dendritic cells; tumor antigens

Previously it has been observed that certain human tumors, particularly melanoma and renal cell carcinoma (RCC), can occasionally undergo spontaneous regression [Gromet et al., 1978; Tihan and Filippa, 1996]. Examination of such regressing lesions implicated the immune system, and in particular tumor-specific CD8+ T lymphocytes, as mediating these anti-tumor effects. Three lines of evidence implicate CD8+ cytotoxic T cells as major anti-tumor effectors in humans. First, immunosuppressed transplant recipients display higher incidences of non-viral tumors, such as melanomas, colon, lung, pancreas, bladder, kidney, and endocrine system cancers than immunocompetent control populations [Penn, 1996]. Second, the presence of lymphocytes within the tumor is often a positive prognostic indicator of patient survival [Clemente et al., 1996]. Third, a minority of cancer patients (<5%) are able to develop spontaneous innate and acquired immune responses to the tumors they bear [Boon and van der Bruggen, 1996; Old and Chen, 1998]. It is for these reasons that stimulation of tumor-specific CD8+ T lymphocytes has become the focus of many clinical trials.

Since the first description of a molecularly defined human tumor-associated antigen (TAA) recognized by cytotoxic CD8+ T cells [van der Bruggen et al., 1991], advances in understanding the nature of tumor-specific immune responses and mechanisms of tolerance induction have encouraged researchers and clinicians alike to develop a more refined approach to immune-mediated therapies. Studies utilizing expression cloning of TAA cDNAs have been integrated with novel strategies such as reverse immunology, biochemical methods, genetic

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approaches, and serological analysis of recombination expression libraries (SEREX) to identify a number of TAAs. Reverse immunology refers to a strategy where epitopes are predicted on the basis of known HLA-binding motifs from an already identified TAA. Biochemical methods involve eluting and fractionating TAA peptides naturally expressed on tumor cells in the context of HLA molecules by reverse-phase high-performance liquid chromatography (HPLC) and mass spectrometry. Genetic approaches are used to identify tumor genes coding for the epitopes recognized by isolated patient cytotoxic T cell clones reactive against autologous tumors. SEREX is based on the recognition of tumor antigens by cancer patient's autologous sera. All of these strategies have successfully been utilized to identify a number of TAA that can be presented by tumor cells or by antigen presenting cells (APCs) in the context of major histocompatibility complex (MHC) molecules on their cell surfaces [Van den Eynde and van der Bruggen, 1997; Jager et al., 1999; Wang, 1999].

According to the pattern of expression in neoplastic and normal tissues, TAAs can be classified into four major categories (Table I). The first category is cancer-testis antigens. These are proteins encoded by genes expressed in various tumors but not in normal tissues, except for testis and placenta. Antigens that belong to this group are MAGE, GAGE, and BAGE families, as well as NY-ESO-1 and its alternative ORF products LAGE and CAMEL. The second group represents differentiation antigens that are shared between tumors and the normal tissue from which the tumor arose. Most identified to date are expressed in melanoma and normal melanocytes, such as tyrosinase, Melan-A/MART-1, gp100, TRP-1, and TRP-2. The third category is tumor-specific antigens. These antigens are generated by point mutations (e.g., p53, Ras, CDK4, β-catenin) [Jager et al., 1999; Wang, 1999] or tumor-specific splicing aberrations in genes that are ubiquitously expressed [e.g., TRP-2/INT2; Lupetti et al., 1998], and are expressed only in tumors from the patient from whom they were identified (unlike cancer-testis antigens). These molecular changes are associated with neoplastic transformation and/or progression. The fourth group of antigens is widely occurring, over-expressed TAA. These are proteins that have been detected in histologically different types of tumors (often with no preferential expression on a certain type of cancer) as well as in many normal tissues, generally with lower expression levels. Some of the antigens belonging to this group include survivin, MUC1/2, α-fetoprotein (AFP) and EphA2, among others [Butterfield et al., 1999].

Constructing an effective peptide-based vaccination strategy against tumors has proven to be a challenging undertaking. Since most TAA used for active specific immunotherapy are considered to be "self"-antigens, one of the main challenges facing immunologists is to develop methods that can effectively and safely break tolerance to TAA. In order to activate CD8+ T cells, the peptide–epitopes they recognize must first be identified (sometimes screened via MHC class I binding rules, other times via peptide library screening) and then tested for immunogenicity and confirmed to be naturally processed and presented by APC and tumors. Peptides do provide a quick, simple and inexpensive strategy for vaccination, to utilize the host's endogenous APC to present TAA peptides, and are generally delivered in an adjuvant.

Table II lists seven examples of peptide-based clinical trials published over the last 10 years, 1997–2007 [Cormier et al., 1997; Rosenberg et al., 1998a; Slingluff et al., 2001, 2004; Schaed et al., 2002; Speiser et al., 2005; Hamid et al., 2007]. These trials began with safety dose ranging trials and have quickly progressed

TABLE I. Summary of Tumor Antigen Categories

TAA categories	Antigen characteristics	Genes
Cancer—testis	Expressed in various tumors but not normal tissues except in testis and placenta	MAGE, GAGE, BAGE, NY-ESO-1
Differentiation	Antigens shared between tumors and normal tissues from which they arose	Tyrosinase, Melan-A/MART-1, gp100, TRP-1, and TRP-2
Tumor-specific	Antigens generated by point mutations or splicing aberrations in ubiquitous genes	p53, Ras, CDK4, β-catenin, TRP-2/INT2
Widely occurring over-expressed	Proteins over-expressed in histologically different types of tumors	Survivin, MUC1/2, AFP and EphA2

TABLE II. Clinical Trial Summary

Strategy[a]	Author/year[b]	Patients[c]	Setting[d]	Responses[e]	Comments[f]
Peptides					
MART-1_{27-35} peptide IFA	Cormier/1997	23	s.c. ×4 dose escalation	No clinical responses. Some immune responses	Immune monitoring w/ IVS
$Gp100_{209}$ native or 2M peptide/IFA	Rosenberg/ 1998a,b	9, native 11 subst. 31 +IL-2	Peptide/IFA s.c., IL-2 was high dose i.v.	1 PR/9 3 MR/11 13 PR + 4 MR/31 $Gp100_{209/2M}$ more immunogenic	Immune monitoring w/ IVS
$Gp100_{280}$ peptide, ±helper tetanus peptide, two different adjuvant	Slingluff/2001	22 high risk stage 2–4	s.c. ×7	Improved PFS over expected, adjuvants similar, too few to see if "help" helped	No ex vivo immune response, with IVS 3+/21
$Tyrosinase_{368/370D}$ + $gp100_{209/2M}$ in IFA, QS21, or GM-CSF adjuvants	Schaed/2002	31, 27 evaluable	s.c. ×3	30% tyros. immune responses	Immune monitoring w/ IVS, IFA worse
Multi-peptide/IFA in GM-CSF + low dose IL-2 together or as delayed boost	Slingluff/2004	40 high risk	s.c and i.d., PBL and SIN assessment	75% immune response+, trend for better survival. Delayed IL-2 better survival	SIN had higher frequencies than PBL, IVS used, IL-2 worse for immune response
MART-1 (ELA mod.) peptide/IFA ± CpG7909	Speiser/2005	16	s.c. ×4	CpG group 8/8 immune response+, 4/8 w/o CpG	Native and modified peptide targets recognized
Multi-peptides/IFA ± IL-12 alum, ± GM-CSF	Hamid/2007	60 stage II–IV	s.c. ×9	IL-12 at either dose was superior to IL-12 + GM-CSF	Native and modified peptide targets recognized, modified better
Antigen presenting cells/DC					
MART-1 peptide-pulsed PBMC + rhIL-12	Peterson/2003	20	s.c. ×3 × 3 cycles	2 CR, 5 MR	Ex vivo ELISPOT correlated with clinical responses
MAGE-1 peptide-pulsed GM-cultured APC	Mukherji/1995; Hu/1996	3	i.d. and i.v. ×4	Distant metastatic site responses	Immune monitoring w/ IVS
MAGE 1/3 pulsed dendritic cells (DC) ± KLH	Toungouz/2001	23	s.c. and i.v. ×4	6/8 immune response to pep/DC, 6/12 in pep/DC + KLH	KLH group not superior
CD34-DC + multi-peptides +Flu + KLH	Banchereau/2002	18	s.c. ×4	16/18 immune responses, 10/17 "non-progressors"	Overall immune score correlates w/clinical response
Multi-class I and II MAGE peptide-pulsed DC + KLH	Schuler-Thurner/ 2002	16	s.c. ×5	1 CR, 8 SD	Ex vivo CD4+ TAA immune responses
DC + lysate + KLH	Chang/2002	14	i.d. ×3	1 PR, 1 MR	3/17 immune response
MART-1 peptide-pulsed DC	Butterfield/2003	18	i.v or i.d ×3, dose escalation	1 CR, 2 SD/10 metastatic pt.	CR had determinant spreading, i.d. better immune response
MART-1 peptide-pulsed DC	Ribas/2004	10	i.d ×3, dose expansion trial	1 CR	CR had determinant spreading
Multi-peptide pulsed DC or lysate pulsed, +KLH	Hersey/2004	33	14 pep/DC 19 lysate/DC Intra-l.n. ×7	3 PR, 1MR all lysate	DTH correlated with clinical response, no IFNg immune response
MART-1, gp100 peptide pulsed DC	Grover/2006	6	Intra-lymphatic	1 PR, little blood immune response	Pronounced rashes in sun-exposed skin with TAA-specific T cells
DC+ killed allogeneic melanoma cells	Palucka/2006	20	s.c. ×4–8	1 CR, 1 PR	3/13 immune response
DC trafficking	Quillies/2006		i.d., intra-l.n., intra-lymphatic	i.d only 1–2% to l.n.	i.lymphatic strongest l.n. response i.l.n. similar
Tumor cells					
Autologous tumor + GM-CSF	Liuten/2005	64	High and low GM dose vaccine	2 SD (1 high, 1 low). 3/14 high dose immune response+	88% vaccines made, many progressed early
Genetic immunization					
Plasmid DNA gp100, ±GM-CSF (gene gun)	Cassaday/2007	18	6 gp100, 6 GM, 6 both	1/6 SD, 2 groups	More local DC with GM-CSF, minimal gp100 response
Adenovirus AdVMART or AdVgp100, ±IL-2	Rosenberg/ 1998a,b	54	s.c., i.m., dose escalation	1/16 CR, AdVMART only, others maybe due to addition of IL-2	3 more CR + 2 PR with IL-2 addition

(*Continued*)

TABLE II. (*Continued*)

Strategy[a]	Author/year[b]	Patients[c]	Setting[d]	Responses[e]	Comments[f]
Fowlpox gp100	Rosenberg/2003	46	Native cDNA, mod. cDNA,miniepitope gene, i.v., i.m.	1/7 native, 10/14 mod. cDNA, 12/16 minigene immune responses	1/46 PR mod. cDNA, 0/13 cDNA + IL-2, 6/12 (3 CR, 3 PR) minigene +IL-2, i.v. = i.m.
Canarypox gp100(mod.) prime, gp100 peptide boost ± tetanus toxoid	Spaner/2006	42	6 designs, i.l.n., s.c.	8/18 immune response to pox, 0/6 peptide, i.l.n. >s.c. 2 SD	Tetanus toxoid did not improve responses
Vaccinia and fowlpox tyrosinase prime boost ± high or low dose IL-2	Lindsey/2006	64	4 arms,each virus × 2 i.m.	7 PR in +IL-2 group 12 MR (most w/IL-2). 3/23 + immune response by PCR	Clinical responses expected from IL-2 alone
Adoptive transfer					
$Gp100_{209}$-specific CD8 T cell adoptive transfer	Dudley/2001	13 pt for cells, 11 pt + IL-2 s.c or i.v.	Previous gp100 peptide pt. T cells cloned	2 MR with IL-2 (pt. previously failed IL-2 alone)	T cells were detectable for 2 weeks
MART-1 specific CTL adoptive transfer	Mackensen/2006	11	i.v. ×3 infusion of 4× IVS expanded CTL	1 CR, 1 PR, 1 MR	T cells detected for 2 weeks, also eosinphilia
Gp100 CTL adoptive transfer after lymphdepletion +IL-2 +vaccine	Powell/2006	9	Previous gp100 vaccinated pt. lymphodepleted, adoptive CTL transfer +IL-2 and either gp100 pox or peptides	No clinical response	Cells persisted longer than 1 month
MART-1 TCR-transfected PBL transfer after lymphodepletion, +IL-2	Morgan/2006	17	Bulk CD4+ and CD8+ cells transferred, shorter IVS time	2 PR, 1 MR	Long T cell persistence

[a]IFA, incomplete Freund's adjuvant; IL, interleukin; GM-CSF, granulocyte-macrophage colony stimulating factor; TIL, tumor infiltrating lymphocytes; PBL, peripheral blood lymphocytes.
[b]First author and year of publication.
[c]Number of patients.
[d]Routes of administration: s.c., subcutaneous; i.v., intra-venous; i.l.n., intra-lymph node, intra-lymphatically; i.d., intradermal, ×number of injections.
[e]PR, partial response; MR, mixed response; CR, complete response.
[f]IVS, in vitro stimulation; KLH, keyhole limpet hemocyanin; DTH, delayed-type hypersensitivity.

(perhaps due to the simplicity of the setting) to test peptides with optimized sequences, comparison of adjuvants, addition of heterologous helper peptides, and inclusion of cytokines. Importantly, multiple trials have observed objective clinical responses in a small cohort of treated patients, proving the principle of peptide-based immunizations. The clear answer to some questions asked in randomized trials have been limited by small samples sizes, a common issue for immunotherapy trials. This is an issue for the question of whether heterologous "helper" proteins like KLH improve the peptide-specific CD8+ T cell response. An important outcome of these trials is the demonstration that clinical responses often correlate with some aspects of the induced immune responses; not strictly the peripheral blood frequency of peptide-specific T cells induced by the vaccine (as tested by MHC tetramer or IFNg ELISPOT), but sometimes DTH, antigen breadth or determinant spreading.

A second common strategy used to promote TAA-specific responses employs the potent immunostimulatory capacity of autologous APC, generally dendritic cells (DC), pulsed with different TAA, often based on the same peptide epitopes recognized by CD8+ and/or CD4+ T lymphocytes used in adjuvant. These defined epitopes are restricted for presentation to T cells by specific MHC class I and II alleles, and therefore may only be used productively to immunize or monitor a limited cohort of patients that express these MHC alleles. Therefore, additional strategies, including pulsing DC with autologous (and uncharacterized) tumor lysate, have also been tested. Table II gives examples of 11 trials testing APC (9/11 with DC) pulsed with antigens, published over the last 12 years [Mukherji et al., 1995; Hu et al., 1996; Banchereau et al., 2001; Toungouz et al., 2001; Chang et al., 2002; Schuler-Thurner et al., 2002; Butterfield et al., 2003; Peterson et al., 2003; Hersey et al., 2004; Ribas et al., 2004; Quillien et al., 2005; Grover et al., 2006; Palucka et al., 2006]. One important complication is that the field of DC biology has evolved considerably since the initiation of these trials, and the "immature" monocyte-derived myeloid DC (cultured for 7 days in GM-CSF and IL-4) have expanded to include those derived from CD34+ progenitors, highly purified DC "matured" to different degrees in uncharacterized patient-specific cocktails (monocyte-conditioned medium) or characterized cytokine cocktails in order to improve cell surface phenotype and T cell activation function. Immature and mature DC have only been compared directly in mice (in which mature DC are superior) but the extent of maturation needed, the optimal maturation for a particular injection site or antigen loading method is unknown. This is impacted by the cost to prepare these cells, in the range of $10,000–$20,000 per patient, which can restrict the number of patients per arm in a clinical trial.

To date, these DC vaccines are safe and immunogenic, capable of inducing immune responses and clinical responses, again, which often correlate with each other by some measures. Initial safety studies have moved on to vaccines including multiple peptides, addition of stimulation for CD4 helper T cells (defined peptides or full-length proteins in lysate), and testing of different loading strategies, maturation cocktails and injection routes. There has been evidence from murine models that the site of antigen encounter can imprint homing preferences on resultant activated T cells. In melanoma, the skin melanocytes are the originating cancer cell, hence many trials have used s.c. or i.d. vaccine administration. Despite resultant T cell activation, only 1–2% of injected DC are observed to traffic to draining lymph nodes [Barratt-Boyes et al., 1997, 2000; Quillien et al., 2005]. In humans, i.v. does not appear optimal, but choices among other routes are unclear. In order to optimize delivery of DC to lymph nodes, intra-nodal and intra-lymphatic delivery has been tested. These routes result in superior localization of DC in the lymph nodes [Quillien et al., 2005], but one trial testing responses from intra-lymphatic delivery showed little T cell response. Randomized trials comparing routes are clearly needed to resolve this issue.

Genetic immunization has been tested in which full-length native antigen, epitope-modified full-length antigen or minigene epitope constructs have been directly administered to patients [Rosenberg et al., 1998b, 2003; Lindsey et al., 2006; Spaner et al., 2006; Cassaday et al., 2007]. Despite presence of full-length antigen, and, in the case of viral vectors, the presence of immunogenic foreign viral proteins, the resultant immune and clinical responses have not been superior to that seen with DC or peptides. When constructs were compared directly, a mini-gene epitope in a viral vector appeared

superior to full-length antigen in the same vector [Rosenberg et al., 2003].

Direct administration of some viral vectors can be inhibited by systemic neutralizing antibodies and the inability to deliver multiple doses. Progress in genetic immunization has also been hampered by critical differences between pre-clinical murine models and human (lack of previous exposure to immunogenic viral vectors in mice, differences in TLR9 expression and CpG motif responses from DNA constructs, among others). Murine data indicates that plasmid DNA can be an effective immunogen, while primate studies have shown that multiple injections with enormous doses (5 mg) of plasmid DNA are required to detect successful vaccination with DNA alone. Additional stimulation, for example, boosting with more immunogenic antigen-encoding virus can improve immunity [Meng et al., 2001; Shiver et al., 2002; Casimiro et al., 2003].

Most recently, adoptive transfer of ex vivo-cultured effectors has been tested [Dudley et al., 2001; Mackensen et al., 2006; Morgan et al., 2006; Powell et al., 2006]. This area has evolved from the long history of testing adoptive transfer of tumor-infiltrating lymphocytes (TIL) expanded ex vivo, pioneered by the Rosenberg group at the NCI Surgery branch. In order to better characterize the cells transferred, strategies tested include transfer of peptide-specific clones in high numbers, cells cultured for shorter times in lower numbers (to reduce differentiation), systemic cytokine support, lymphodepletion of patients, in vivo restimulation with vaccine administration post-transfer, and cells engineered to express a specific TCR. Early efforts were hampered by lack of persistence of the transferred cells after 2 weeks, which has been improved by both cytokine support and delivery into a lymphopenic host. Detailed study of the clinically effective subsets of cells, along with further addition of elements such as Treg depletion or anti-CTLA-4 antibody may allow these effectors to be more clinically efficacious.

FUTURE DIRECTIONS

Immune Response Enhancement Through Determinant Spreading

The correlation between different aspects of immune response and clinical outcome in several trials suggests that the breadth of the antigen-specific response is a critical element. The logic of this is clear. For the tumor, if loss of expression of one antigen occurs (or class of antigens coordinately regulated) other expressed TAA can be targeted. It is possible that long term expression of certain antigens may lead to tolerance or deletion of high avidity responder T cell clones, but this might be circumvented by expanding T cells specific to multiple expressed tumor antigens, particularly those whose expression has increased with tumor progression. From an immune regulatory stand point, induction of multi-antigen, broad immunity, directly from the vaccine may lead to self-regulation of that response, explaining the observation of transient detection of vaccine-activated lymphocytes. In the case of determinant spreading, a "driver clone" [Sercarz et al., 1993; perhaps induced by the vaccine], is sufficiently potent to lead to antigen-specific tumor cell lysis in an immuno-stimulatory milieu (perhaps created by cytokine secretion by infiltrating lymphocytes or local APC). The release of tumor cell contents, including TAA and endogenous "danger signals" allows for a new round of antigen presentation and potential for sequential waves of lymphocyte activation. This would allow for potentiation of anti-tumor immunity, instead of transient activation and down-regulation. This might be enhanced by targeting antigens whose expression is not simply coincident with tumor progression, but is functionally important for the tumor.

Enhancement of TAA-Specific T Cell Responses Using Epitope Analogues

In the past it was believed that individual T cell clones were capable of distinguishing and responding to a unique epitope sequence presented in the context of an autologous MHC complex. Recent studies have instead suggested that a fair degree of T cell cross-reactivity exists and is in fact necessary to maintain an immune system with sufficient flexibility to adapt to a continuously changing antigenic environment. Indeed, T cell clones thought to be specific for an antigen have been shown to recognize peptides differing considerably in their amino acid sequences [Hemmer et al., 1998; Mason, 1998; Kohrt et al., 2005]. "Analogue" or "heteroclitic" peptides refer to those peptides that share a high degree of homology with naturally occurring, wild-type tumor epitopes, and induce cross-reactive T cells to their homologues. Most

of these studies examined CD8+ T cell responses against 9-mer peptides. While the anchor residues at positions 2 and 9 of HLA-A2 MHC class I-presented epitopes have been shown to be highly restricted, the other amino acids of reactive peptides differed at as many as six or seven of the remaining positions. Importantly, a large proportion (one-third to one-half) of analogue peptide-stimulated T cells to produce IFNγ at peptide concentrations far lower than that of the native peptide, suggesting the higher functional avidity of TCR for analogue peptides presented by MHC class I molecules. In fact, analogue epitopes have been shown to be more effective at breaking immunological tolerance than cognate wild-type epitopes [Hoffmann et al., 2002]. Some in vivo studies further substantiated these observations. One study showed that immunization with a $gp100_{209-217}$ (210M) heteroclitic melanoma antigen peptide promoted the development of circulating effector-memory T cells that were reactive against the wild type $gp100_{209-217}$ epitope [Chiong et al., 2004]. Individual amino acid substitutions have also been associated with differential cytokine responses in one study of MART-1_{26-35}-specific CD8+ T lymphocytes. Substitution of the N-terminal amino acid of this 10-mer dictated whether the T cell response would be Type-1 or Type-0 [both Type-1 and Type-2 cytokines secreted; Nielsen et al., 2000]. Data from clinical trials which tested anchor-modified peptides found, however, that not all analogue-specific T cells from all patients recognize tumors presenting native peptides.

Analogue peptides have also been observed in nature, and these cross-reactive epitopes have been coined "epitope mimics". Epitope mimicry has been described as a potential mechanism underlying the induction of autoimmune diseases due to pathologic T cells primed against infectious microorganisms that cross-react against host proteins in susceptible individuals. Diseases such as viral myocarditis, lyme disease, rheumatoid arthritis [Davies, 1997], multiple sclerosis [Brocke et al., 1998], and virus-induced autoimmune diabetes [Hudrisier et al., 2001; Moriyama et al., 2002] have long been considered to be initiated or exacerbated by microbial pathogens. From this observation came an idea that immunotolerance to TAA could be broken by employing mimicking epitopes to stimulate TAA-specific lymphocytes. A study performed by Loftus, et al. showed that the HLA-A2-presented MART-1_{27-35} epitope bears sufficient sequence or conformational homology to peptides derived from microbial proteins (to which many individuals may have become naturally primed), allowing for functional T cell-mediated cross-reactivity [Loftus et al., 1996].

One way to take advantage of this cross-reactivity to better promote cancer immunity is to utilize pathogen-specific T cells which recognize TAA and purposefully activate them to promote potentially tumor-specific responses. Recently, a *Mycoplasma penetrans* HF-2 permease-derived epitope (MPHF2) that is highly homologous to the novel promiscuous, HLA-DR-restricted peptide encoded within the 172–187 amino acid region of MAGE-A6 (MAGE-$A6_{172-187}$) was identified. MPHF2 peptide-primed CD4+ T cells cross-recognized autologous monocytes pulsed with the MAGE-$A6_{172-187}$ peptide or recombinant MAGE-A6 protein. This cross-reactivity appeared to be on a clonal level as HLA-DR4-restricted, CD4+ T cell clone obtained by limiting dilution from the bulk culture of MPHF2 peptide-primed CD4+ T cells specific for MPHF2 peptide effectively cross-recognized both the MAGE-$A6_{172-187}$ peptide and HLA-matched MAGE-A6+ melanoma cell lines (Vujanovic et al., 2007, in preparation). Therefore, from these studies one may hypothesize that there is a limited flexibility of TCR antigenic specificity that could potentially be exploited for immunomonitoring strategies or more importantly to stimulate TAA-specific lymphocyte responses in patients who may have become functionally tolerant to their TAA.

CONCLUSIONS

Melanoma cancer vaccines trials have progressed to test many strategies in many trial settings. The principle that these vaccines can induce clinically relevant anti-tumor immunity has been proven. Harnessing of determinant spreading may be critical for further efficacy of cancer vaccines in order to have multiple waves of responses to multiple antigens. Testing of non-antigen-specific boosting (by systemic cytokines like IFNα or blocking of CTLA-4) in conjunction with tumor antigen-specific vaccines may be a promising area for new vaccine trials. Similarly, taking advantage of the available, cross-reacting T cells which can also recognize tumor and which may be of higher

avidity, may allow for improved responses to cancer vaccines, while still allowing the mechanistic dissection that a defined antigen approach allows.

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