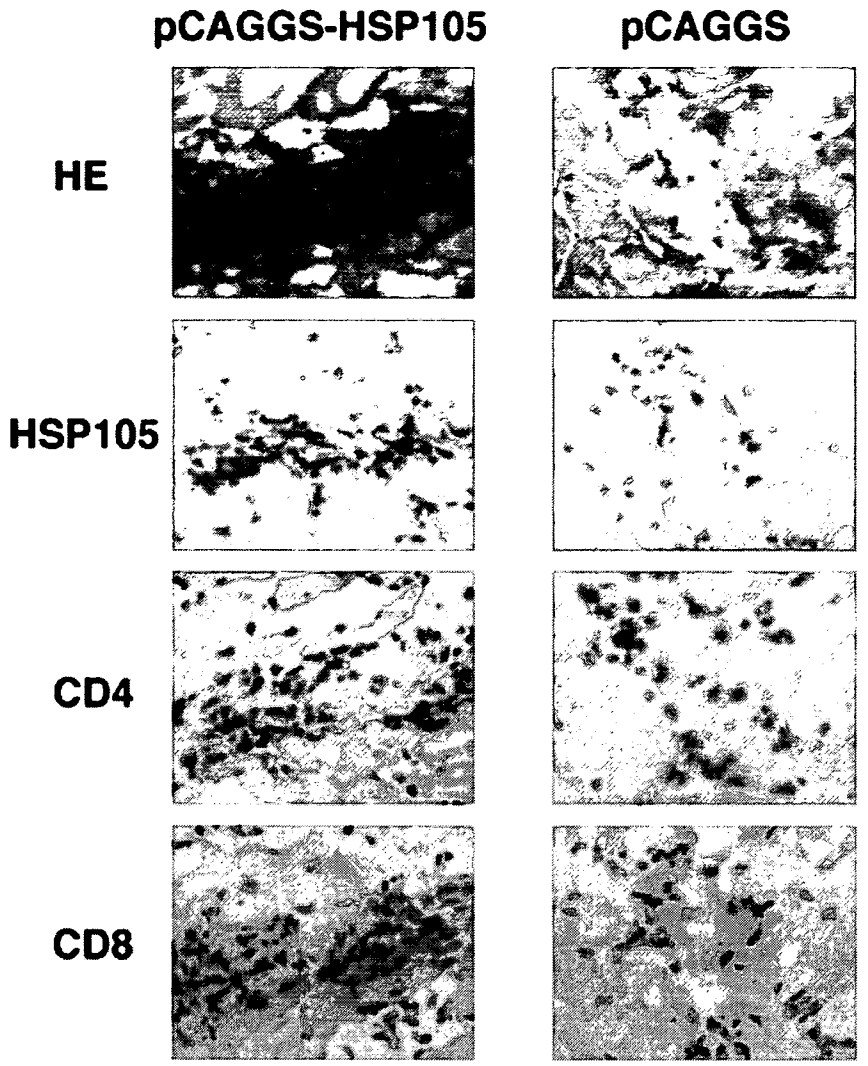

Fig. 3. Expression of HSP105 protein and infiltration of $CD4^+$ T cells and $CD8^+$ T cells in the *HSP105* DNA vaccine-injected sites. To observe HSP105 expression and infiltrating cells in muscles injected with the *HSP105* DNA vaccine, we carried out intramuscular immunizations with pCAGGS-DNA into the right anterior tibialis muscle, and with pCAGGS-*HSP105* DNA into the left anterior tibialis muscle in four mice. After 48 h, we killed the mice and studied their muscle tissue by using HE staining and histological analysis, and immunohistochemical analysis of HSP105, CD4, and CD8. Representative results are shown. Objective magnification was 400×.

lower than those in C26 (C20) tumor cells, which suggests a low risk of damage to normal tissue as a result of immune responses to the HSP105 antigen. To evaluate the risk of autoaggression by immunization against self-HSP105, the tissues of mice immunized with *HSP105* DNA were histologically examined. All mice were apparently healthy, and without abnormalities, suggesting autoimmunity for, for example, dermatitis, arthritis, or neurological disorders. The brain, liver, lung, heart, kidney, and spleen tissues of *HSP105*-immunized mice were critically scrutinized and compared with those of normal mice. These tissues had normal structure and cellularity for each of the two groups examined, and pathological changes caused by immune response, such as infiltrations of $CD8^+$ or $CD4^+$ T cells, or tissue destruction and repair, were not present (Fig. 4b). Although $CD4^+$ T cells and $CD8^+$ T cells infiltrated into the C26 tumor (Fig. 4a), infiltration of $CD4^+$ T cells or $CD8^+$ T cells was not observed in any of the normal adult tissues examined (Fig. 4b). These results indicate that T cells stimulated with the *HSP105* DNA vaccine do not recognize normal cells that express HSP105 at physiological levels.

Anti-C26 tumor adoptive immunity elicited by injection with $CD4^+$ T cells or $CD8^+$ T cells from *HSP105* DNA-vaccinated mice

Antitumor responses could be augmented by homeostatic T cell proliferation in the periphery, involving the expansion of T cells recognizing MHC/tumor antigenic peptide ligands.[21–23] To ascertain that the tumor rejections induced by *HSP105* DNA vaccination were mediated through the activation of $CD8^+$ T cells or $CD4^+$ T cells, in a homeostatic lymphocyte proliferation model, we subcutaneously inoculated BALB/c mice with C26 cells (3×10^4) 6 days after sublethal irradiation (5 Gy). We intravenously injected 1.5×10^7 whole spleen cells or 3×10^6 $CD8^+$ T cells, $CD4^+$ T cells, NK cells, or $CD8^-$ $CD4^-$ NK^- cells derived from each untreated or *HSP105* DNA-vaccinated mouse on day 3 before the tumor inoculation (Fig. 5a). Measurements of tumor size were continued for 22 days after inoculation with the tumor cells (Fig. 5b). Each group consisted of four mice. Inoculation with whole spleen cells or $CD8^+$ T cells, $CD4^+$ T cells, NK cells, or $CD8^-$ $CD4^-$ NK^- cells derived from untreated mice, and with NK cells, or $CD8^-$ $CD4^-$ NK^- cells derived from *HSP105* DNA-vaccinated

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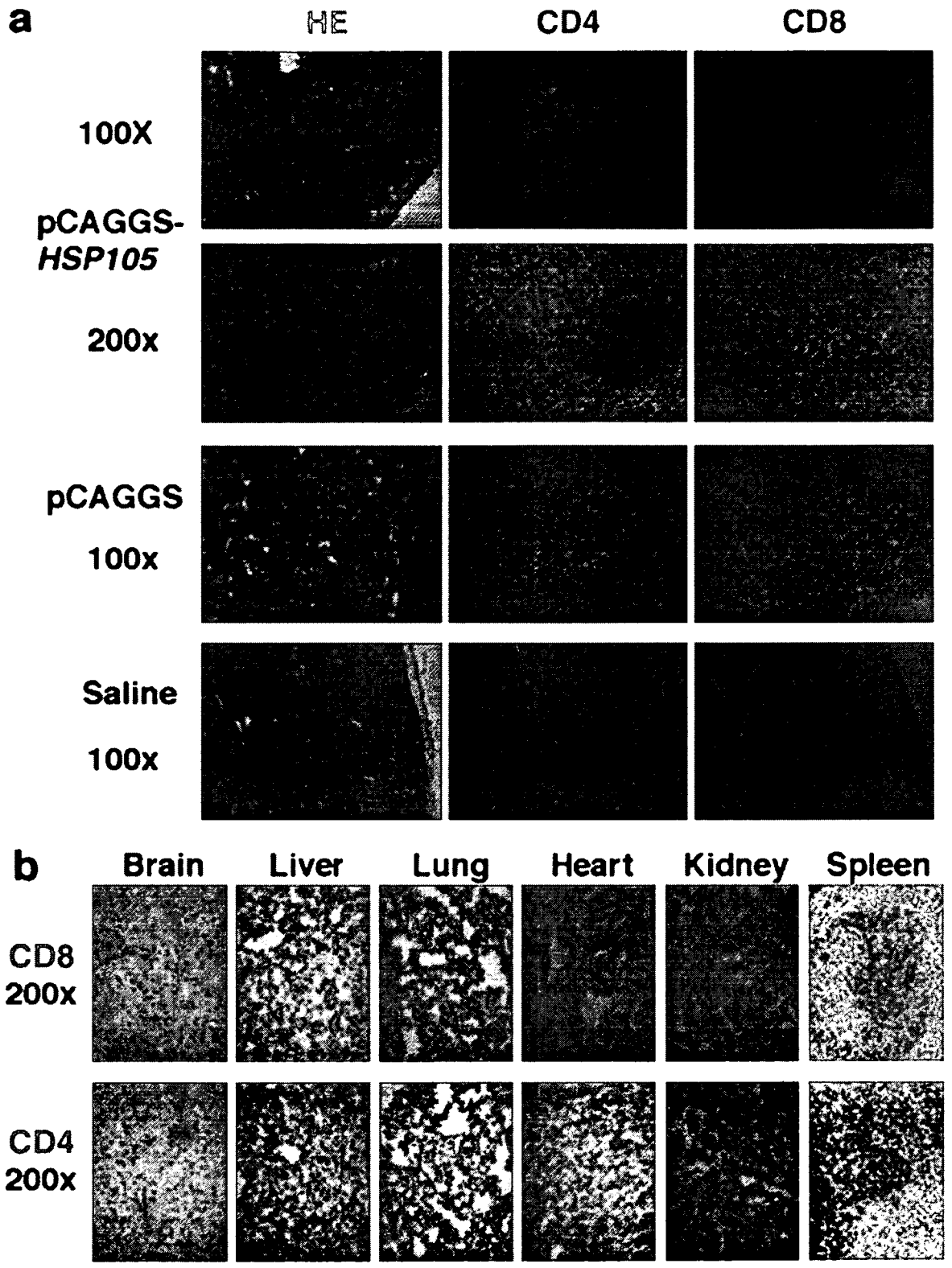

Fig. 4. Vaccination with *HSP105* DNA induced infiltration of both $CD4^+$ T cells and $CD8^+$ T cells into C26 tumors, but not into normal tissues. (a) Subcutaneous C26 tumors removed from two *HSP105* DNA-immunized mice, a saline-inoculated mouse, and a pCAGGS-immunized mouse that did not reject the tumor challenges were analyzed using immunohistochemical staining with anti-CD4 mAb and anti-CD8 mAb. (b) Normal tissues of mice vaccinated with *HSP105* DNA were histologically and immunohistochemically examined. Objective magnification was 200×. The spleen was used as a positive control for staining of both CD4 and CD8.

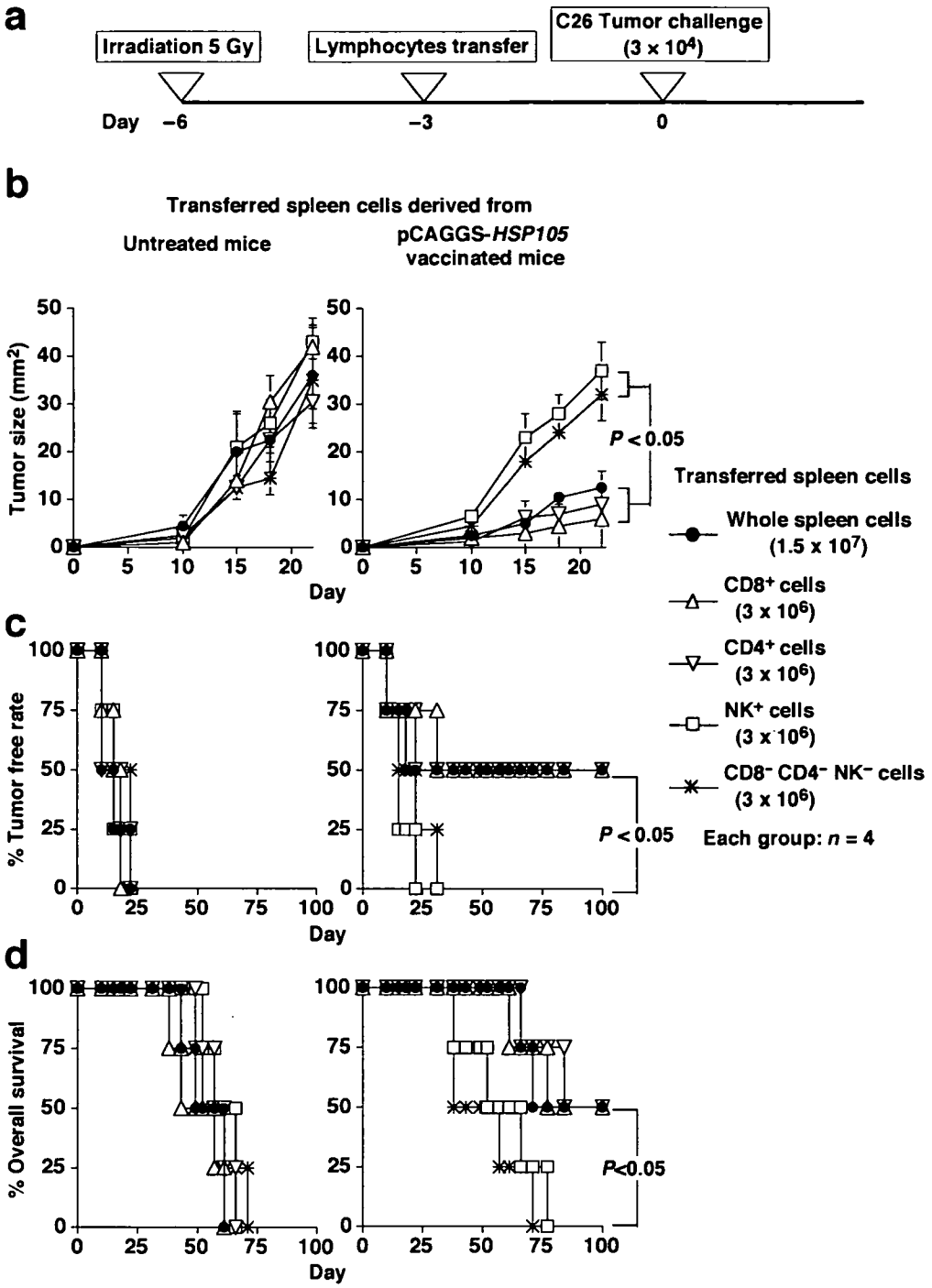

Fig. 5. Injection of either CD4⁺ T cells or CD8⁺ T cells sensitized with *HSP105* DNA vaccine into sublethally irradiated mice elicited effective antitumor adoptive immunity. (a) Experimental protocol; each group consisted of four mice. (b) Suppression of the growth of HSP105-expressing C26 tumors inoculated subcutaneously into mice transferred with each group of spleen cells. Tumor area was calculated as the product of width and length. The result is presented as the mean area of tumor ± SE, and we evaluated the statistical significance using the unpaired *t*-test. (c,d) Percentage tumor free rate (c) and percentage overall survival (d) were calculated using the Kaplan–Meier method, and the statistical significance of differences in survival time between groups was evaluated using Wilcoxon's test.

mice did not cause the mice to reject challenges with C26 cells (3×10^4). Conversely, two of the four mice (50%) that were treated with whole spleen cells, CD8⁺ T cells, or CD4⁺ T cells derived from *HSP105* DNA-vaccinated mice completely rejected challenges with C26 cells (3×10^4; Fig. 5b–d). Thus, sublethally irradiated lymphopenic mice transfused with CD4⁺ T cells or CD8⁺ T cells derived from *HSP105* DNA-vaccinated mice displayed tumor growth inhibition. These results suggest that both CD4⁺ and CD8⁺ T cells play critical roles in antitumor immunity induced by immunization with

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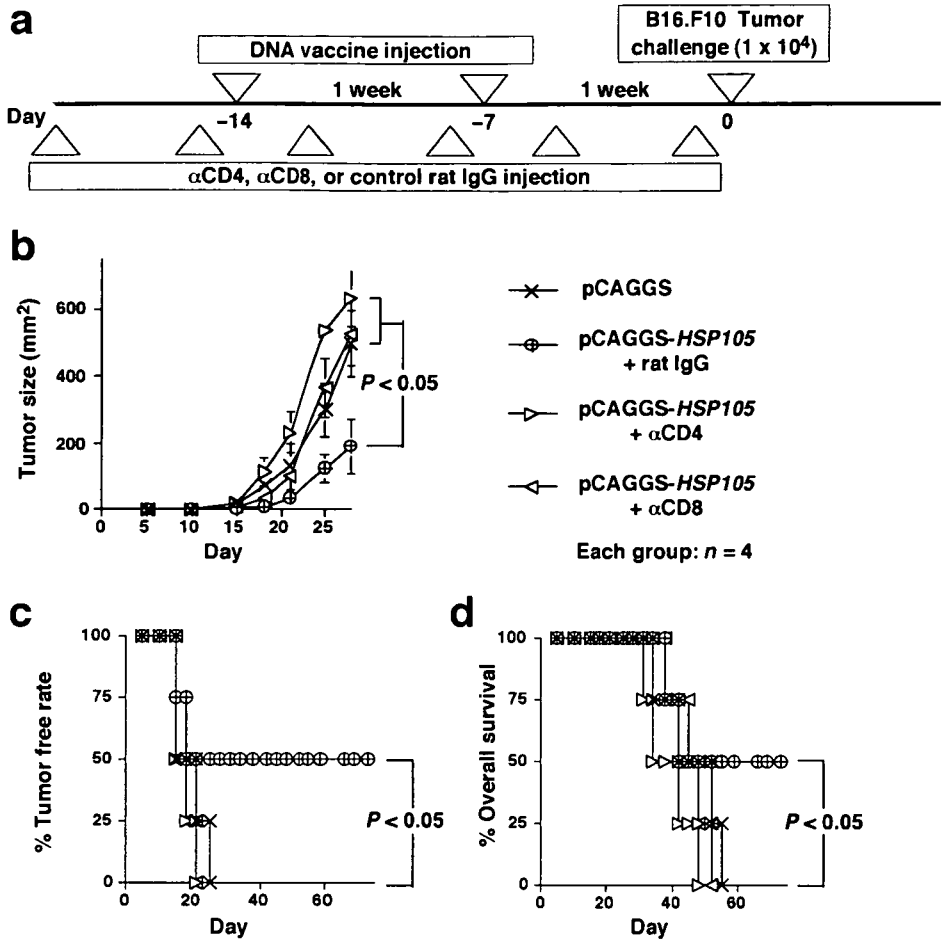

Fig. 6. Involvement of both CD4⁺ T cells and CD8⁺ T cells in protection against B16.F10 induced by vaccination with *HSP105* DNA. (a) Experimental protocol for *in vivo* depletion of CD4⁺ T cells and CD8⁺ T cells. Each group consisted of four mice. (b) Suppression of the growth of HSP105-expressing B16.F10 tumors inoculated subcutaneously into mice vaccinated with *HSP105* DNA. Tumor area was calculated as the product of width and length. Data are presented as mean area of tumor ± SE, and we evaluated the statistical significance using the unpaired *t*-test. (c,d) Percentage tumor free rate (c) and percentage overall survival (d) were calculated using the Kaplan–Meier method, and the statistical significance of differences in survival time between groups was evaluated using Wilcoxon's test.

the *HSP105* DNA-vaccine. The mice shown in Figure 5 were killed more than 100 days after lymphocyte transfer, respectively. All mice were apparently healthy and without abnormalities, suggesting autoimmunity for, for example, dermatitis, arthritis, or neurological disorders. The brain, liver, lung, heart, kidney, and spleen tissues of *HSP105* DNA-immunized mice were critically scrutinized and compared with those of normal mice. These tissues had normal structures and cellularity for each of the two groups examined, and pathological changes caused by immune response, such as CD8⁺ or CD4⁺ T lymphocyte infiltration or tissue destruction and repair, were not present, as shown in Figure 4b. These results indicate that T cells stimulated with HSP105 do not recognize normal cells that express HSP105 at physiological levels.

Involvement of both CD4⁺ T cells and CD8⁺ T cells in protection against B16.F10 induced by *HSP105* DNA-vaccination

To determine the role of CD4⁺ T cells and CD8⁺ T cells in the protection against B16.F10 tumor cells induced by *HSP105* DNA-vaccination, we depleted mice of CD4⁺ T cells or CD8⁺ T cells by treatment with anti-CD4 or anti-CD8 mAb *in vivo*. More than 90% of CD4⁺ T cells or CD8⁺ T cells were depleted (data not shown). During this procedure, mice were immunized with DNA vaccine and challenged with B16.F10 cells (Fig. 6a). Depletion of either CD4⁺ T cells or CD8⁺ T cells almost totally abrogated the protective immunity induced by immunization with *HSP105* DNA vaccine (Fig. 6b–d). These results suggest that both CD4⁺ T cells and CD8⁺ T cells play critical roles in antitumor immunity induced by immunization with *HSP105* DNA vaccine.

Discussion

Advances in molecular biology and tumor immunology have paved the way for identification of a large number of genes encoding TAA and antigenic peptides recognized by tumor-reactive CTL, hence peptide-based cancer immunotherapy has been the focus of much research.[24–26] However, current clinical trials for peptide-based immunotherapy have rarely resulted in tumor regression.[27] The immunogenicity of these

tumor antigenic peptides or the vaccination strategy may be sufficient to induce CTL responses but not to elicit $CD4^+$ T cells.

DNA-based immunization is potentially a powerful method for immunizing against microbial, viral, and tumor antigens through both humoral and cell-mediated immune responses.[28] The generation of T-cell immunity involves local target cell transfection and protein antigen production, which is taken up by host APC, leading to cross-presentation in draining lymph nodes; in addition, direct DNA transfection into APC in peripheral tissue has also been demonstrated.[29] Compared with orthodox vaccines consisting of tumor proteins or viral components, DNA vaccination stimulates host immunity against transgene-encoding proteins without the processes related to protein purification. In the present study, a DNA vaccine was used to activate HSP105-specific tumor immunity.

Although the SEREX method facilitated the identification of tumor antigens that could be recognized by antibodies and $CD4^+$ Th cells, few of their T cell epitopes have been determined.[2,30] We previously reported that HSP105, identified by SEREX of pancreatic adenocarcinoma, was overexpressed specifically in a variety of human cancers, including pancreatic and colon adenocarcinoma.[1,5] Other investigators identified HSP105 by SEREX using other cDNA libraries derived from tissues including colorectal cancer, melanoma, and normal testis. HSP105 are complexes associated with HSP70/HSC70,[31,32] which negatively regulate HSP70/HSC70 chaperone activity.[33] In addition, HSP105 protects neuronal cells against the apoptosis induced by various stresses.[34] HSP105 consists of HSP105α and HSP105β. HSP105α is a constitutively expressed 105-kDa HSP that is induced by a variety of stresses, whereas HSP105β is a 90-kDa HSP that is specifically induced by heat shock at 42°C. HSP105β is a truncated form of HSP105α.[12] We used in this study the mouse HSP105α DNA and protein. Recently, Subjeck and colleagues reported that recombinant HSP110 and cancer antigens such as Her2/*neu* or gp100 complexes are powerful cancer vaccines.[8,9,35] Their HSP110[11] and our HSP105α are in fact the same protein.

Although they noted that HSP110 did not have immunogenic properties, we emphasize in this study that HSP105 does have a strong immunogenic action. Although we did not identify the HSP105-derived epitope peptides of $CD8^+$ T-cells or $CD4^+$ T-cells in this study, we did prove that HSP105 itself could induce both $CD4^+$ T-cells and $CD8^+$ T-cells to become reactive to tumor cells expressing HSP105. As shown in Figure 5, in a homeostatic lymphocyte proliferation model, we demonstrated that adoptive transfer of either $CD4^+$ T cells or $CD8^+$ T cells alone into sublethally irradiated mice was sufficient to reject C26 cells that do not express MHC class II molecules. To ascertain whether this is also true for B16.F10 that express both MHC class I and II molecules in the presence of interferon (IFN)-γ, further investigation is needed. As shown in Figure 6, we demonstrated that both $CD4^+$ T cells and $CD8^+$ T cells were required for rejection of B16.F10 in the induction phase. In terms of the mechanism for the rejection of C26 tumors, we have other data relating to vaccination with HSP105 protein-pulsed BM-DC instead of *HSP105* DNA vaccination. In those experiments, we also demonstrated that both $CD4^+$ T cells and $CD8^+$ T cells were required for rejection of not only B16.F10 but also C26 in the induction phase by depleting $CD4^+$ T cells and $CD8^+$ T cells using the *in vivo* administration of antibodies (unpublished data). Therefore, both HSP105-specific $CD4^+$ T cells and $CD8^+$ T cells seem to be important for the rejection of HSP105-expressing tumors in the induction phase, and either $CD4^+$ T cells or $CD8^+$ T cells can independently exert anti-C26 tumor effects in the effector phase in a homeostatic lymphocyte proliferation model.

It has been reported that antigen-specific $CD4^+$ T-cell help is required to activate memory $CD8^+$ T cells to fully functional effector killer cells.[36] The peptides derived from exogenous antigens acquired by endocytosis are typically presented on MHC class II molecules on the surface of APC, and activate $CD4^+$ T cells. We observed in this study that $CD4^+$ T cells specific to HSP105, in fact, have an important role in tumor rejection, even when tumors do not express MHC class II molecules, such as the C26 tumors used in this study. It was recently reported that tumor-specific $CD4^+$ T cells may have a pivotal role in preventing early tumorigenesis by secreting IFN-γ and stimulating the classical macrophage-activation pathway. This results in the inhibition of tumor cell growth, even when tumor cells themselves do not express MHC class II molecules.[37] To better understand the mechanism of C26 tumor rejection by HSP105-specific $CD4^+$ T cells, further studies are needed. Furthermore, peptides derived from exogenous self-antigen, HSP105, acquired by endocytosis are possibly presented by MHC class I molecules on the surface of APC by cross-presentation to activate $CD8^+$ T cells.

Because HSP are present in all organisms, low levels of human HSP-derived peptides serve as harbingers of autoimmune responses after CTL have been primed to respond to bacterial HSP-derived peptides.[38] However, because many cancers overexpress HSP, CTL-based vaccines that elicit an anti-HSP response might be effective against many different tumors.[39] Indeed, in this study, HSP105 itself evoked T-cell-mediated tumor rejection without autoimmune reactions. In the present paper, all results shown in the figures were obtained using female mice, but we have carried out the same experiment using male mice. *HSP105* DNA vaccination did not induce T-cell infiltration or damage in testis tissue (in which HSP105 is highly expressed). Furthermore, *HSP105* DNA vaccination was also able to induce antitumor immunity in male mice (data not shown), indicating that male mice did not acquire immunological tolerance to HSP105 expressed in testis tissue.

To substantiate the specificity for HSP105, we searched for mouse cancer cell lines derived from BALB/c mice and C57BL/6 mice that do not express HSP105. However, all cancer cell lines we examined strongly expressed HSP105. BALB/3T3 fibroblasts expressed HSP105 relatively weakly, but these cells unfortunately did not form tumors in mice. Further investigations are needed to clarify whether *HSP105* DNA vaccination affects the growth of some tumors that do not express HSP105.

We showed in this study that *HSP105* DNA vaccination can prime T cells to be reactive to tumor cells expressing HSP105 *in vivo*, and that growth of C26 and B16.F10 cells expressing HSP105 was prevented without inducing autoimmune destruction in murine subcutaneous CRC and melanoma models. We believe that *HSP105* DNA vaccination is a novel strategy for the prevention of CRC and melanoma in patients treated surgically who are at high risk of recurrence

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of CRC or melanoma. Whether or not HSP105 is an ideal target for immunotherapy in human cancers will continue to be investigated in our laboratory.

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Usefulness of the Novel Oncofetal Antigen Glypican-3 for Diagnosis of Hepatocellular Carcinoma and Melanoma

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Contents

Abstract

Glypican-3 (GPC3) mRNA and protein are expressed in >80% of human hepatocellular carcinomas (HCC) but not in normal tissues except for placenta and fetal liver. The oncofetal antigen GPC3 is a glycosylphosphatidyl inositol-anchored membrane protein and may be secreted. It is a novel tumor marker for human HCC: GPC3 protein was present in sera from 40–50% of HCC patients, but was not detected in sera from patients with liver cirrhosis or chronic hepatitis, or in sera from healthy individuals. α-Fetoprotein (AFP) and PIVKA-II (protein induced by vitamin K absence or antagonist-II), are well known major tumor markers for HCC. Generally, AFP shows high positivity for HCC but also high false-positivity in detection assays. *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein (AFP-L3) is a recently described marker of HCC. Detection of AFP-L3 shows a much higher specificity than AFP, but a lower sensitivity. On the other hand, detection of PIVKA-II shows a lower false-positivity, but is not always sensitive enough to detect low levels secreted by small HCCs. There was no correlation between the three tumor markers, AFP, PIVKA-II, and GPC3 in terms of their presence in HCC cells. All three tumor markers showed similar positivity in patients with HCC, detecting 80% of patients with the disease.

GPC3 is also a novel tumor marker for the diagnosis of human melanoma, especially in the early stages of the disease. Expression of GPC3 mRNA and protein was evident in tumor cells from >80% of patients with melanoma and melanocytic nevus, which is a common benign lesion. GPC3 protein was detected in sera from

40% (36/91) of melanoma patients, but not in sera from those with large congenital melanocytic nevus, or from healthy donors. Surprisingly, we detected serum GPC3 even in patients with stage 0, *in situ* melanoma. The positive detection rate of serum GPC3 at stage 0, I, and II (44.4%, 40.0%, 47.6%, respectively) was significantly higher than that of 5-S-cysteinyldopa, a well known tumor marker for melanoma (0.0%, 8.0%, and 10.0%, respectively).

Interestingly, GPC3 was highly immunogenic in mice and elicited effective anti-tumor immunity with no evidence of autoimmunity. Thus, GPC3 is useful for diagnosis of HCC and melanoma and may also have a role in immunotherapy or tumor prevention. However, studies in humans are warranted.

Primary hepatocellular carcinoma (HCC) is one of the most common solid malignancies in the world and accounts for about 1 million deaths each year. Numerically, most of these cases occur in the Far East and are related to chronic infection with the hepatitis B virus (HBV) although, proportionally, chronic hepatitis C (HCV) is more important in developed Western countries.[1] Because of the global pandemic of hepatitis B and C viral infections, the incidence of HCC is increasing rapidly in Asian and Western countries,[2] and this trend is expected to continue for the next 50 years because of the long latency between infection and the development of HCC. The prognosis of patients with advanced HCC remains poor, and novel treatment and diagnostic strategies are needed urgently. There are several tumor markers, including carcinoembryonic antigen (CEA),[3,4] carbohydrate antigens (CA) 19-9,[5] and α-fetoprotein (AFP),[6] which may be used in different settings in cancer patients, including screening measures, differentiating between malignant and benign lesions, monitoring the response to treatment, and detecting recurrences. AFP and PIVKA-II (protein induced by vitamin K absence or antagonist-II)[7] are well known tumor markers for HCC. Generally, detection of AFP shows a high sensitivity but also shows a high false-positivity. Serum AFP levels are often increased in patients with benign liver diseases such as chronic hepatitis (CH) and liver cirrhosis (LC). *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein (AFP-L3) is a recently described marker of HCC. Detection of AFP-L3 shows a much higher specificity than AFP, but a lower sensitivity. On the other hand, PIVKA-II shows a lower false-positivity, but is not always sensitive enough to detect low levels of PIVKA-II in small HCCs.

Age-adjusted incidence rates for melanoma have been increasing in most fair-skinned populations in recent decades. The annual increase in incidence rate varies between populations but generally has been in the order of 37% for fair-skinned Caucasian populations. Annual incidence rates vary from >40 per 100 000 persons in Australia to <5 per 100 000 in countries of low insolation in Northern Europe. The increase in incidence rates among non-European people with darker skin has not been as consistent, although the incidence rates are generally very low and trends are difficult to determine. The incidence in these populations varies from 0.1 to 3 per 100 000 persons per year depending on the skin type and latitude.[8] In the last decade, several molecules have been evaluated as tumor markers to detect melanoma, including melanin metabolites, adhesion molecules, cytokines, and melanoma-associated antigens.[9-11] In melanoma, several tumor markers have been evaluated for use as prognostic variables, to monitor response to therapy, and to detect recurrence.[12-14] Several investigators have reported[15-18] that 5-S-cysteinyldopa (5-S-CD) is useful as a marker for melanoma progression or for monitoring metastatic melanoma. 5-S-CD is often used as a tumor marker for melanoma in Japan and the usefulness of melanoma-inhibitory activity (MIA) as a tumor marker has also been reported.[19,20] However, detection of 5-S-CD often gives a false-positive result. Serum 5-S-CD levels are often increased in patients with a large congenital melanocytic nevus, which is a benign tumor.[21] There is no available tumor marker that can detect primary melanomas at early stages, that are small, and without metastasis. In addition, current methods are not sensitive enough to detect organ metastasis at early stages. A simple, inexpensive, and non-invasive method with high sensitivity to detect a serum tumor marker would aid the management of high-risk patients who have already had the disease but are at high risk of recurrence.

1. Novel Strategies for Identification of Tumor-Associated Antigens

Cloning of the human melanoma antigen (*MAGE*) gene with cDNA expression cloning methods, has indicated that the human immune system can recognize cancer as a foreign body and can respond to it.[22] This 'genetic approach' to T-cell epitope cloning led to the identification of a large number of genes encoding tumor antigens and antigenic peptides that are recognized by tumor-reactive cytotoxic T lymphocytes (CTLs), thereby enhancing the possibility of antigen-specific cancer immunotherapy.[23-26] In

1995, tumor-derived cDNA expression libraries were screened for identification of tumor-associated antigens. The antigens were recognized by high-titered IgG antibodies present in sera of cancer patients, so called serologic identification of antigens by recombinant expression cloning (SEREX). This method allows for the systematic identification of many human cancer-associated antigens, indeed, over 1500 types of tumor antigen have been identified using the SEREX method.[27-33] cDNA microarray technology, by which investigators can obtain comprehensive data regarding gene-expression profiles, is progressing rapidly. Studies have demonstrated the usefulness of this technique for identification of novel cancer-associated genes and for classification of human cancers at the molecular level.[34-39]

2. Identification and Expression of Glypican (GPC)-3 in Hepatocellular Carcinoma (HCC) and Melanoma

2.1 Identification by cDNA Microarray Analysis of the GPC3 Gene Over-Expressed in HCC

Antigens ideal for HCC tumor immunotherapy are those that are strongly expressed in almost all HCCs, but not in normal adult tissues (except for immune-privileged tissues such as testis and placenta or fetal organs). To identify such HCC antigens, two kinds of data were used from cDNA microarrays containing 23 040 genes. One was a comparison of expression profiles between 20 HCCs (10 cases were HBV-positive and 10 were HCV-positive) and corresponding adjacent non-cancerous liver tissues.[40] The other data was from various normal human tissues.[41]

GPC3 was identified as a gene over-expressed specifically in HCC.[42] In 16 of 20 HCCs, the expression of *GPC3* mRNA in the cancer tissue was ≥5-fold higher than that in non-cancerous tissues. The *GPC3* gene was found to be over-expressed in most HCCs and the expression was not related to HBV or HCV infection. *GPC3* mRNA is highly expressed in the placenta, fetal liver, fetal lung, and fetal kidney and expression is low in most normal adult tissues. Similar observations on GPC3 have been published by other investigators, based on northern blotting studies.[43,44] Thus, like AFP, GPC3 is a novel oncofetal antigen present in HCC.

2.2 Limited Expression of GPC3 Protein in Human HCC, Melanoma, and Fetal Tissues

GPC3 has been found to be overexpressed in HCC [32,42-48] and melanoma;[49] immunohistochemical analysis of GPC3 has been conducted using various human tissues (table I).[50] Immunohistochemical staining of GPC3 in HCC and melanoma tumor cells usually had a coarsely granular pattern located near the cell membrane. Strong membrane staining was also observed in several cases. Occasionally, there was diffuse nongranular staining of the cytoplasm. In >80% of HCC, melanoma, and melanocytic nevus tumor samples there was evident expression of GPC3 mRNA and protein.[42,49,50] GPC3 protein was expressed in placenta and fetal liver, but little or no expression was observed in all normal adult human tissues tested, including brain, lung, heart, liver, kidney, mammary gland, spleen, and thymus (table I).[50]

3. Detection of GPC3 in Patients

3.1 Detection of Soluble GPC3 in Sera from Patients with HCC

GPC3 is a glycosylphosphatidyl inositol (GPI)-anchored membrane protein and may be secreted. Using an enzyme-linked immunosorbent assay (ELISA), soluble GPC3 protein could be detected in culture supernatants from four of five HCC cell lines and in sera from 40% of patients with HCC.[42] The quantification by ELISA of GPC3 protein in sera from 40 HCC (27 HCV, 8 HBV, 6 non HBV or HCV), 13 LC (8 HCV, 4 HBV, 1 non HBV or HCV), 34 CH (31 HCV, 3 HBV) patients, from other patients, and from 60 healthy donors (HDs) is indicated in table II. We detected and quantified GPC3 protein in the sera from 16 of 40 HCC patients, but not in sera from patients with LC, CH, autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), HD, and other kinds of cancers (colon, gastric, pancreatic, biliary, esophageal, lung, and breast).

Table I. The expression levels of glypican-3 protein in various human tissues as determined by immunohistochemical analysis

Expression level	Tissue
Very strong	HCC, melanoma
Strong	Placenta, fetal liver
Weak	Lung, mammary gland
Very weak or none	Liver, brain, heart, kidney, pancreas, spleen, thymus, stomach, small intestine, colon, ovary, uterus, prostate, testis

HCC = hepatocellular carcinoma.

Table II. Clinical profiles of serum donors and detection of glypican (GPC)-3 using ELISA

Disease	Mean age (y)	Sex (m/f)	UICC stage[a]					GPC3 positive rate (% of patients)
			0	I	II	III	IV	
HCC	66	36/4		1	15	14	10	16/40 (40)
Liver cirrhosis	65	6/7						0/13 (0)
Chronic hepatitis	60	15/19						0/13 (0)
Autoimmune hepatitis	65	0/2						0/2 (0)
Primary biliary cirrhosis	79	0/1						0/1 (0)
Melanoma	66	43/48	9	25	21	18	18	36/91 (40)
Large congenital melanocytic nevus	21	3/2						0/5 (0)
Healthy donors	40	25/35						0/60 (0)
Cancers								
colon	66	16/5		1	6	5	9	0/21 (0)
gastric	71	9/5		7	3	4	0	0/14 (0)
pancreatic	58	6/5		0	0	0	11	0/11 (0)
biliary	70	2/4		0	3	1	2	0/6 (0)
esophageal	59	6/0		1	0	2	3	0/6 (0)
lung	64	7/0		3	0	0	4	0/7 (0)
breast	50	0/10		4	2	2	2	0/10 (0)

a UICC classification; TNM classification of malignant tumors.

f = female; **HCC** = hepatocellular carcinoma; **m** = male; **TNM** = tumor, nodes, metastasis; **UICC** = International Union Against Cancer; **y** = year.

3.2 Comparison of Serum Concentrations of GPC3, α-Fetoprotein (AFP), and PIVKA-II in Patients with HCC

There was no correlation between the three tumor markers AFP, PIVKA-II, and GPC3 in terms of their presence in HCC cells.[42] In our study, the sensitivity of AFP, AFP-L3, PIVKA-II, and GPC3 was 20/40 (50%), 10/36 (27.7%), 20/40 (50%), and 16/40 (40%), respectively. We could not diagnose 28 of 40 (70%) HCC patients using AFP and PIVKA-II without using GPC3. However, with GPC3 we could identify an additional four patients with HCC among 12 patients; three were classified as being in a relatively early disease stage (International Union Against Cancer [UICC] stage II), hence GPC3 may be useful for diagnosis of early-stage HCC. We could diagnose 80% of our patients with HCC using AFP, PIVKA-II, and GPC3.[42]

3.3 Detection of Soluble GPC3 in Sera from Patients with Melanoma

Soluble GPC3 protein could be detected in culture supernatants of 5 of 11 melanoma cell lines and in sera from 40% of patients with melanoma.[49] The quantification by ELISA of GPC3 protein in sera of 91 preoperative patients with melanoma, five patients with large congenital melanocytic nevus, and 60 HDs who had many small melanocytic nevi was performed.[49] We detected and quantified GPC3 protein in the sera of 36 of 91 melanoma patients (39.6%), but more importantly, not in sera of patients with large congenital melanocytic nevus and HDs, whereas GPC3 mRNA and protein were expressed in melanocytic nevus tissues.[49]

There is no convincing correlation between levels of secreted GPC3 as measured by ELISA and levels of *GPC3* mRNA and protein expression determined by RT-PCR and immunohistochemical analysis in HCC and melanoma cell lines, or in HCC and melanoma tissues. About 40% of HCC and melanoma patients showed characteristics of GPC3 secretion, irrespective of GPC3 expression levels. The mechanisms of secretion of GPC3 from HCC and melanoma cells remain to be elucidated.

3.4 Comparison of Serum Concentrations of GPC3, 5-S-CD, and Melanoma Inhibitory Activity in Patients with Melanoma Classified by Stage

We compared the serum concentrations of GPC3, 5-S-CD, and MIA in patients with melanoma classified by stage.[49] Although serum concentrations of 5-S-CD and MIA increased markedly in patients with stage IV disease, percentages of serum GPC3-positive patients were almost equal among the five clinical stages. To

our surprise, we detected GPC3 in the sera of patients with very small melanomas, such as stage 0 or I. There was no correlation between the positive state of three tumor markers, GPC3, 5-S-CD, and MIA.[49] More importantly, 27 of 36 GPC3-positive patients were negative for both 5-S-CD and MIA, and many were classified as having relatively early UICC stage 0, I, and II disease. Thus, GPC3 is very useful for the diagnosis of melanoma at early stages. Finally, we could diagnose 59 of 91 (64.8%) cases of melanoma using 5-S-CD, MIA, and GPC3.

3.5 GPC3 Protein in the Sera of HCC and Melanoma Patients Disappeared After Surgical Treatments

Changes in serum levels of GPC3 before and after surgical treatments were seen in 15 pre-operative GPC3-positive patients (three HCC patients and 12 melanoma patients). For example, GPC3 protein was detectable in three patients with HCC prior to surgery, but GPC3 was not detectable after the surgery. GPC3 protein was detected in sera of 12 melanoma patients prior to surgery, but not so after the surgery, except for one patient, who could not be followed after postoperative day 27. Thus, GPC3 is useful for monitoring the response to treatment. Taken together, these results indicate that GPC3 may prove appropriate for diagnosing patients with HCC and melanoma and determining the outcome of therapy.

4. Known Biologic Properties of GPC3

In 1996, Pilia et al.[51] reported that *GPC3*, which encodes one member of the glypican family, is mutated in patients with Simpson-Golabi-Behmel syndrome. This syndrome is an X-linked disorder characterized by pre- and post-natal overgrowth, and a broad spectrum of clinical manifestations that vary from a very mild phenotype in carrier females, to infantile lethal forms in some males.[52] The list of clinical manifestations of this syndrome includes a distinct facial appearance, cleft palate, syndactyly, polydactyly, supernumerary nipples, cystic and dysplastic kidneys, and congenital heart defects.[53,54] Most *GPC3* mutations are point mutations or small deletions encompassing a varying number of exons.[55,56] Given the lack of correlation between patient phenotype and location of the mutations, it has been proposed that Simpson-Golabi-Behmel syndrome is caused by the lack of a functional GPC3 protein, with additional genetic factors being responsible for the intra- and inter-familial phenotypic variation. The development of *GPC3*-deficient mice added strong support for this hypothesis.[57] These mice have several abnormalities found in Simpson-Golabi-Behmel syndrome patients, including overgrowth, and cystic and dysplastic kidneys.

Furthermore, it was reported that GPC3 could induce apoptosis in certain types of tumor cells.[58] Some reports indicated that GPC3 expression is downregulated in tumors of different origin. They showed that, although GPC3 is expressed in normal ovary, mammary gland, and mesothelial cells, the expressions are undetectable in a significant proportion of ovarian and breast cancer, and mesothelioma cell lines.[59] In all cases where GPC3 expression was lost, the *GPC3* promoter was hypermethylated, and mutations were nil in the coding region. GPC3 expression was restored by treatment with a demethylating agent. In addition, the authors demonstrated that ectopic expression of GPC3 inhibits colony-forming activity in several of these cancer cell lines. Collectively, these data suggest that GPC3 can act as a negative regulator of growth in these cancers. As the expression of GPC3 is reduced during tumor progression in cancers originating from tissues that are GPC3-positive in adults, this reduction seems to play a role in generation of the malignant phenotype.

However, in the case of HCC tumors originating from tissues that express GPC3 only in the embryo, GPC3 expression tends to reappear with malignant transformation. Whether or not re-expression of GPC3 plays a role in progression of these tumors is unknown, i.e. why is GPC3 upregulated only in HCC and melanoma? Whether GPC3 is involved in oncogenesis in melanoma and HCC is under investigation in our laboratory.

4.1 Mouse Homolog of a Human GPC3 Evokes T Cell-Mediated Tumor Rejection Without Autoimmune Reactions in Mice

GPC3, expressed in almost all HCCs and melanomas, but not in normal tissues except for placenta or fetal liver, is an ideal tumor antigen for immunotherapy. Very recently, we reported that GPC3 could be highly immunogenic in mice, eliciting effective antitumor immunity with no evidence of autoimmunity in mice.[50] In this study, we identified a mouse GPC3-derived and K^d-restricted CTL epitope peptide in BALB/c mice. Inoculation of these GPC3 peptide-specific CTLs into subcutaneous Colon26 cancer cell tumors transfected with the mouse *GPC3* gene (C26/GPC3) led to rejection of the tumor *in vivo*. In addition, intravenous inoculation of these CTLs into sublethaly irradiated mice markedly inhibited growth of an established subcutaneous tumor. Inoculation of bone-marrow-derived dendritic cells pulsed with this peptide prevented the growth of subcutaneous and splenic C26/GPC3 tumors accompanied with massive infiltration of CD8+ T cells into tumors.

Evidence of autoimmune reactions was not observed in surviving mice that had rejected tumor cell challenges.

Thus, GPC3 is useful, not only for the diagnosis of HCC and melanoma, but also for possible immunotherapy or prevention of these tumors.

5. Conclusion

The novel oncofetal protein GPC3 appears to be a novel tumor marker useful for the diagnosis of HCC and melanoma, especially in early stages of the disorder. Furthermore, we found GPC3 to be highly immunogenic in mice and elicited effective anti-tumor immunity with no evidence of autoimmunity. Thus, GPC3 may be useful not only for diagnosis of HCC and melanoma, but also for possible immunotherapy or tumor prevention. The next step is to introduce GPC3 into the clinic as a tumor marker and as a cancer antigen for immunotherapy. We are making a GPC3 ELISA kit for the diagnosis of HCC and melanoma, and are planning to conduct a clinical trial of immunotherapy for HCC using GPC3 as a cancer antigen.

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臨床免疫学（上）

—基礎研究の進歩と最新の臨床—

基礎編

I. 概　　論

癌免疫療法研究の新展開

吉武義泰　　中面哲也　　西村泰治

癌免疫療法研究の新展開

New era of tumor immunotherapy

吉武義泰　中面哲也　西村泰治

Key words：癌，免疫療法，腫瘍拒絶抗原，CTL

はじめに

近年，分子標的治療が脚光を浴びているが，腫瘍免疫の分野においても癌特異的に発現する分子を標的とした治療法や，T細胞活性化に必要な分子を標的とした治療法などが盛んに行われるようになってきた．例えば癌細胞に特異的な抗原分子やレセプター分子を標的とするヒト化抗体が次々と開発され，臨床に応用され効果が認められつつある．

また，1991年にBoonらによりメラノーマ抗原MAGEの遺伝子が同定され，ヒトのT細胞が癌を異物として認識し，排除し得ることに科学的な根拠が与えられた[1]．すなわち，癌化に関連して特異な蛋白質が産生されると，これらに由来するペプチドがHLAクラスI分子に結合して細胞の表面に提示され，$CD8^+$細胞傷害性T細胞(cytotoxic T lymphocyte: CTL，キラーT細胞)がこれらを識別して活性化され，癌細胞を破壊するというメカニズムが存在する(図1)．現在までに，様々な腫瘍拒絶抗原およびペプチドが同定され，それらを用いた臨床試験が世界中で行われている．しかし，癌の免疫療法が治療法の一つとして再び脚光を浴びたのとは裏腹に，転移性進行癌を対象にしたペプチドワクチンを中心とした多くの臨床試験，トランスレーショナル・リサーチの結果は，我々の期待を満たすものではなかった．すなわち，腫瘍に反応するCTLは癌患者の体内で誘導できるが，癌の拒絶にまでは至らないといった結果がほとんどであった．しかし，手術(外科治療)・抗がん剤(化学療法)・放射線療法の癌の3大治療の適応外の患者に対しては，癌の縮小を期待すること自体が難問である．それよりも腫瘍に反応するCTLが確かに誘導できたという事実にこそ，我々は着目すべきではないだろうか．

本稿では，最初にcancer immunoeditingの概念を述べ，癌を免疫療法で治療するためには，どのようなアプローチが必要であるのかを考えていくことにする．

1. 腫瘍と免疫系の攻防：cancer immunoediting

従来より，形質転換した細胞は免疫系により認識され破壊される，という癌に対する免疫監視機構の概念がBurnetとThomasにより提唱されてきた．しかし，その詳細は明らかではなかったのだが，近年，その存在や癌が免疫系から逃避するメカニズムが明らかになりつつある．

腫瘍の免疫監視機構からの逃避は，3段階で行われると考えられる(図2)．まず第1段階では，ナチュラルキラー細胞(natural killer cell: NK細胞)やナチュラルキラーT細胞(natural killer T cell: NKT細胞)，またCTLや$CD4^+$ヘル

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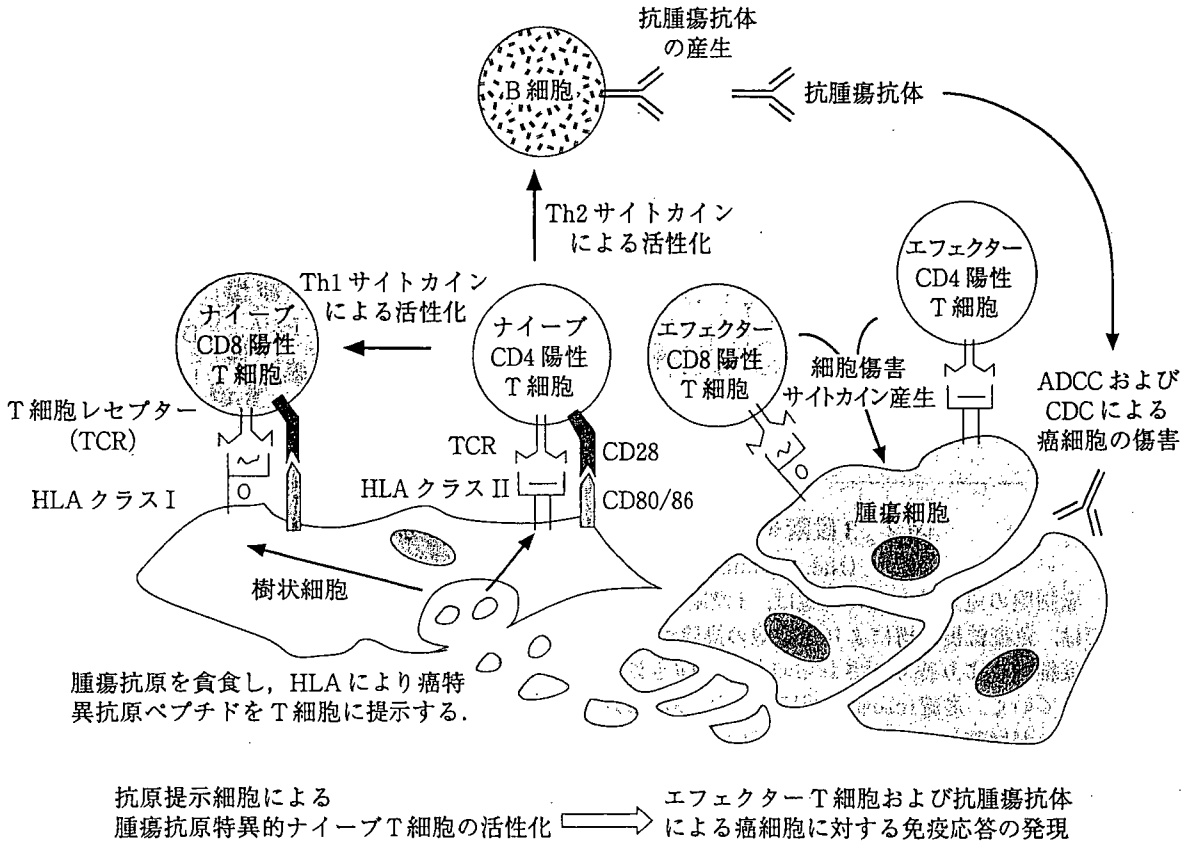

図1 腫瘍抗原特異的免疫反応の成り立ち

局所においてネクローシスあるいはアポトーシスにより破壊された癌細胞に由来する蛋白質を貪食した未熟な樹状細胞が，HLAクラスII分子やCD80/86などの共刺激分子を発現した成熟樹状細胞へと分化しながら所属リンパ節へと移行する．そこでナイーブTh細胞およびCTLを活性化し，エフェクターT細胞へと分化させる．活性化されたTh細胞は，IL-2などのTh1サイトカインを産生し活性化されたCD8陽性T細胞の増殖を促進するとともに，抗原提示細胞を活性化する．エフェクターCTLは，癌細胞のHLAクラスIに提示されている腫瘍抗原ペプチドを認識し，これを傷害する．HLAクラスIIを発現している癌細胞に対しては，エフェクターTh細胞も提示されている腫瘍抗原を認識することができ，これを傷害したりサイトカインの産生などを行う．また，活性化CD4陽性T細胞はIL-4やIL-6などのTh2サイトカインを産生することにより，B細胞を抗体産生細胞へと分化させ，抗腫瘍抗体が産生されるようになる．このような抗体により，抗体依存性細胞傷害活性(ADCC)や補体依存性細胞傷害活性(CDC)が誘導されて，癌細胞が破壊される．以上のような機序により，腫瘍抗原特異的免疫反応が生じていると考えられている．

パーT細胞(Th細胞)により攻撃され，癌細胞は排除されていく(elimination)．第2段階では，免疫系により繰り返される攻撃から生き延びることのできる多様性をもった癌細胞が現れるようになり(equilibrium)，第3段階として，そのような癌細胞が増殖し，腫瘍を形成していく(escape)，という流れである[2]．この多様性こそが，癌細胞のもつ免疫逃避の手段なのである．例えば，癌細胞が突然変異によりMHCクラスI分子を発現しなくなると，CTLによる攻撃から逃避できるため優勢を占めるようになる．実際に，癌細胞においてすべてのクラスI，あるいは特定の対立遺伝子の喪失が少なからず発生している．

一般的に，クラスIが喪失している細胞に対してはNK細胞やNKT細胞が細胞傷害を示すことが知られているが，これまで行われてきたCTL，Th細胞単独での免疫療法に加えて，今後はNKおよびNKT細胞も含めた免疫療法を考えていく必要があると考えられる．

Rockenらは，クラスIの発現が低い癌細胞に対して，NK細胞が反応してIFN-γを分泌する

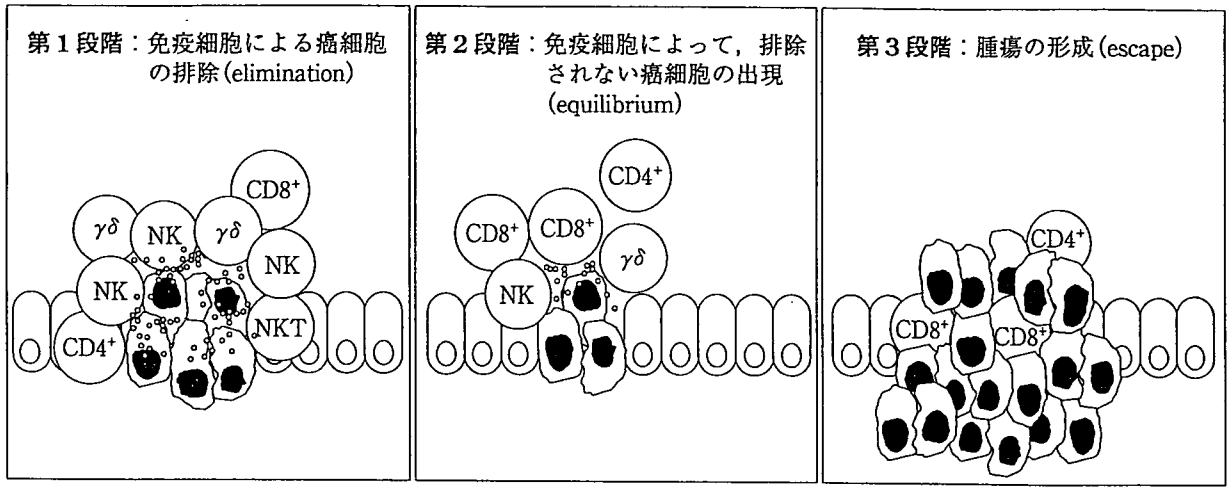

図2 3段階を経た癌細胞の免疫監視機構からの逃避機構

(Nature Immunology 2002より改変)

癌細胞の免疫系からの逃避は，3段階で行われると考えられている．通常排除(elimination)期では，免疫監視機構により癌細胞の排除が行われている．平衡(equilibrium)期では，癌細胞は，免疫細胞により繰り返される攻撃から生き延びることができるように，自身を様々に形質転換させていく．逃避(escape)期になると，最終的に免疫機構から攻撃されないように形質転換した癌細胞が増殖し，腫瘍を形成していくことになる．

ことにより，樹状細胞(dendritic cell: DC)が活性化されIL-12を分泌するようになり，更にCTLが活性化され，クラスIの発現が低くても癌細胞を傷害できるようになることを報告した[3]．また，最近のトピックスとして，非古典的クラスI分子の一つで，通常は妊娠中のトロホブラスト(栄養膜)や胸腺上皮細胞にしか発現せず，それらの細胞を免疫系の攻撃から守っているHLA-Gが癌細胞に発現することにより，癌細胞がNK細胞やCTLの攻撃から逃避しているという報告が相次いでいる．この現象には癌細胞における古典的クラスI分子の発現喪失が相関しており，IL-10が関与しているらしい[4]．

Strandらは，肝細胞癌がFasの発現を落とすことにより，CTLに発現するFasLの攻撃から逃れているだけでなく，癌細胞自身がFasLを発現することにより，リンパ球を傷害している可能性があると報告している[5]．その後，大腸癌，乳癌，胆管細胞癌，血管肉腫にもFasLが発現しており，リンパ球を傷害しているという報告がなされている．PD-1は，細胞死の誘導時に発現が増強される遺伝子として単離され，T細胞応答を負に制御しており，免疫寛容の維持にも重要な役割を果たしていると考えられている分子である．最近，湊らを含む複数の研究室から，PD-1のリガンドの一つであるPD-L1が腫瘍細胞に発現しており，免疫系からの逃避に関与しているとの報告がなされている[6]．

2. 腫瘍拒絶抗原が備えているべき性質

免疫療法への応用を考える場合には，①多くの患者のすべての癌細胞に高発現しているという汎用性，②癌細胞および腫瘍組織だけに発現しているという特異性，③免疫原性が強く抑制性免疫応答を起こしにくい性質，すなわち腫瘍拒絶能が高く，癌化に関連しているために抗原消失を起こしにくく，更に自己免疫などの有害事象誘導の危険性が低いなどの観点から各抗原の特徴をとらえる必要がある．実際にこれらすべての条件を満たす抗原が見つかっているわけではないが，WT-1[7]，hTERT[8]，survivin[9]などは比較的上記の基準を満たしており，腫瘍拒絶抗原として臨床試験も進められている．

3. ヒト癌抗原の分類と同定方法

現在までに同定されているヒト癌抗原を分類

表1 ヒト癌抗原の同定方法

1) 癌化と関連した腫瘍抗原候補に対するT細胞応答の解析 細胞の癌化に関連した癌遺伝子や癌抑制遺伝子産物の突然変異部分，融合蛋白の境界部分，あるいはウイルス抗原に由来するペプチドを特異的に認識するT細胞の証明(変異Ras，変異p53，BCR/ABLほか)
2) 癌細胞に特異的に反応するT細胞株(クローン)を利用した，癌細胞由来のcDNA発現ライブラリーのスクリーニング(MAGE-1/3，チロシナーゼ，gp100，Melan-A/MART-1，SART-1ほか多数)
3) 癌患者血清中の抗腫瘍抗原IgG抗体を利用した，癌細胞由来のcDNA発現ライブラリーのスクリーニング(SEREX法)(NY-ESO-1，KM-HN-1，SSX-2ほか多数)
4) cDNAマイクロアレイ解析による，遺伝子発現の組織特異性が抗腫瘍免疫の誘導に適している腫瘍抗原遺伝子の探索(GPC3，PP-RPほか現在進行中)

すると，①組織特異抗原(分化抗原や癌胎児性抗原)，②cancer-testis(CT)抗原，③癌細胞で過剰発現している蛋白質に由来する抗原，④発癌に関連した癌遺伝子，癌抑制遺伝子，融合遺伝子の産物に由来する抗原，⑤癌細胞特異的変異ペプチド抗原(RNAスプライシングやORF異常などによるもの)，⑥癌細胞に特異的なプロセシングにより生じる抗原ペプチド，⑦発癌関連ウイルスに由来する抗原，などがあげられる．また，その代表的な同定方法として表1に示す4つがあげられる．その中でも近年精力的に行われている2つの方法について詳述する．

a. SEREX法(serological identification of antigens by recombinant expression cloning)による腫瘍拒絶抗原の同定

この10年間に，癌細胞由来のcDNAライブラリーを大腸菌に発現させ，その蛋白質を患者の血清中の抗腫瘍抗原IgG抗体でスクリーニングするSEREX法により数多くの癌抗原の候補が同定されている．著者らが膵癌のSEREXで同定したheat shock protein 105(HSP105)の蛋白は，成人の正常臓器では精巣で高発現する以外ほとんど発現しないが，膵癌・大腸癌・食道癌・甲状腺癌・乳癌などの癌ではほとんどの症例で高発現を示すことがわかった[10,11]．しかも，HSP105は癌細胞におけるアポトーシスの抑制にかかわっていることが示された．

CT抗原と総称される抗原は，名前が示すとおりに種々の癌組織および正常組織では免疫系から隔離されたtestis(精巣，睾丸)，卵巣，胎盤のみに発現する抗原群で，ヒトのメラノーマで同定された最初の癌抗原MAGE，SEREXで同定されたNY-ESO-1が代表的なCT抗原である．発現パターンから免疫療法の理想的なターゲットと考えられており，同定されたCT抗原をターゲットとした抗腫瘍免疫療法の臨床試験が既に行われている．効率よくSEREX法でCT抗原を同定する工夫として，著者らは，頭頸部癌患者の血清IgGに反応する精巣cDNAライブラリーの検索を行い，3種類の新規CT抗原を同定した[12]．このうちKM-HN-1は頭頸部癌以外にも多様な癌細胞に発現を認め，また血清中にKM-HN-1に対するIgG抗体価が，健常人ではほとんど検出されないのに比べ，様々な癌患者の40-100%において検出されることも発見し，その腫瘍マーカーとしての有用性を示した[13]．

b. cDNAマイクロアレイ解析による理想的な腫瘍拒絶抗原の探索と同定

遺伝子発現を網羅的にスクリーニングすることのできるcDNAマイクロアレイ解析を利用することにより，組織特異抗原，癌精巣抗原，癌胎児性抗原また，癌高発現抗原などを同定することが可能となった．著者らは，東大医科研・ヒトゲノムセンターの中村祐輔博士との共同研究により，cDNAマイクロアレイを利用した2万種類を越える遺伝子の肝細胞癌(hepatocellular carcinoma: HCC)と正常組織における発現

解析データを用いて，上記の理想的な癌抗原としてふさわしいHCC特異的な新規癌胎児性抗原としてglypican-3(GPC3)を同定した．GPC3は膜蛋白で分泌され，既知のαフェトプロテイン(AFP)およびPIVKA-IIに次ぐHCCの第3の腫瘍マーカーとしての有用性も示された[14]．また更に，GPC3のメラノーマにおける腫瘍マーカーとしての有用性も示された[15]．著者らは食道癌においても同様の方法により，食道癌に特異的に高発現して発癌にも関連する新規抗原，proliferation potential-related protein(PP-RP)を同定した[16]．これらの抗原はいずれもCTLに認識され，マウスにおける*in vivo*実験で腫瘍拒絶効果を誘導することが示された[17]．

4. CTLにより認識される汎用性の高い腫瘍抗原ペプチドの同定

欧米白人ではHLA-A2の頻度が高いこと，メラノーマの患者が多いことなどの理由により，癌拒絶抗原ペプチドとして，メラノーマ由来のHLA-A2拘束性のペプチドが圧倒的に多く同定されている．日本人においてもHLA-A2の発現頻度は42.9％と比較的高いが，HLA-A分子の中で日本人に最も頻度が高いのは57.9％を占めるHLA-A24(A*2402)である．どちらも有していない日本人は16.4％にすぎない．したがって，著者らが同定したような，ほとんどの癌患者の癌細胞において高発現している癌抗原に関して，HLA-A24あるいは-A2拘束性の癌拒絶抗原ペプチドを同定することにより，多くの日本人癌患者を対象としたペプチドワクチンやDCワクチン，更には養子免疫療法(adoptive-cell-transfer therapy: ACT therapy)などの免疫療法が可能となると考えられる．また，HLA-A24に強く結合するペプチドは，BALB/cマウスのクラスI分子であるK^dにもよく結合することがわかっており，癌抗原のアミノ酸配列の中で，ヒトとマウスに共通な(HLA-A24にもK^dにも結合し得る)ペプチドを合成して，それぞれの末梢血単核細胞を刺激することにより，ヒトとマウスの両方でCTLの誘導を試みることができる．これを利用して，マウスモデルにおける*in vivo*腫瘍拒絶効果を検討することができる．

5. Th細胞を活性化する腫瘍抗原

HLAクラスII分子は，抗原提示細胞や活性化Tリンパ球などを除き，通常の体細胞には発現していないが，癌細胞に発現する場合も決して少なくはない[18]．マウスではナイーブCTLが抗原による初回感作を受ける際に，Th細胞が存在しないと強い二次免疫応答を示すメモリーCTLが誘導されないことが報告されている[19]．これらの事実は，癌の免疫療法を考えるうえで，MHCクラスIIおよびTh細胞についても考慮することが重要であることを示している．ところが，CTLのエピトープペプチドの決定と比べて，Th細胞のエピトープを決めるのは容易ではない．過去に同定されたCTLエピトープの数に比べ，Th細胞のエピトープの報告は圧倒的に少ない[20]．クラスII分子に結合するペプチドのモチーフはクラスI分子ほど法則性もなく，クラスI分子のそれに比べ解析が遅れていることが一因である．

また，クラスIにおけるA2やA24のように日本人集団において高頻度に観察されるクラスII分子としては，60％が陽性のDR53(DRB4*0103)くらいしか存在しない．したがってDR53拘束性エピトープを決める意義はあるが，その他の多様なクラスII分子により提示されるエピトープペプチドを決めたとしても汎用性に乏しい．腫瘍抗原特異的Th細胞を誘導するためには，合理性だけを考えると，癌抗原遺伝子あるいは蛋白質まるごとを用いたDNAワクチン，蛋白ワクチン更には，それらを導入あるいは貪食させたDCワクチンが有効であると考えられる．そういう意味では，担癌患者にIgG産生を誘導するSEREX法で同定された癌抗原は，*in vivo*で少なくともTh細胞に認識されることが保証されていることから，腫瘍抗原特異的Th細胞の誘導に有用であると考えられる．

6. 抗体を利用した腫瘍免疫療法

1975年にハイブリドーマ法が開発されたこ

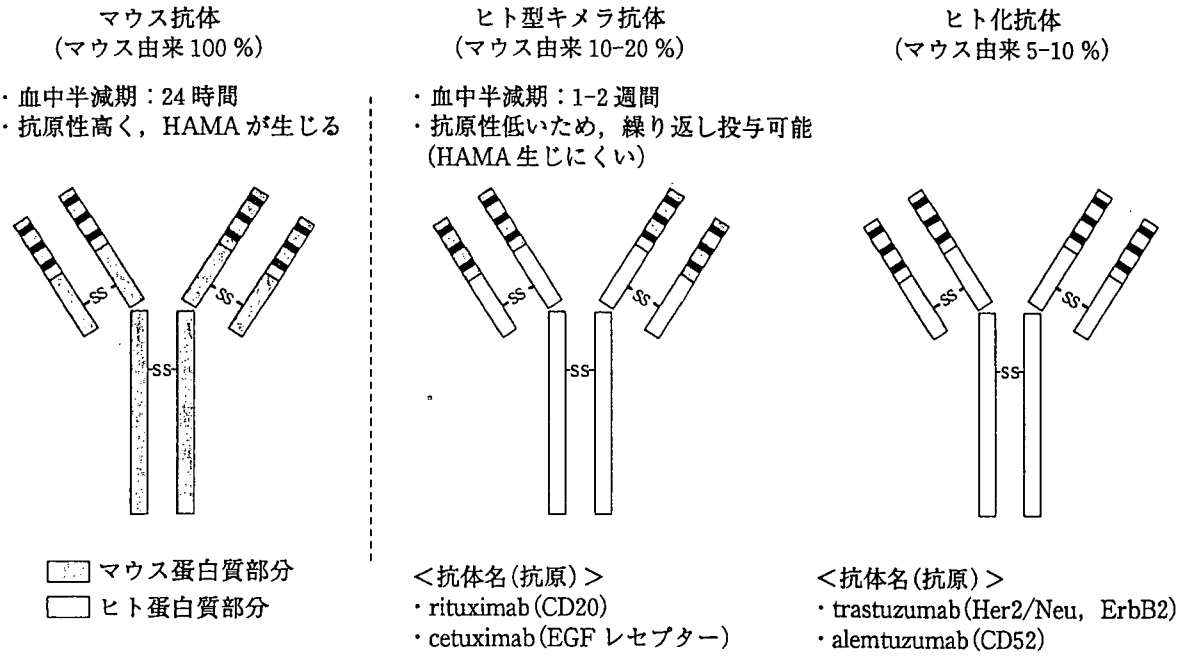

図3　腫瘍の免疫療法に利用されているモノクローナル抗体の構造と特徴

遺伝子改変によって，マウス抗体のV領域をヒト抗体のC領域に結合させたヒト型キメラ抗体が作製された．次いで，抗体のH鎖およびL鎖のV領域は，抗原との結合に直接関与している各3カ所の相補性決定領域CDR(complementarity determining region)とそれ以外の領域(フレームワーク：FR)に分けられるが，CDRのみがマウス抗体由来でFRはヒト抗体に置き換えてあるヒト化抗体が開発された．

とにより，マウスモノクローナル抗体を作製することが可能になり，医薬品としての抗体が開発されるようになった．1980年代には癌特異的なマウスモノクローナル抗体を用いた臨床試験が行われたが，十分な治療効果を得ることはできなかった．その最大の原因は，マウス抗体がヒト免疫系に異物として認識されてしまい，ヒト抗マウス抗体(human anti-mouse antibody: HAMA)が誘導されてしまうからであった．HAMAが誘導されると，投与した抗体が血中より短時間で消失してしまい治療効果が消失してしまうことに加えて，抗体の投与を繰り返し行うことによりアナフィラキシーなどのショック症状を起こす危険性が生じる．したがって，抗体の抗原性の低減が必要となった．1980年代の半ばに，抗体の10-20％がマウス抗体由来で残りはヒト抗体由来であるヒト型キメラ抗体が開発され，次いで，その5-10％のみがマウス抗体由来であるヒト化抗体が開発された(図3)．これまでに複数の抗体が医薬として発売され有効性が示唆されている．現在ではヒト抗体遺伝子トランスジェニックマウスやヒト抗体遺伝子ファージディスプレイ法により，完全ヒト型抗体の作製も可能となってきている．抗体の癌細胞除去のメカニズムは，抗体依存性細胞傷害活性(antibody-dependent cellular cytotoxicity: ADCC)と補体依存性細胞傷害活性(complement-dependent cytotoxicity: CDC)であると考えられている．

現在様々な癌抗体医薬の臨床開発が進行しているが，その幾つかについて，紹介したい．

(1) rituximab(CD20に対する抗体)：CD20はB細胞に特異的に発現しており，更に活性化されたB細胞では静止期のB細胞に比べて発現量が増加する．rituximabはADCCとCDCによりCD20を発現している活性化したB細胞を除去することができる．B細胞非ホジキンリンパ腫再発患者の約50％に奏効し，更に化学療法を併用することでより高い奏効率が得られることも報告されている[21]．

(2) trastuzumab(Her2に対する抗体)：Her2はErbBチロシンキナーゼ受容体ファミリーの