Antigenic Peptide Vaccination: Provoking Immune Response and Clinical Benefit for Cancer

Tomohide Tsukahara[1,2], Toshihiko Torigoe[1], Yasuaki Tamura[1], Takuro Wada[2], Satoshi Kawaguchi[2], Tetsuhiro Tsuruma[3], Koichi Hirata[3], Toshihiko Yamashita[2] and Noriyuki Sato[*,1]

[1]*Department of Pathology, Sapporo Medical University School of Medicine, South-1, West-17, Chuo-ku, Sapporo, 060-8556, Japan*

[2]*Department of Orthopaedic Surgery, Sapporo Medical University School of Medicine, South-1, West-16, Chuo-ku, Sapporo, 060-8543, Japan*

[3]*Department of Surgery, Sapporo Medical University School of Medicine, South-1, West-16, Chuo-ku, Sapporo, 060-8543, Japan*

Abstract: Recent immunotherapy depends largely on understanding of the molecular interactions between T cell receptors (TCR) on cytotoxic T lymphocytes (CTL) and peptide/MHC class I complexes on tumor cells. Many tumor antigens identified by cDNA library expression cloning methods, especially from malignant melanoma, have greatly contributed to clarifying such mechanisms and led to peptide vaccination trials, mainly for patients with melanoma. Although the objective tumor regression rate mediated by peptide vaccination is still low compared to adoptive cell transfer therapy, antigenic peptide vaccination can cause a constant objective response generally evaluated as stable disease or decreased serum levels of tumor markers. In addition, recent trials in the adjuvant setting showed some suppressive effects against recurrence. Therefore, peptide vaccination still has potential for clinical benefits in patients with various cancers. For further improvement of peptide vaccination, we considered that (i) novel antigenic peptides, (ii) effective adjuvants, (iii) more sensitive immunological monitoring and (iv) drugs up-regulating HLA class I molecules might be important.

Keywords: Peptide vaccination, tumor-associated antigen, CTL epitope, clinical trial.

INTRODUCTION

Recent immunotherapy depends largely on understanding of the molecular interactions between T cell receptors (TCR) on cytotoxic T lymphocytes (CTL) and peptide/MHC class I complexes on tumor cells. Many tumor antigens identified by cDNA library expression cloning, especially from malignant melanoma, have greatly contributed to clarifying such mechanisms and made feasible vaccinations, mainly for patients with melanoma. Various vaccination approaches, including those with antigenic peptides [1], recombinant viruses encoding antigenic genes [2], dendritic cells and antigenic proteins [3] were reported. Recent adoptive transfer of *ex vivo* expanded autologous tumor-infiltrating lymphocytes following chemotherapeutic lymphodepletion combined with total body irradiation [4] and adoptive transfer of T lymphocytes in which antigen-specific TCR is genetically engineered [5] resulted in strong clinical responses. Nevertheless, we are still focusing on peptide-based vaccination and have identified novel antigenic peptides by forward and reverse immunological approaches. In this review, we describe the recent status of the field of peptide-based vaccination immunotherapy and future perspectives on the basis of our work.

*Address correspondence to this author at the Department of Pathology, Sapporo Medical University School of Medicine, South-1, West-17, Chuo-ku, Sapporo, 060-8556, Japan; Tel: +81-11-611-2111; Fax: +81-11-643-2310; E-mail: nsatou@sapmed.ac.jp

IDENTIFICATION OF TUMOR ANTIGENS FOR PEPTIDE VACCINATION

Many tumor-associated antigenic genes and peptides recognized by CTLs have been identified since 1991 when the first CTL-defined tumor antigen, *MAGE,* was found [6]. Mainly in melanoma studies, tumor antigens were cloned by cDNA library expression cloning using CTL lines reacting with autologous tumor cells. This strategy is called the 'forward immunological approach.' The forward immunological approach can detect 'true' antigens naturally priming the cellular immune system of the patient. However, especially in non-melanocytic tumors, the establishment of autologous pairs of tumor cell-CTL lines is very difficult [7]. On the other hand, recent many antigenic tumor genes were screened by 'the reverse immunological approach', on the basis of the tumor-specific expression profiles obtained from cDNA microarrays and various bioinformatics databases, followed by *in vitro* stimulation of CTLs reacting with candidate antigen-derived peptides and natural tumor cells [8, 9]. This approach does not require a CTL line reacting with autologous tumor cells and makes feasible identification of tumor antigens associated with various cancers.

From melanoma studies, tumor antigens were categorized on the basis of their expression profiles in tumor tissues and normal organs into five groups: (i) cancer-testis antigens, (ii) melanoma-melanocyte differentiation antigens, (iii) mutated (unique) antigens, (iv) shared overexpression antigens and (v) ubiquitous antigens. This categorization is also adaptable

1573-3955/08 $55.00+.00

for non-melanocytic tumors including antigens associated with epithelial cancer and sarcoma. The comprehensive database of CTL-defined tumor antigens and peptides in the context of HLA class I and class II is constantly updated by the Ludwig Institute for Cancer Research, Brussels Branch, Belgium (available at http://www.cancerimmunity.org/links/databases.htm). Considering the tumor-specific expression status of target antigens, CT antigens, differentiation antigens and overexpression antigens can be used as target molecules. We are still focusing on the identification of novel tumor antigens and antigenic peptides by forward and reverse immunological approaches [10]. Candidate tumor antigens and peptides we previously identified are shown in Table **1** [11-19].

CLINICAL STUDY: PEPTIDE VACCINATION AND ADOPTIVE CELL TRANSFER

Adoptive Cell Transfer: Strong Clinical Response

Since the first vaccination trial of a tumor-associated antigenic peptide in 1995 [20], much work on identification of CTL epitopes derived from tumor antigens has been conducted to promote clinical vaccination trials and immunomonitoring [1]. In the beginning, the immunological and clinical results suggested that peptide vaccination therapy was a promising modality against metastatic melanoma [21]. After one decade, Rosenberg *et al*. reviewed the past vaccination trials and concluded that this strategy could hardly mediate the objective response [22]. Although this pessimistic judgment of vaccination trials has been criticized [23, 24], general attention shifted from peptide vaccination to adoptive transfer. Adoptive tumor-infiltrating lymphocyte (TIL) transfer therapy, which started from 1996, reached an objective response of ≥50% in patients with metastatic melanoma in combination with lymphodepletion chemotherapy [25, 26]. This approach was augmented by total body irradiation for the further depletion of regulatory T cells and stimulation of innate immunity *via* Toll-like receptor (TLR) 4 [27, 28]. Moreover, adoptive transfer of T lymphocytes in which antigen-specific TCR was genetically engineered was performed in patients with metastatic melanoma [5]. This approach could be applicable for non-melanocytic cancer, for which there is limited availability of *ex vivo* expanded TIL [29]. Adoptive transfer of T lymphocytes activated *ex vivo* showed that adequate effector status of T cells is essential in addition to a sufficient number of T cells to kill the solid tumor mass. At present, adoptive effector cell transfer might be the most effective strategy mediating the objective regression of solid tumors graded by the RECIST criteria [30]. However, the adoptive cell transfer strategy still has the following limitations: (i) lymphodepletion chemotherapy can cause severe infectious disease and (ii) the requirement for special institutes meeting the criteria of the GMP grade for handling the T lymphocytes ex vivo limits the popularization of this approach.

Peptide Vaccination: Weak But Certain Clinical Response

On the other hand, peptide vaccination trials are still continuing and attempts have been made to trigger immunological responses and clinical responses. In addition to melanoma studies using the MAGE family and melanoma-melanocyte differentiation antigens (gp100, tyrosinase and Melan-A/MART-1), many tumor-associated antigens identified from non-melanocytic cancers were targeted to elicit T cell proliferation and activation (Table **2**) [31-47]. The target diseases for studies have also been expanded to non-melanocytic cancers. Although the precursor frequency of anti-vaccine CTLs in peripheral blood was still low for *in situ* detection using tetramers, cytokine ELISA and ELISPOT, anti-vaccine CTL responses *in vivo* were detected in many clinical studies including non-melanocytic cancers. The rate of objective tumor regression (CR or PR) was also estimated to be low, though antigenic vaccination could cause certain objective responses against disseminated cancers, including reduction of tumor masses, which was generally evaluated as SD, and reduction of serum tumor markers from the beginning of the vaccination trial [21]. Peptide vaccines have some advantages compared with adoptive T lymphocyte transfer therapy: (i) side effects more than grade 3

Table 1. Tumor-Associated Antigens and Candidate Peptides for Vaccination Trial

Antigen	Peptide	HLA	Disease	Vaccination Trial	Ref.
Forward Immunological Approach					
c98	YSWMDIITIC	A31	Gastric cancer		[11]
PBF	CTACRWKKACQR	B55	Osteosarcoma		[12]
	AYRPVSRNI	A24	Osteosarcoma	Planned	[13]
	ALPSFQIPV	A2	Osteosarcoma	Planned	[14]
Reverse Immunological Approach					
Survivin	AYACNTSTL	A24	Lung, gastric, colorectal, pancreatic and breast cancers	Ongoing	[15]
Livin	KWFPSCQFLL	A24	Lung cancer		[16]
Recoverin	QFQSIYAKFF	A24	Lung cancer		[17]
SYT-SSX	GYDQIMPKK	A24	Synovial sarcoma	Ongoing	[18]
	GYDQIMPKI*	A24	Synovial sarcoma	Ongoing	[19]

*Aggretope-substituted peptide.

Table 2. Phase I/II Clinial Trials of Antigenic Peptide Vaccination Since 2004

						Anti-Vaccine CTL Response		Clinical Response				
Target Antigen	**Peptide Vaccine**	**HLA Restriction**	**Adjuvant**	**Disease**	**n**	**Method**	**Response**	**Criteria**	**Response**	**Adverse Effect ‡**	**Correlation†**	**Ref.**
NY-ESO-1	SLLMWITQV* WITQCFLPVFLA QPPSGQRA	A2 DP4	IL-2	Melanoma	37	ELISA	100%	RECIST	PR; 3%	2%	ND	[31]
gp100	GRAMLGTHT MEVTV	A2, (DR53, DQ6)	IFA GM-CSF	Melanoma	28	Tetramer	57%	RECIST	SD; 4%	None	No	[32]
gp100	IMDQVPFSV	A2	IL-2	Melanoma	26	ELISPOT Tetramer	65% 31%	RECIST	SD; 31%	27%	No	[33]
hTERT	YLFFYRKSV * RLFFYRKSV	A2 A2	IFA	NSCLC	22	ELISPOT Pentamer	88% 90%	RECIST	SD; 36%	None	Yes	[34]
hTERT	YLFFYRKSV * RLFFYRKSV	A2 A2	IFA	Various	19	Tetramer	93%	WHO	SD; 21%	None	ND	[35]
WT1	CMTWNQMNL CYTWNQMNL*	A24	IFA	Various	26	Tetramer Intracellular FACS	50%	Tumor marker Number of blast cells	Reduction; 76%	12%	Yes	[36]
Survivin	AYACNTSTL	A24	None	Colorectal cancer	15	Tetramer	50%	RECIST Tumor marker	MR; 7% Reduction; 40%	None	ND	[37]
Survivin	AYACNTSTL	A24	IFA	Breast cancer	14	Tetramer ELISPOT	50%	RECIST	SD; 14%	None	ND	[38]
SYT-SSX	GYDQIMPKK	A24	None	Synovial sarcoma	6	Tetramer	50%	RECIST	SD; 17%	None	No	[39]
CA9	EYRALQLHL AYEQLLSRL RYFQYEGSL	A24	IFA	RCC	23	ELISA	76%	WHO	PR; 13% SD; 26%	None	Yes	[40]
Multiple (12 antigens)	Multiple (48 peptides)	A2, A24	IFA	RCC	10	ELISA	5%	RECIST	SD; 60%	None	ND	[41]
Multiple (9 antigens)	Multiple (16 peptides)	A24	IFA	Prostate cancer	16	ELISA	57%	Serum PSA level	Reduction; 100%	None	ND	[42]
Multiple (7 antigens)	Multiple (14 peptides)	A24	IFA	Prostate cancer	10	ELISA	50%	Serum PSA level	Reduction; 20%	None	No	[43]
Multiple (8 antigens)	Multiple (16 peptides)	A2	IFA	Prostate cancer	10	ELISA	40%	Serum PSA level	Reduction; 30%	None	No	[44]
Adjuvant setting												
NY-ESO-1	SLLMWITQC	A2	IFA	Ovarian cancer	9	Tetramer ELISPOT	78%	Recurrence-free rate at 22 months	33%	None	No	[45]
HER2/neu	KIFGSLAFL	A2, A3	GM-CSF	Breast cancer	186	Immuno-globlin dimer assay	ND	Recurrence-free rate at 20 months	Vaccinated group; 94.4% Non-vaccinated group; 85.8%	2%	ND	[46]
Multiple (6 antigens)	Multiple (4 peptides)	A1, A2, A3	IFA tetanus helper peptide GM-CSF	Melanoma	52	ELISPOT	87%	Overall survival at 24 months	89%	37%	Yes	[47]

*Aggretope-substituted peptide.

†Correlation between immunological response and clinical response.

‡The proportion of reactions scaled as more than grade 3, according to the National Cancer Institute Common Toxicity Criteria.

are merely observed and generally tolerable, (ii) there is no requirement for special institutes, and (iii) costs for manufacturing and vaccination are relatively low. Recent studies of adjuvant vaccination with MAGE3 protein increased the 5-year survival rate in patients with non-small-cell lung cancer (NSCLC) [48, 49]. In addition, peptide vaccination trials in the adjuvant setting were also performed [45-47]. These results have encouraged many researchers.

Peptide Vaccination: Current Problems

(i) Status of Circulating Anti-Vaccine CTLs: Function and Frequency

Discrepancies between immunological responses and clinical responses remain unsolved. With regard to the immunological aspect, we support the idea that thorough monitoring is still required to detect the immunological status provoked by vaccination and to improve the current vaccination strategy for the next generation [23, 24]. In cases in which anti-vaccine CTLs positively detected by tetramers, cytokine ELISPOT or ELISA could not mediate tumor regression, the functional status of CTLs *in vivo* was altered from effector-memory or memory to effector by manipulation with *in vitro* stimulation. Adoptive T lymphocyte transfer studies also supported the idea that adequate *ex-vivo* activated T cells could reject large tumor masses. Speiser *et al*. reported that *ex-vivo* five-cell PCR of sorted tetramer-positive cells from peripheral blood showed that cytokine profiles affecting the natural status were provoked by vaccination [50, 51].

On the other hand, immunosuppressive cells might affect the effector function of CTLs. Regulatory T cells (Treg) have been reported and reviewed in detail as the critical suppressive factor in peripheral blood and the tumor microenvironment in patients bearing cancer [52]. Several drugs depleting Treg, including denileukin diftitox (ONTAK), the anti-cytotoxic T-lymphocyte antigen-4 (CTLA-4) antibody and anti-folate receptor 4 antibody have been shown to have potential for the enhancement of anti-vaccine CTLs in *in vivo* studies [53-55]. Recently, myeloid-derived suppressor cells (MDSC) were focused on with regard to immune escape. In the peripheral lymphoid organs, MDSC present antigens to antigen-specific T lymphocytes and induce nitration of TCR and CD8 molecules on the T-lymphocyte surface. This results in conformational changes in these molecules and induces loss of their ability to bind to the peptide-MHC complex on tumor cells [56]. The blockade of peroxynitrite generation, which could induce nitration, might have the possibility to enhance the anti-tumor immunity.

Considering that antigenic peptides are derived from self-antigens, most anti-vaccine CTLs might have low- or moderate-affinity TCR because of clonal deletion of T lymphocytes reacting to self-antigens with high affinity TCR in the thymus, which is called central tolerance. Recently, Janicki *et al*. reported that CTLs having high affinity TCR could form tumor-infiltrating lymphocytes, although they lose effector function. Meanwhile, T lymphocytes recognizing self-antigens could become tolerant as a result of the conformational change of TCR modified by addition of inhibitory or removal of activating molecules [57]. This suggests that expansion of the anti-vaccine CTLs having adequate characteristics of TCR by active peptide vaccination is still difficult. However, we think that novel tumor antigens, epitopes and vaccination have some possibility to induce effective CTLs having such TCR. Adjuvants also might be able to alter the clonal diversity of TCR repertoire [58].

If anti-vaccine CTLs cannot be detected by standard monitoring procedures in spite of positive clinical responses, more sensitive procedures are required to detect them at extremely low frequencies. Limiting dilution (LD)/mixed lymphocyte peptide culture (MLPC) followed by tetramer-based frequency analysis is the most sensitive method now available [59-62]. Collected peripheral blood mononuclear cells (PBMCs) are stimulated with antigenic peptides *in vitro* under limiting dilution conditions (200,000 cells/well of 96-well microculture plates), followed by detection of tetramer-positive anti-vaccine CTLs. With many internal negative pools, the positive pools including tetramer-positive cells are carefully identified. This procedure could provide the sensitivity to detect anti-vaccine CTLs under the 10^{-7} level in non-vaccinated patients and healthy donors. Moreover, the sensitivity might be increased by increasing the amount of PBMCs. We analyzed the precursor frequency of CTLs against osteosarcoma antigen papillomavirus binding factor (PBF)-derived peptide in the context of HLA-A24 and A2 by LD/MLPC/tetramer analysis [13, 14]. Among non-vaccinated patients with osteosarcoma, the peripheral frequency of anti-PBF CTLs was detected at between $5x10^{-7}$-$7x10^{-6}$ and $2x10^{-7}$-$5x10^{-6}$ in HLA-A*2402-positive patients and HLA-A*0201-positive patients, respectively. In addition, the frequency of anti-PBF CTLs was detected at between $8x10^{-7}$-$5x10^{-6}$ and $1x10^{-7}$-$5x10^{-7}$ in HLA-A*2402-positive and HLA-A*0201-positive healthy donors, respectively (Tsukahara *et al*. unpublished observation 2008). However, this procedure requires intensive laboratory work [63, 64].

(ii) Status of Tumor Cells: The Loss of Antigens and HLA Class I Molecules

With regard to tumor biology, the problem of tumor escape after vaccination remains. Tumor escape results from the loss of antigens and the loss of antigen-presenting HLA class I molecules. The loss of antigens is easy to resolve by using multiple peptides or targeting molecules essential for tumor cell survival. We performed vaccination trials targeting the inhibitor of apoptosis protein survivin, which plays a key role in resistance to various apoptotic stimuli [15, 37, 38]. As described above, we consider that intensive laboratory work to identify novel tumor-associated antigens and related peptides is still required. The loss or down-regulation of HLA class I molecules is another classic but important problem. It is well known that tumor cells can lose HLA class I molecules on the cell surface and escape from immune pressure [65-67]. We observed that the loss or down-regulation of HLA class I molecules occurred in 100% and 45% of non-responders and responders to survivin-derived peptide vaccination, respectively (Torigoe *et al*. unpublished observation 2007). Although the sample size was very small, the expression of HLA class I was negative in 3 of 3 synovial sarcoma specimens. The propensity of synovial sarcoma cells to lose HLA class I may also serve as an obstacle for immunotherapeutic trials such as one we undertook using SYT-SSX fusion gene-derived peptide vaccine [39]. We also observed that epigenetic silencing of beta2-microglobulin was the key point to explain the loss or down-regulation of

HLA class I. Moreover, oral administration of the histone deacetylase inhibitor valproic acid caused retrieval of the HLA class I expression on xenograft tumors in mouse models (Torigoe *et al*. unpublished observation 2007). In addition, the correlation between the loss or down-regulation of HLA class I molecules and poor prognosis in renal cell cancer [68], NSCLC [69] and osteosarcoma [70] also supports the important role of HLA class I expression in the immune escape of various tumors.

Peptide Vaccination in the Future: Augmentation with TLR Agonists

To strengthen the vaccine-mediated immunological response, novel adjuvant drugs are highly desirable. Some candidates were already described above. On the basis of studies regarding TLR signaling in innate immunity, TLR agonists were introduced as adjuvants for the activation of antigen-presenting dendritic cells by vaccination. Many drugs, including TLR agonists, were reviewed and scored in the NCI Immunotherapy Workshop Proceedings (available at the NCI-Frederick web site; http://web.ncifcrf.gov/research/brb/workshops.asp). In addition to CpG (a TLR9 agonist) and poly I:C (a TLR3 agonist), monophosphoryl lipid-A (MPLA; a TLR4 agonist) was introduced as a novel adjuvant candidate. MPLA is a low-toxicity derivative of lipopolysaccharide (LPS; a component of the bacterial wall) and could trigger production of type I interferon (interferon-alpha and -beta) and T cell proliferation equal to LPS [71]. We used interferon-alpha as an adjuvant in peptide vaccination trials and found a strong immune response and clinical response (PR graded by RECIST) in one patient with recurrent pancreatic cancer (Iwayama *et al.* unpublished observation, 2007). Although it is still unclear what adjuvant is optimal to activate and expand anti-vaccine T lymphocytes, the finding of additional novel TLR agonists as adjuvants is anticipated.

OUR FUTURE PERSPECTIVES

Our further projects are composed of (i) a PBF-derived peptide vaccination trial for patients with osteosarcoma, and (ii) peptide vaccination with heat-shock protein as a novel adjuvant. As described above, without these further approaches, it seems to be difficult to enhance anti-vaccine CTLs having adequate TCR avidity and effector function. The adjuvant effects of TLR ligands, drugs depleting Treg and cytokines should be clinically assessed. Nevertheless, in the future, we believe that antigenic peptide vaccination with strong adjuvants will provoke immune responses and objective responses against cancer.

New Target: Osteosarcoma Antigen PBF

Osteosarcoma is a high-grade malignancy originating from mesenchymal cells. Before 1970, the 5-year survival rate of patients with osteosarcoma was less than 10%. To develop new treatment modalities, vaccination trials for osteosarcomas were initially conducted for patients with osteosarcoma during 1970s [72]. Surprisingly, autologous tumor lysate vaccination showed some effect to increase the survival rate [73]. However, during the same period, multidrug adjuvant chemotherapy including high dose methotrexate was demonstrated to raise the 5-year survival to 60-70% [74, 75]. Although vaccination could not outperform chemotherapy, its potential to trigger the host immune system and reject tumor cells conferring metastasis, especially in the adjuvant setting, is certainly present. As the first step, we identified osteosarcoma-associated antigen PBF using an autologous pair comprised of an osteosarcoma cell line and a CTL clone [12, 76]. PBF is a nuclear-cytoplasmic shuttling transcription factor that regulates apoptosis [77]. PBF protein was expressed in 92% of primary osteosarcoma tissues. Moreover, PBF-positive osteosarcomas conferred a poorer prognosis than those with negative expression of PBF [13]. Therefore, PBF might be a candidate target for peptide vaccination clinical trials. As the next step, we analyzed the frequency and function of anti-PBF CTLs in peripheral blood of patients with osteosarcoma [13, 14]. Among non-vaccinated patients with osteosarcoma, the peripheral frequency of anti-PBF CTLs was between $5x10^{-7}$-$7x10^{-6}$ and $2x10^{-7}$-$5x10^{-6}$ in HLA-A*2402-positive patients and HLA-A*0201-positive patients, respectively. The low frequency of anti-PBF CTLs might support the evidence that spontaneous regression of osteosarcoma is extremely rare [78, 79]. Now we are planning a phase I study of PBF-derived peptide vaccination with IFA or interferon-alpha in end-stage patients with osteosarcoma. Although strong objective clinical responses in many peptide vaccination trials for various cancers could hardly be observed, vaccination targeting a novel tumor-associated antigen PBF for osteosarcoma might have a certain possibility to induce some objective responses in addition to immunological responses. Considering the early study of vaccination with autologous tumor lysates [73], PBF-derived peptide vaccination trials in adjuvant or neoadjuvant settings seem attractive.

New Adjuvant: Heat-Shock Protein

As mentioned above, new adjuvants are expected to elicit strong immune responses. Activation of innate immunity in addition to acquired immunity against a vaccine might be essential to further increase efficacy. We focused on molecular chaperone heat-shock protein 90 (hsp90), which could elicit anti-tumor CTL responses in mouse models [80]. Our preclinical study demonstrated that DCs could take up the exogenous hsp90-peptide vaccine complex and present the peptide on DCs in the context of HLA class I molecules *via* a cross-presentation pathway. As a result, the hsp90-antigenic peptide complex could elicit anti-vaccine CTLs [81]. Moreover, hsp90 could induce the production of inflammatory cytokines (TNF-alpha, IL-1, IL-6 and IL-12) *via* TLR-2 and -4 signaling pathways [82]. Therefore, hsp90 might be promising for an adjuvant effect in the peptide vaccination strategy.

ACKNOWLEDGEMENTS

This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant No. 16209013 to N. Sato, No. 20390403 to T. Wada), Practical Application Research from the Japan Science and Technology Agency (Grant No. H14-2 to N. Sato), the Ministry of Health, Labor and Welfare (Grant No. H17-Gann-Rinsyo-006 to T. Wada), Postdoctoral Fellowship of the Japan Society for the Promotion of Science (Grant No. 02568 to T. Tsukahara), Northern Advancement Center for Science and Technology (Grant No. H18-Waka-075 to T. Tsukahara) and The Uehara Memorial Foundation (Grant No. H19-Kenkyu-Syorei to T. Tsukahara).

REFERENCES

[1] Mocellin S, Mandruzzato S, Bronte V, Lise M, Nitti D. Part I: Vaccines for solid tumours. Lancet Oncol 2004; 5: 681-9.

[2] van Baren N, Bonnet MC, Dreno B, *et al*. Tumoral and immunologic response after vaccination of melanoma patients with an ALVAC virus encoding MAGE antigens recognized by T cells. J Clin Oncol 2005; 35: 9008-21.

[3] Thurner B, Haendle I, Roder C, *et al*. Vaccination with MAGE-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med 1999; 190: 1669-78.

[4] Dudley ME, Rosenberg SA. Adoptive cell transfer therapy. Semin Oncol 2007; 34: 524-31.

[5] Morgan RA, Dudley ME, Wunderlich JR, *et al*. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 2006; 314: 126-9.

[6] Van der Bruggen P, Traversari C, Chomez P, *0*. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 1991; 254: 1643-7.

[7] Kawaguchi S, Wada T, Tsukahara T, *et al*. A quest for therapeutic antigens in bone and soft tissue sarcoma. J Transl Med 2005; 3: 31.

[8] Van der Bruggen P, Zhang Y, Chaux P, *et al*. Tumor-specific shared antigenic peptides recognized by human T cells. Immunol Rev 2002; 188: 51-64.

[9] Kawakami Y, Fujita T, Matsuzaki Y, *et al*. Identification of human tumor antigens and its implications for diagnosis and treatment of cancer. Cancer Sci 2004; 95: 784-91.

[10] Tsukahara T, Kawaguchi S, Nagoya S, *et al*. Novel approach to immunotherapy for epithelial cancers, bone and soft-tissue sarcomas. Ann Cancer Res Ther 2004; 12: 53-70.

[11] Sahara H, Nabeta Y, Torigoe T, *et al*. A gene encoding human gastric signet ring cell carcinoma antigen recognized by HLA-A31-restricted cytotoxic T lymphocytes. J Immunother 2002; 25: 235-42.

[12] Tsukahara T, Nabeta Y, Kawaguchi S, *et al*. Identification of human autologous cytotoxic T-lymphocyte-defined osteosarcoma gene that encodes a transcriptional regulator, papillomavirus binding factor. Cancer Res 2004; 64: 5442-8.

[13] Tsukahara T, Kawaguchi S, Torigoe T, *et al*. Prognostic impact and immunogenicity of a novel osteosarcoma antigen, papillomavirus binding factor, in patients with osteosarcoma. Cancer Sci 2008; 99: 368-75.

[14] Tsukahara T, Kawaguchi S, Torigoe T, *et al*. HLA-A*0201-restricted CTL epitope of a novel osteosarcoma antigen, papillomavirus binding factor, submitted 2008.

[15] Hirohashi Y, Torigoe T, Maeda A, *et al*. An HLA-A24-restricted cytotoxic T lymphocyte epitope of a tumor-associated protein, survivin. Clin Cancer Res 2002; 8: 1731-9.

[16] Hariu H, Hirohashi Y, Torigoe T, *et al*. Aberrant expression and potency as a cancer immunotherapy target of inhibitor of apoptosis protein family, Livin/ML-IAP in lung cancer. Clin Cancer Res 2005; 11: 1000-9.

[17] Maeda A, Ohguro H, Nabeta Y, *et al*. Identification of human antitumor cytotoxic T lymphocytes epitopes of recoverin, a cancer-associated retinopathy antigen, possibly related with a better prognosis in a paraneoplastic syndrome. Eur J Immunol 2001; 31: 563-72.

[18] Sato Y, Nabeta Y, Tsukahara T, *et al*. Detection and induction of CTLs specific for SYT-SSX-derived peptides in HLA-A24(+) patients with synovial sarcoma. J Immunol 2002; 169: 1611-8.

[19] Ida K, Kawaguchi S, Sato Y, *et al*. Crisscross CTL induction by SYT-SSX junction peptide and its HLA-A*2402 anchor substitute. J Immunol 2004; 173: 1436-43.

[20] Rosenberg SA. Development of cancer immunotherapies based on identification of the genes encoding cancer regression antigens. J Natl Cancer Inst 1996; 88: 1635-44.

[21] Rosenberg SA, Yang JC, Schwartzentruber DJ, *et al*. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med 1998; 4: 321-7.

[22] Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med 2004; 10: 909-15.

[23] Mocellin S, Mandruzzato S, Bronte V, Marincola FM. Cancer vaccines: pessimism in check. Nat Med 2004; 10: 1278-9; author reply 9-80.

[24] Manjili MH, Kmieciak M, Keeler J. Comment on "Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific CD8+ T cells in patients with melanoma". J Immunol 2006; 176: 4511; author reply -2.

[25] Dudley ME, Wunderlich JR, Robbins PF, *et al*. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002; 298: 850-4.

[26] Dudley ME, Wunderlich JR, Yang JC, *et al*. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol 2005; 23: 2346-57.

[27] Paulos CM, Kaiser A, Wrzesinski C, *et al*. Toll-like receptors in tumor immunotherapy. Clin Cancer Res 2007; 13: 5280-9.

[28] Paulos CM, Wrzesinski C, Kaiser A, *et al*. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells *via* TLR4 signaling. J Clin Invest 2007; 117: 2197-204.

[29] Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer 2008; 8: 299-308.

[30] Restifo NP, Rosenberg SA. Use of standard criteria for assessment of cancer vaccines. Lancet Oncol 2005; 6: 3-4.

[31] Khong HT, Yang JC, Topalian SL, *et al*. Immunization of HLA-A*0201 and/or HLA-DPbeta1*04 patients with metastatic melanoma using epitopes from the NY-ESO-1 antigen. J Immunother 2004; 27: 472-7.

[32] Celis E. Overlapping human leukocyte antigen class I/II binding peptide vaccine for the treatment of patients with stage IV melanoma: evidence of systemic immune dysfunction. Cancer 2007; 110: 203-14.

[33] Roberts JD, Niedzwiecki D, Carson WE, *et al*. Phase 2 study of the g209-2M melanoma peptide vaccine and low-dose interleukin-2 in advanced melanoma: Cancer and Leukemia Group B 509901. J Immunother 2006; 29: 95-101.

[34] Bolonaki I, Kotsakis A, Papadimitraki E, *et al*. Vaccination of patients with advanced non-small-cell lung cancer with an optimized cryptic human telomerase reverse transcriptase peptide. J Clin Oncol 2007; 25: 2727-34.

[35] Mavroudis D, Bolonakis I, Cornet S, *et al*. A phase I study of the optimized cryptic peptide TERT(572y) in patients with advanced malignancies. Oncology 2006; 70: 306-14.

[36] Oka Y, Tsuboi A, Taguchi T, *et al*. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. Proc Natl Acad Sci USA 2004; 101: 13885-90.

[37] Tsuruma T, Hata F, Torigoe T, *et al*. Phase I clinical study of anti-apoptosis protein, survivin-derived peptide vaccine therapy for patients with advanced or recurrent colorectal cancer. J Transl Med 2004; 2: 19.

[38] Tsuruma T, Iwayama Y, Ohmura T, *et al*. Clinical and immunological evaluation of anti-apoptosis protein, survivin-derived peptide vaccine in phase I clinical study for patients with advanced or recurrent breast cancer. J Transl Med 2008; 6: 24.

[39] Kawaguchi S, Wada T, Ida K, *et al*. Phase I vaccination trial of SYT-SSX junction peptide in patients with disseminated synovial sarcoma. J Transl Med 2005; 3: 1.

[40] Uemura H, Fujimoto K, Tanaka M, *et al*. A phase I trial of vaccination of CA9-derived peptides for HLA-A24-positive patients with cytokine-refractory metastatic renal cell carcinoma. Clin Cancer Res 2006; 12: 1768-75.

[41] Suekane S, Nishitani M, Noguchi M, *et al*. Phase I trial of personalized peptide vaccination for cytokine-refractory metastatic renal cell carcinoma patients. Cancer Sci 2007; 98: 1965-8.

[42] Noguchi M, Itoh K, Yao A, *et al*. Immunological evaluation of individualized peptide vaccination with a low dose of estramustine for HLA-A24+ HRPC patients. Prostate 2005; 63: 1-12.

[43] Sato Y, Maeda Y, Shomura H, *et al*. A phase I trial of cytotoxic T-lymphocyte precursor-oriented peptide vaccines for colorectal carcinoma patients. Br J Cancer 2004; 90: 1334-42.

[44] Noguchi M, Itoh K, Suekane S, *et al*. Phase I trial of patient-oriented vaccination in HLA-A2-positive patients with metastatic hormone-refractory prostate cancer. Cancer Sci 2004; 95: 77-84.

[45] Diefenbach CS, Gnjatic S, Sabbatini P, *et al*. Safety and Immunogenicity Study of NY-ESO-1b Peptide and Montanide ISA-51 Vaccination of Patients with Epithelial Ovarian Cancer in High-Risk First Remission. Clin Cancer Res 2008; 14: 2740-8.

[46] Peoples GE, Holmes JP, Hueman MT, *et al*. Combined clinical trial results of a HER2/neu (E75) vaccine for the prevention of recurrence in high-risk breast cancer patients: U.S. Military Cancer Institute Clinical Trials Group Study I-01 and I-02. Clin Cancer Res 2008; 14: 797-803.

[47] Slingluff CL, Jr., Petroni GR, Chianese-Bullock KA, *et al*. Immunologic and clinical outcomes of a randomized phase II trial of two multipeptide vaccines for melanoma in the adjuvant setting. Clin Cancer Res 2007; 13: 6386-95.

[48] Brichard VG, Lejeune D. GSK's antigen-specific cancer immunotherapy programme: pilot results leading to Phase III clinical development. Vaccine 2007; 25 (Suppl 2): B61-71.

[49] Atanackovic D, Altorki NK, Cao Y, *et al*. Booster vaccination of cancer patients with MAGE-A3 protein reveals long-term immunological memory or tolerance depending on priming. Proc Natl Acad Sci USA 2008; 105: 1650-5.

[50] Speiser DE, Lienard D, Rufer N, *et al*. Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. J Clin Invest 2005; 115: 739-46.

[51] Speiser DE, Baumgaertner P, Barbey C, *et al*. A novel approach to characterize clonality and differentiation of human melanoma-specific T cell responses: spontaneous priming and efficient boosting by vaccination. J Immunol 2006; 177: 1338-48.

[52] Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008; 133: 775-87.

[53] Dannull J, Su Z, Rizzieri D, *et al*. Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. J Clin Invest 2005; 115: 3623-33.

[54] Keilholz U. CTLA-4: negative regulator of the immune response and a target for cancer therapy. J Immunother 2008; 31: 431-9.

[55] Yamaguchi T, Hirota K, Nagahama K, *et al*. Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. Immunity 2007; 27: 145-59.

[56] Nagaraj S, Gabrilovich DI. Tumor escape mechanism governed by myeloid-derived suppressor cells. Cancer Res 2008; 68: 2561-3.

[57] Teague RM, Greenberg PD, Fowler C, *et al*. Peripheral CD8+ T cell tolerance to self-proteins is regulated proximally at the T cell receptor. Immunity 2008; 28: 662-74.

[58] Malherbe L, Mark L, Fazilleau N, McHeyzer-Williams LJ, McHeyzer-Williams MG. Vaccine adjuvants alter TCR-based selection thresholds. Immunity 2008; 28: 698-709.

[59] Coulie PG, Karanikas V, Colau D, *et al*. A monoclonal cytolytic T-lymphocyte response observed in a melanoma patient vaccinated with a tumor-specific antigenic peptide encoded by gene MAGE-3. Proc Natl Acad Sci U S A 2001; 98: 10290-5.

[60] Karanikas V, Lurquin C, Colau D, *et al*. Monoclonal anti-MAGE-3 CTL responses in melanoma patients displaying tumor regression after vaccination with a recombinant *canarypox virus*. J Immunol 2003; 171: 4898-904.

[61] Germeau C, Ma W, Schiavetti F, *et al*. High frequency of antitumor T cells in the blood of melanoma patients before and after vaccination with tumor antigens. J Exp Med 2005; 201: 241-8.

[62] So T, Hanagiri T, Chapiro J, *et al*. Lack of tumor recognition by cytolytic T lymphocyte clones recognizing peptide 195-203 encoded by gene MAGE-A3 and presented by HLA-A24 molecules. Cancer Immunol Immunother 2007; 56: 259-69.

[63] Coulie PG, Connerotte T. Human tumor-specific T lymphocytes: does function matter more than number? Curr Opin Immunol 2005; 17: 320-5.

[64] Boon T, Coulie PG, Van den Eynde BJ, van der Bruggen P. Human T cell responses against melanoma. Annu Rev Immunol 2006; 24: 175-208.

[65] Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol 2000; 74: 181-273.

[66] Algarra I, Garcia-Lora A, Cabrera T, Ruiz-Cabello F, Garrido F. The selection of tumor variants with altered expression of classical and nonclassical MHC class I molecules: implications for tumor immune escape. Cancer Immunol Immunother 2004; 53: 904-10.

[67] Bubenik J. MHC class I down-regulation: tumour escape from immune surveillance? Int J Oncol 2004; 25: 487-91.

[68] Kitamura H, Torigoe T, Honma I, *et al*. Effect of human leukocyte antigen class I expression of tumor cells on outcome of intravesical instillation of bacillus calmette-guerin immunotherapy for bladder cancer. Clin Cancer Res 2006; 12: 4641-4.

[69] Kikuchi E, Yamazaki K, Torigoe T, *et al*. HLA class I antigen expression is associated with a favorable prognosis in early stage non-small cell lung cancer. Cancer Sci 2007; 98: 1424-30.

[70] Tsukahara T, Kawaguchi S, Torigoe T, *et al*. Prognostic significance of HLA class I expression in osteosarcoma defined by anti-pan HLA class I monoclonal antibody, EMR8-5. Cancer Sci 2006; 97: 1374-80.

[71] Mata-Haro V, Cekic C, Martin M, *et al*. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Science 2007; 316: 1628-32.

[72] Campbell CJ, Cohen J, Enneking WF. Editorial: New therapies for osteogenic sarcoma. J Bone Joint Surg Am 1975; 57: 143-4.

[73] Marcove RC. A clinical trial of autogenous vaccines in the treatment of osteogenic sarcoma. Beitr Pathol 1974; 153: 65-72.

[74] Rosen G, Marcove RC, Caparros B, *et al*. Primary osteogenic sarcoma: the rationale for preoperative chemotherapy and delayed surgery. Cancer 1979; 43: 2163-77.

[75] Ferrari S, Smeland S, Mercuri M, *et al*. Neoadjuvant chemotherapy with high-dose Ifosfamide, high-dose methotrexate, cisplatin, and doxorubicin for patients with localized osteosarcoma of the extremity: a joint study by the Italian and Scandinavian Sarcoma Groups. J Clin Oncol 2005; 23: 8845-52.

[76] Nabeta Y, Kawaguchi S, Sahara H, *et al*. Recognition by cellular and humoral autologous immunity in a human osteosarcoma cell line. J Orthop Sci 2003; 8: 554-9.

[77] Sichtig N, Silling S, Steger G. Papillomavirus binding factor (PBF)-mediated inhibition of cell growth is regulated by 14-3-3beta. Arch Biochem Biophys 2007; 464: 90-9.

[78] Ogihara Y, Takeda K, Yanagawa T, Hirasawa Y. Spontaneous regression of lung metastases from osteosarcoma. Cancer 1994; 74: 2798-803.

[79] Bacci G, Palmerini E, Staals EL, *et al*. Spontaneous regression of lung metastasis from osteosarcoma: a case report. J Pediatr Hematol Oncol 2008; 30: 90-2.

[80] Tamura Y, Peng P, Liu K, Daou M, Srivastava PK. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 1997; 278: 117-20.

[81] Kurotaki T, Tamura Y, Ueda G, *et al*. Efficient cross-presentation by heat shock protein 90-peptide complex-loaded dendritic cells *via* an endosomal pathway. J Immunol 2007; 179: 1803-13.

[82] Tsan MF, Gao B. Heat shock protein and innate immunity. Cell Mol Immunol 2004; 1: 274-9.

Received: May 29, 2008 Revised: August 4, 2008 Accepted: August 6, 2008