Cancer Chemother Pharmacol (2000) 46 (Suppl): S46–S51 

Alexander Knuth · Dirk Jäger · Elke Jäger

# Cancer immunotherapy in clinical oncology

**Abstract** The identification of tumor-associated antigens recognized by cellular or humoral effectors of the immune system has opened new perspectives for cancer therapy. Different groups of cancer-associated antigens have been described as targets for cytotoxic T lymphocytes (CTLs) in vitro and in vivo: 1) cancer-testis (CT) antigens, which are expressed in different tumors and normal testis; 2) melanocyte differentiation antigens; 3) point mutations of normal genes; 4) antigens that are overexpressed in malignant tissues; and 5) viral antigens. Clinical studies with peptides derived from these antigens have been initiated to induce specific CTL responses in vivo. Immunological and clinical parameters for the assessment of peptide-specific reactions have been defined, i.e., delayed-type hypersensitivity (DTH), CTL, autoimmmune, and tumor regression responses. Preliminary results demonstrate that tumor-associated peptides alone elicit specific DTH and CTL responses leading to tumor regression after intradermal injection. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was proven effective in enhancing peptide-specific immune reactions by amplification of dermal peptide-presenting dendritic cells. Long-lasting complete tumor regressions have been observed after induction of peptide-specific CTLs. However, in single cases with disease progression after an initial tumor response, either a loss of the respective tumor antigen targeted by CTLs or of the presenting major histocompatibility complex (MHC) class I allele was detected as a mechanism of immune escape under immunization. Based on these observations, cytokines to enhance antigen and MHC class I expression in vivo are being evaluated to prevent immunoselection. Recently, a strategy utilizing spontaneous antibody responses to tumor-associated antigens (SEREX) has led to the identification of a new CT antigen, NY-ESO-1, which is regarded as one of the most immunogenic antigens known today inducing spontaneous immune responses in 50% of patients with NY-ESO-1-expressing cancers. Clinical studies involving antigenic constructs that induce both antibody and CTL responses will show whether these are more effective for immunotherapy of cancer.

**Key words** Tumor antigens · T cell responses · Peptide immunization

Work presented at the 15th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "New Immunological Approach to Cancer Treatment," 10–11 September 1999, Nagoya, Japan

A. Knuth (✉) · D. Jäger · E. Jäger
II Medizinische Klinik, Hämatologie-Onkologie,
Krankenhaus Nordwest, Steinbacher Hohl 2-26,
60488 Frankfurt am Main, Germany
Tel.: +49-(0)69-7601-3380; Fax: +49-(0)69-769932

## Introduction

Spontaneous regressions of human tumors have been reported in different types of cancer, especially in melanoma and renal cell carcinoma [3, 19], but also in other types of cancer, such as non-small cell lung cancer, bladder carcinoma, and breast cancer. These observations suggest the interaction of the immune system with antigenic determinants presented by the tumor [37, 58]. Early attempts to activate the immune system against tumor growth were based on studies with cultured melanoma cells that were lysed by autologous $CD8^+$ T lymphocytes in vitro. The clinical translation of this observation was applied to single patients with metastatic melanoma who received irradiated autologous tumor cells as a vaccine. Two patients (SK-29 and MZ-2) with recurrent metastatic melanoma refractory to standard chemotherapy have been observed by our group since 1978 and 1982, respectively [34, 37]. Both patients received intradermal immunizations with irradiated autologous tumor cells for an extended period of time. Complete regression of all tumor manifestations was documented after prolonged immunization with autologous tumor cells. The patients have remained free

of disease for 20 and 15 years, respectively. Based on this favorable clinical outcome, a systematic search was initiated to identify and characterize the cancer antigens and immune effector mechanisms mediating these tumor regressions in vivo [35, 36].

## Human tumor antigens recognized by the immune system

### Cancer-testis antigens

Cytotoxic T lymphocytes (CTLs) were first isolated from melanoma patients and shown to lyse melanoma cells effectively in vitro [36]. Antigenic peptides have been identified which are presented by major histocompatibility complex (MHC) class I and II molecules. The first antigen recognized by CTLs in the context of human leukocyte antigen (HLA)-A1 was isolated from a melanoma cell line derived from patient MZ-2, designated as MAGE-1 [56]. Later, a family of MAGE-1-related genes (MAGE-1, MAGE-3, BAGE, GAGE) was identified which encodes antigens that are expressed in melanomas and several other tumors, but not in normal tissues except testis [5, 17, 54, 55, 56]. Therefore antigens with this pattern of expression are designated as cancer-testis (CT) antigens. More recently, the new CT antigen NY-ESO-1 was identified from an esophageal cancer using a serological approach (SEREX) that is based on the screening of recombinant tumor cDNA libraries for specific interactions with autologous serum antibodies [10, 49]. HLA-A2 binding peptides derived from NY-ESO-1 were characterized that elicit strong CTL responses in vitro [29]. Since NY-ESO-1 and members of the MAGE gene family are frequently expressed in different types of cancer, these antigens represent attractive targets for specific immunotherapy in cancer patients.

### Melanocyte differentiation antigens

A second category of antigens derived from melanocyte differentiation antigens are recognized by autologous CTLs in melanomas, which are also expressed in normal melanocytes [2, 6, 12, 32]. Several epitopes derived from these self-antigens such as Melan A/MART-1, tyrosinase, gp100/Pmel17, and gp75/TRP-1 have been found to be targets for CTLs and tumor infiltrating lymphocytes (TILs) in the context of HLA-A2.1 and other MHC class molecules [30, 33, 59], resulting in objective tumor regressions in some patients. Phase I clinical trials in melanoma patients with peptides derived from these antigens have shown that specific delayed-type hypersensitivity (DTH) reactions can be elicited after intradermal peptide injection [23]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) used as a systemic adjuvant strongly enhanced peptide-related DTH reactions in single patients [25]. In contrast to phase I clinical trials with MAGE-derived peptides, the induction of peptide-specific CTLs was observed after immunization with peptides derived from Melan A and tyrosinase [23, 25]. Furthermore, objective tumor regressions were observed in single patients under continued immunization [23, 24].

### Point mutations

Another group of potential cancer antigens is defined by point mutations of constitutive cellular proteins. These point mutations may induce strong CTL responses against tumor cells in cancer patients or experimental animals [11, 40, 60]. In breast cancer, mutations of the proteins p53 and ras have been reported. Humoral immune responses to the mutated and to the wild-type proteins occurring spontaneously in patients with breast, lung, and gastrointestinal cancer have been detected [13, 51]. In women with a family history of breast cancer, antibody responses to p53 occur at a higher incidence compared to controls (11% vs 1%) [13]. Since the majority of p53 antibodies detected are of the IgG class, a preceding CD4+ T cell response to p53 can be predicted. In single patients with breast cancer showing accumulation of p53 in primary tumors, a lymphoproliferative CD4+ T cell response to wild-type p53 was demonstrated [53]. From these observations made in a limited number of patients, it may be concluded that immune responses occur after mutation of oncoproteins. These may also be directed against nonmutated portions of the proteins. To date it is unknown whether p53 as an intracellular protein is available at the cancer cell surface or in the extracellular cancer environment to serve as an immunotherapeutic target for humoral and/or cellular effectors to mediate tumor regression.

In animal models, mutant p53 has been shown to elicit specific CTL responses that mediate lysis of the transformed cells. In a murine sarcoma model, it was demonstrated that vaccination with p53 peptides combined with interleukin (IL)-12 leads to regression of p53-expressing advanced Meth A sarcomas [44]. In many human cancers, accumulation of wild-type p53 in the cytosol can be detected. It is assumed that accumulated p53 is effectively presented by MHC class I molecules in amounts sufficient to elicit specific CTL responses. Therefore immune responses against wild-type p53 may be useful in protecting against cancers with p53 accumulation.

Ras mutations described so far involve single amino acid substitutions, mostly at positions 12 and 61. These are less complex than in p53 and thus easier to evaluate. $CD4^+$ and $CD8^+$ T cell responses leading to tumor lysis can be elicited by immunization with ras peptides containing the mutant segment in animal models [16]. In humans, it remains to be determined whether wild-type or mutant ras protein is a useful target for active or passive therapeutic immune interventions. In a limited number of patients with pancreatic cancer, immunization with MHC class I-restricted ras peptides led to proliferative T cell responses [18].

Other mutation-based antigens, which have been defined primarily via CTL recognition, i.e., MUM-1 and mutated CDK4, have been shown to generate new peptide epitopes that are presented by MHC class I molecules. It is unknown whether these antigens would be useful targets for CTL-based vaccines in a larger patient population [11, 60].

### Overexpressed antigens

Some tumor types constitutively express normal "self"-proteins in abundance. The most extensively studied self-antigens that serve as targets for active and passive immunotherapy are Melan A, a melanocyte differentiation antigen present in melanoma and normal melanocytes, and HER-2/neu, a growth factor receptor overexpressed in 30% of breast and ovarian cancers and a variety of other adenocarcinomas [13]. Immune reactions directed against this type of antigen theoretically result in destruction of normal tissues. However, preliminary experiences with peptide immunization in patients with Melan A-expressing melanomas have not shown prominent toxicity except the development of vitiligo in single patients [23]. Patients with HER-2/neu-expressing tumors have been shown to produce spontaneous humoral and cellular immune responses that may be amplified by appropriate routes of immunization toward a therapeutic response, leading to tumor regression [13].

### Viral antigens

Some human malignancies are associated with defined viral diseases, i.e., Burkitt lymphoma and Epstein-Barr virus [43], hepatocellular carcinoma and hepatitis B and C viruses [39, 46], cervical and anal carcinoma and human papillomavirus [15], and human T lymphotropic virus and T cell leukemia [38]. Independent of whether the viral infection becomes the oncogenic agent, it was shown that viral antigens are expressed in the associated tumors and can be used as targets for preventive or therapeutic vaccination [38].

## Development of immunotherapeutic strategies

### Peptides derived from CT antigens as active immunogens?

MAGE-1- and MAGE-3-derived peptides have been used as a vaccine in HLA-A1-positive patients with tumors expressing the respective antigens to assess toxicity and immunological responses. Tumor regression responses have been observed in more than 30% of melanoma patients after immunization with the MAGE-3-derived, HLA-A1-restricted peptide [4]. CTLs against MAGE-3-expressing target cells, however, could not be identified in response to the vaccine in these patients [41]. In a subsequent study using systemic GM-CSF to improve antigen presentation by enhancement of CD1a+ dermal Langerhans cells followed by intradermal administration of MAGE-1 and MAGE-3 peptides, a partial regression of liver and lung metastases was achieved in a melanoma patient within 3 months of immunization [Jäger E. et al. unpublished results]. In parallel with this remarkable clinical development, MAGE-1- and MAGE-3-specific CTLs were detected which showed an increase in frequency subsequent to immunization. Based on these promising results, phase I studies are currently being initiated to evaluate immune reactions to peptide vaccination in patients with other MAGE-expressing carcinomas.

CTLs against the HLA-A1-restricted MAGE-1- and MAGE-3-derived peptides were repeatedly isolated from the peripheral blood of patient MZ2, the patient from whom the MAGE-1 and MAGE-3 genes were cloned [17, 56]. It is strongly suggested that antigen-specific CTLs are effective mediators of tumor regression, since this patient experienced a complete regression of metastatic MAGE-1- and MAGE-3-positive melanoma metastases after repeated immunization with autologous MAGE-1/MAGE-3-positive tumor cells. During the course of repeated tumor cell vaccination, an increased frequency of CTLs recognizing autologous tumor cells was detected in the peripheral blood of this patient [20]. The specificity of CTL responses, however, could not be assessed at that time, since the structure of the autologous tumor-associated antigens was not known.

The rare detection of CTLs against MAGE genes in patients with MAGE-positive melanoma may be attributed to either low immunogenicity of MAGE genes or to a low frequency of CTL precursors. Different methods for the assessment of MAGE-specific CTL responses are being evaluated. One promising approach appears to be the ELISPOT assay, an enzyme-linked immunosorbent assay that visualizes the direct antigen/T cell receptor interaction by staining of the spot-like liberation of $\gamma$-interferon or other cytokines by the T cell reacting to a defined antigen.

## Targeting differentiation antigens in melanoma

Objective tumor responses in single melanoma patients have been observed after adoptive transfer of TIL lines recognizing gp100/Pmel17-, tyrosinase-, and gp75-derived epitopes, suggesting that differentiation antigens can serve as tumor rejection antigens [2, 31, 48]. To study further the effects of T cell interactions with melanocyte differentiation antigens in vitro and in vivo, we determined: 1) the spontaneous CTL reactivity against HLA-A2-restricted peptides derived from the differentiation antigens Melan A/MART-1, tyrosinase, and gp100/Pmel17 in HLA-A2-positive melanoma patients and healthy individuals [28]; 2) cellular immune responses to melanoma-associated peptides administered

intradermally as a vaccine to HLA-A2-positive melanoma patients [23, 49]; and 3) changes in expression of melanoma-associated antigens and peptide-presenting MHC class I molecules in melanoma tissues showing regression or progression in the presence or absence of antigen-specific CTL responses in vivo [27].

The baseline CTL reactivity against melanoma-associated peptides was determined in melanoma patients and healthy individuals as a basis for the development of active immunotherapeutic strategies using antigenic peptides. Spontaneous CTL reactivity against the differentiation antigens Melan A/MART-1, tyrosinase, and gp100/Pmel17 is frequently detected in melanoma patients and healthy individuals, without any differences in intensity and frequency of CTL responses [28, 47, 57]. In healthy individuals, Melan A-specific CTLs reactive with Melan A-expressing melanoma cells were isolated from vitiligo areas [45]. These findings suggest that CTL responses against self-antigens occur spontaneously in individuals, and may be amplified by appropriate vaccination.

Antigenic peptides derived from Melan A/MART-1, tyrosinase, or gp100/Pmel17 were shown to induce DTH reactions and specific $CD8^+$ CTL responses after intradermal immunization. The induction of objective clinical responses was associated with measurable CTL responses to the vaccine. Toxic side effects of the vaccine were not observed. Some patients with beneficial clinical development, however, developed reversible vitiligo [23, 49]. In a single patient, a clonal expansion of a Melan A-specific T cell receptor V$\beta$16 was isolated from T cell cultures stimulated with Melan A peptide, from Melan A-specific DTH reactions, and from vitiligo areas after immunization with Melan A peptide for an extended period of 5 years [24].

Dermal antigen-presenting cells, such as dendritic Langerhans cells, can be stimulated by GM-CSF in vivo [7]. Combined administration of melanoma-associated peptides and GM-CSF resulted in enhanced DTH reactions and $CD8^+$ CTL responses. Immunohistochemical characterization of DTH constituting elements showed infiltrates of $CD4^+$ and $CD8^+$ T lymphocytes and strong expression of IL-2 and $\gamma$-interferon, suggesting the activation of $CD4^+$ Th1 and $CD8^+$ CTLs by peptides presented by MHC class I molecules of dermal antigen-presenting cells [49].

## Immunoselection in vivo mediated by peptide-specific CTLs

The development of monoclonal antibodies used for immunohistochemical staining of melanocyte differentiation antigens expressed in melanoma tissues has set the basis for studying the microheterogeneity of defined antigens in tumor lesions under specific immunotherapy [8, 9]. In HLA-A2-positive melanoma patients immunized with Melan A-, tyrosinase-, and gp100-derived peptides combined with GM-CSF, we observed, after an initial phase of tumor regression in some patients, progressive disease in the presence of detectable peptide-specific CTLs that efficiently lysed HLA-matched melanoma cell lines in vitro [27]. Biopsies were obtained from lesions in the phase of progressive tumor growth and compared with the initially described homogenous antigen expression, a highly heterogenous distribution of antigens was observed in response to increased peptide-specific CTL responses. Furthermore, a loss of expression of MHC class I molecules as detected by immunohistochemistry was found in single cases, which represents an additional mechanism of immune escape from antigen-specific T cell recognition.

Future clinical studies involving antigen-specific T cell reactions in cancer patients should analyze the prognostic implication of the heterogeneity of MHC class I- and tumor-associated antigen expression in tumor tissues for T cell-based immunotherapy. Cytokines, i.e., IL-12-inducing interferon gamma, will be of interest in future trials to show whether these can mediate the upregulation of antigens and antigen-presenting molecules in tumor tissues.

## Immunotherapy in cancer: Perspectives

Different types of cancer expressing defined tumor-associated antigens may become targets for immunotherapeutic interventions. The growing number of tumor antigens detected and the lessons learned from immunotherapy in malignant melanoma have set a solid foundation for the development of immunotherapeutic strategies in cancer patients. CT antigens are regarded as promising targets for specific CTL responses induced by peptide or protein vaccines. The detection of spontaneous antibody responses to CT antigens in sera of cancer patients [52] and the correlation of antibody titers with the development of the disease [22] suggest also the spontaneous stimulation of $CD4^+$ T cells against peptides presented by MHC class II molecules on the surface of tumor cells [21]. The characterization of these antigens as targets for $CD4^+$ T cell responses will allow concurrent immunization with MHC class I and class II epitopes to mount potentially more effective immune responses.

Future perspectives of tumor vaccine development are focused on more potent strategies of immunization. Vaccination with whole proteins containing multiple possibly relevant antigenic epitopes may increase the chance of multidirectional B and T cell activation. Adjuvants may enhance the immunogenicity of peptides and proteins by activating costimulatory factors and mediating the production of cytokines [42]. Autologous dendritic cells pulsed with peptides/proteins in vitro, or transfected with the relevant genes, may effectively activate both class I- and class II-restricted T lymphocytes in vivo [1, 50]. Cytokines have been identified to play a key role in T cell activation. GM-CSF has been shown to induce long-lasting Th1- and $CD8^+$ T cell responses

by efficient activation of dendritic cells in vivo [14]. IL-12 is a potent activator of Th1 and $CD8^+$ T lymphocytes. At low dose levels it has been shown to mediate complete tumor regressions when used as an adjuvant to immunization with a mutant peptide of p53 in an animal model [44]. The identification of further tumor antigens will give a broader basis for polyvalent immunization strategies to prevent the escape of antigen-loss variants [26]. As the clinical effectiveness of cancer vaccination becomes more established, immunotherapy may soon be considered as an alternative modality for adjuvant treatment of cancer patients at high risk for recurrence.

## References


1. Alijagic SP, Müller M, Artuc K, Jurgovsky B, Czarnetzki M, Schadendorf D (1995) Dendritic cells generated from peripheral blood transfected with human tyrosinase induce specific T cell activation. Eur J Immunol 25: 3100
2. Bakker AB, Schreurs MWJ, deBoer AJ, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG (1994) Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. J Exp Med 179: 1005
3. Balch CM, Houghton AN, Milton GW, Soong SJ (1992) Cutaneous melanoma. J.B. Lippincott, Philadelphia
4. Boon T (1998) Tumor antigens recognized by cytolytic T cells. Cancer Vaccine Week October 5–9: S01
5. Brasseur FD, Rimoldi D, Lienard D, Lethe B, Carrel S, Arienti F, Suter L, Vanwijck R, Bourlond A, Humblet Y, Vacca A, Conese M, Lahaye T, Degiovanni G, Deraemaecker R, Beauduin M, Sastre X, Salamon E, Dreno B, Knuth A, Jäger E, Chevreau C, Suciu S, Lachapelle J-M, Pouillart P, Parmiani G, Lejeune F, Cerottini J-C, Boon T, Marchand M (1995) Expression of MAGE genes in primary and metastatic cutaneous melanoma. Int J Cancer 63: 375
6. Brichard V, Van Pel A, Wölfel T, Wölfel C, DePlaen E, Lethe B, Coulie P, Boon T (1993) The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 178: 489
7. Caux C, Dezutter-Dambuyant C, Schmitt D (1992) GM-CSF and TNFα cooperate in the generation of dendritic Langerhans cells. Nature 360: 258
8. Chen YT, Stockert E, Tsang S, Coplan KA, Old LJ (1995) Immunophenotyping of melanomas for tyrosinase: implications for vaccine development. Proc Natl Acad Sci USA 92: 8125
9. Chen Y-T, Stockert E, Jungbluth A, Tsang S, Coplan CA, Scanlan MJ, Old LJ (1996) Serological analysis of Melan A (MART-1), a melanocyte-specific protein homogenously expressed in human melanomas. Proc Natl Acad Sci USA 93: 5915
10. Chen Y-T, Scanlan MJ, Sahin U, Türeci Ö, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ (1997) A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci USA 94: 1914
11. Coulie PG, Lehmann F, Lethe B, Herman J, Lurquin C, Andrawiss M, Boon T (1995) A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. Proc Natl Acad Sci USA 92: 7976
12. Coulie PG, Brichard V, Van Pel A, Wölfel T, Schneider J, Traversari C, Mattei S, DePlaen E, Lurquin C, Szikora J-P, Renauld J-C, Boon T (1994) A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 180: 35
13. Disis ML, Cheever MA (1996) Oncogenic proteins as tumor antigens. Curr Opin Immunol 8: 637
14. Dranoff G, Jaffee E, Lazenbry A, Golumbek P, Levitsky H, Brose K, Jeckson V, Hamada H, Pardoll D, Mulligan R (1993) Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA 90: 3539
15. Feltkamp MC, Smits HL, Vierboom MP, Minnaar RP, De Jongh BM, Drijfhout JW, ter Schegget J, Melief CJ, Kast WM (1993) Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. Eur J Immunol 23: 2242
16. Fenton RG, Keller CJ, Hanna N, Taub DD (1995) Induction of T-cell immunity against Ras oncoprotein by soluble protein or Ras-expressing Escherichia coli. J Natl Cancer Inst 87: 1853
17. Gaugler B, Van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, DePlaen E, Lethe B, Brasseur F, Boon T (1994) Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med 179: 921
18. Gjertsen MK, Bakka A, Breivik J, Saeterdal I, Solheim BG, Soreide O, Thorsby E, Gaudernack G (1995) Vaccination with mutant Ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding Ras mutation. Lancet 346: 1399
19. Gromet MA, Epstein WL, Blois MS (1978) The regressing thin malignant melanoma: a distinctive lesion with metastatic potential. Cancer 42: 2282
20. Herr W, Wölfel T, Heike M, Meyer zum Büschenfelde K-H, Knuth A (1994) Frequency analysis of tumor-reactive cytotoxic T lymphocytes in peripheral blood of a melanoma patient vaccinated with autologous tumor cells. Cancer Immunol Immunother 39: 93
21. Jäger E, Jäger D, Karbach J, Chen Y-T, Ritter G, Nagata Y, Gnjatic S, Stockert E, Arand M, Old LJ, Knuth A (2000) Identification of NY-ESO-1 epitopes presented by HLA-DRB4 0101–0103 and recognized by CD4+ T lymphocytes of patients with NY-ESO-1 expressing melanoma. J Exp Med 191: 625
22. Jäger E, Stockert, Zidianakis Z, Chen Y-T, Karbach J, Jäger D, Arand M, Ritter G, Old LJ, Knuth A (1999) Humoral immune responses of cancer patients against 'Cancer-Testis' antigen NY-ESO-1: correlation with clinical events. Int J Cancer 84: 506
23. Jäger E, Bernhard H, Romero P, Ringhoffer M, Arand M, Karbach J, Ilsemann C, Hagedorn M, 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
24. Jäger E, Maeurer M, Höhn H, Karbach J, Jäger D, Zidianakis Z, Bakhshandeh-Bath A, Orth J, Neukirch C, Necker A, Reichert TE, Knuth A (1999) Clonal expansion of Melan A-specific cytotoxic T lymphocytes (CTL) in a melanoma patient responding to continued immunization with melanoma-associated peptides. Int J Cancer 6: 419
25. Jäger E, Ringhoffer M, Dienes H-P, Arand M, Karbach J, Jäger D, Ilsemann C, Hagedorn M, Oesch F, Knuth A (1996) Granulocyte macrophage colony-stimulating factor enhances immune responses to melanoma associated peptides in vivo. Int J Cancer 67: 54
26. Jäger E, Ringhoffer M, Karbach J, Arand M, Oesch F, Knuth A (1996) Inverse relationship of melanocyte differentiation antigen expression in melanoma: evidence for immunoselection of antigen-loss variants in vivo. Int J Cancer 66: 470
27. Jäger E, Ringhoffer M, Karbach J, Arand M, Oesch F, Jäger D, Knuth A (1997) Immunoselection in vivo: Independent loss of MHC class I and melanocyte differentiation antigen expression in metastatic melanoma mediated by antigen-specific CTL. Int J Cancer 71: 142
28. Jäger E, Ringhoffer M, Arand M, Karbach J, Jäger D, Ilsemann C, Hagedorn M, Oesch F, Knuth A (1996) Cytolytic T cell reactivity against melanoma associated differentiation antigens in peripheral blood of melanoma patients and healthy individuals. Melanoma Res 6: 419

29. Jäger E, Chen Y-T, Drijfhout JW, Karbach J, Ringhoffer M, Jäger D, Arand M, Wada H, Noguchi Y, Stockert E, Old LJ, Knuth A (1998) Simultaneous humoral and cellular immune response against Cancer-Testis Antigen NY-ESO-1: definition of human histocompatibility leucocyte antigen (HLA)-A2-binding peptide epitopes. J Exp Med 187: 265
30. Kang XQ, Kawakami Y, El-Gamil M, Wang R, Sakaguchi K, Yannelli JR, Appella E, Rosenberg SA (1995) Identification of a tyrosinase epitope recognized by HLA-A24-restricted, tumor-infiltrating lymphocytes. J Immunol 155: 1343
31. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Sakaguchi K, Appella E, Yannelli JR, Adema GJ, Miki T, Rosenberg SA (1994) Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. Proc Natl Acad Sci USA 91: 6458
32. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Rivoltini L, Topalian SL, Miki T, Rosenberg SA (1994) Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci USA 91: 3525
33. Kawakami Y, Eliyahu S, Jennings C, Sakaguchi K, Kang XQ, Southwood S, Robbins PF, Sette A, Appella E, Rosenberg SA (1995) Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating lymphocytes associated with in vivo tumor regression. J Immunol 154: 3961
34. Knuth A, Danowski B, Oettgen HF, Old LF (1984) T-cell-mediated cytotoxicity against autologous malignant melanoma: analysis with interleukin-2-dependent T-cell cultures. Proc Natl Acad Sci USA 81: 3511
35. Knuth A, Wölfel T, Meyer zum Büschenfelde K-H (1991) Cellular and humoral responses against cancer: implications for cancer vaccines. Curr Opin Immunol 3: 659
36. Knuth A, Wölfel T, Meyer zum Büschenfelde K-H (1992) T cell responses to human malignant tumours. Cancer Surveys 13: 39
37. Knuth A, Wölfel T, Klehmann E, Boon T, Meyer zum Büschenfelde K-H (1989) Cytolytic T-cell clones against an autologous human melanoma: specificity study and definition of three antigens by immunoselection. Proc Natl Acad Sci USA 86: 2804
38. Koenig S, Woods RM, Brewar YA, Newell AJ, Jones GM, Boone E, Adelsberger JW, Baseler MW, Robinson SM, Jacobson S (1993) Characterization of MHC class I restricted cytotoxic T cell responses to tax in HTLV-1 infected patients with neurologic disease. J Immunol 151: 3874
39. Koziel MJ, Dudley D, Afdhal N, Grakoui A, Rice CM, Choo QL, Houghton M, Walker BD (1995) HLA-class I-restricted cytotoxic T lymphocytes specific for hepatitis C virus. Identification of multiple epitopes and characterization of patterns of cytokine release. J Clin Invest 96: 2311
40. Mandelboim O, Berke G, Fridkin M, Feldman M, Eisenstein M, Eisenbach L (1994) CTL induction by a tumour-associated antigen octapeptide derived from a murine lung carcinoma. Nature 369: 67
41. Marchand M, Weymants P, Rankin E, Arienti F, Belli F, Parmiani G, Cascinelli N, Bourlond A, Vanwjick R, Humblet Y, Canon J-L, Laurent C, Naeyaert J-M, Plagne R, Deraemaeker R, Knuth A, Jäger E, Brasseur F, Herman J, Coulie PG, Boon T (1995) Tumor regression responses in melanoma patients treated with a peptide encoded by gene MAGE-3. Int J Cancer 63: 883
42. Matzinger P (1994) Tolerance, danger, and the extended family. Ann Rev Immunol 12: 991
43. Murray RJ, Kurilla MG, Brooks JM, Thomas WA, Rowe M, Kieff E, Rickinson AB (1992) Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies. J Exp Med 176: 157
44. Noguchi Y, Richards EC, Chen YT, Old LF (1995) Influence of interleukin-12 on p53 peptide vaccination against established Meth A sarcoma. Proc Natl Acad Sci USA 92: 2219
45. Ogg GS, Dunbar RP, Romero P, Chen J-L, Cerundolo V (1998) High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. J Exp Med 188: 1203
46. Rehermann B, Fowler P, Sidney J, Person J, Redeker A, Brown M, Moss B, Sette A, Chisari FV (1995) The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. J Exp Med 181: 1047
47. Rivoltini L, Kawakami Y, Sakaguchi K, Southwood S, Sette A, Robbins PF, Marincola FM, Salgaller ML, Yannelli JR, Appella E, Rosenberg SA (1995) Induction of tumor-reactive CTL from peripheral blood and tumor-infiltrating lymphocytes of melanoma patients by in vitro stimulation with an immunodominant peptide of the human melanoma antigen MART-1. J Immunol 154: 2257
48. Robbins PF, El-Gamil M, Kawakami Y, Rosenberg SA (1994) Recognition of tyrosinase by tumor-infiltrating lymphocytes from a patient responding to immunotherapy. Cancer Res 54: 3124
49. Sahin U, Türeci Ö, Schmitt H, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M (1995) Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci USA 92: 11810
50. Schirmbeck R, Melber K, Reimann J (1995) Hepatitis B virus small surface antigen particles are processed in a novel endosomal pathway for major histocompatibility complex class I-restricted epitope presentation. Eur J Immunol 25: 1063
51. Schlichtholz B, Legros Y, Gille D, Gaillard C, Marthy M, Lane D, Calvo F, Soussi T (1992) Antibodies against p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. Cancer Res 52: 6380
52. Stockert E, Jäger E, Chen Y-T, Scanlan MJ, Gout I, Karbach J, Knuth A, Old LJ (1998) A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 187: 1349
53. Tilkin AF, Lubin R, Soussi T, Lazar V, Janin N, Mathieu MC, Lefrere I, Carlu C, Roy M, Kayibanda M (1998) Primary proliferative T cell response to wild-type p53 protein in patients with breast cancer. Eur J Immunol 25: 1765
54. Traversari C, van der Bruggen P, Luescher IF, Lurquin C, Chomez P, van Pel A, DePlaen E, Amarcostesec A, Boon T (1992) A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T-lymphocytes directed against tumor antigen MZ2-E. J Exp Med 176: 1453
55. Van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T (1995) A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J Exp Med 182: 689
56. Van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 254: 1643
57. Visseren MJW, van Elsas A, van der Voort EIH, Ressing ME, Kast WM, Schrier PI, Melief CJM (1995) CTL specific for the tyrosinase autoantigen can be induced from healthy donor blood to lyse melanoma cells. J Immunol 154: 3991
58. Wölfel T, Klehmann E, Müller C, Schütt KH, Meyer zum Büschenfelde KH, Knuth A (1989) Lysis of human melanoma cells by autologous cytolytic T cell clones. Identification of human histocompatibility leukocyte antigene A2 as a restriction element for three different antigens. J Exp Med 170: 797
59. Wölfel T, Schneider J, Meyer zum Büschenfelde K-H, Rammensee H-G, Rötzschke O, Falk K (1994) Isolation of naturally processed peptides recognized by cytolytic T lymphocytes (CTL) on human melanoma cells in association with HLA-A2.1. Int J Cancer 57: 413
60. Wölfel T, Hauer M, Schneider J, Serrano M, Wölfel C, Klehmann-Hieb E, DePlaen E, Hankeln T, Meyer zum Büschenfelde K-H, Beach D (1995) A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 269: 1281