CHAPTER

ANTIGEN-RECEPTOR GENE-MODIFIED T CELLS FOR TREATMENT OF GLIOMA

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Abstract: Immunological effector cells and molecules have been shown to access intracranial tumor sites despite the existence of blood brain barrier (BBB) or immunosuppressive mechanisms associated with brain tumors. Recent progress in T-cell biology and tumor immunology made possible to develop strategies of tumor-associated antigen-specific immunotherapeutic approaches such as vaccination with defined antigens and adoptive T-cell therapy with antigen-specific T cells including gene-modified T cells for the treatment of patients with brain tumors. An array of recent reports on the trials of active and passive immunotherapy for patients with brain tumors have documented safety and some preliminary clinical efficacy, although the ultimate judgment for clinical benefits awaits rigorous evaluation in trials of later phases. Nevertheless, treatment with lymphocytes that are engineered to express tumor-specific receptor genes is a promising immunotherapy against glioma, based on the significant efficacy reported in the trials for patients with other types of malignancy. Overcoming the relative difficulty to apply immunotherapeutic approach to intracranial region, current advances in the understanding of human tumor immunology and the gene-therapy methodology will address the development of effective immunotherapy of brain tumors.

INTRODUCTION

Central nervous system (CNS) has been considered as an immunological privileged site that may provide a unique difficulty for cancer immunotherapy. However, recent studies have clearly demonstrated that immunological maneuvers such as delivery of effector cells and molecules could target tumor sites in CNS. Currently, advances in

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cancer immunology from multiple aspects have provided strategies of antigen-specific immunotherapy for malignancy. In this article, we briefly overview the recent progress in the understanding of interaction of immune cells and central nerve system, review the recent novel strategies of immunotherapy of cancer with a special focus on adoptive therapy with gene-modified T cells and discuss how these promising strategies can be applied to treat patients with brain tumors.

INTERACTION OF IMMUNE SYSTEM WITH CENTRAL NERVOUS SYSTEM

Historically, the brain has been assumed as an immune-privileged site because of (1) the presence of blood brain barrier (BBB), (2) the lack of lymphatics and conventional dendritic cells (DC) and (3) immunosuppressive environment evidenced by the lack of allograft rejection in the brain. However, recent studies have demonstrated that immune cells do interact with CNS that is strongly evidenced in the diseases such as multiple sclerosis or experimental autoimmune encephalitis. In addition, the immune effector cells and molecules were shown to approach to intracranial tumors in numerous preclinical studies in mouse.[1]

BBB, that consists with the CNS capillary endothelial cells, functions with pericytes, parenchymal membrane, and astrocytic feet as a neurovascular unit (NVU).[2] The BBB in patients with brain tumor appear to be compromised,[3,4] associated with increased edema and/or pericyte swelling. These disruptions are considered to affect the migration of immune cells and the perfusion of effector molecules into the parenchyma. Moreover, it is now well understood that immune cells do move across the intact BBB.[5]

The primary antigen presenting cells (APC) in CNS have been referred to various cell types including vascular endotherial cells, smooth muscle cells, astrocytes, perivascular macrophages, choroid plexus epitherial cells, neurons and DC. Recent work focused on CNS DC as more potent antigen presenting- and T-cell stimulating-APC compared to CNS microglias and macrophages.[6] Other reported the suppressing activity of plasmacytoid DC, the major population of CNS DC, suggesting a regulatory role for plasmacytoid DC in T-cell activation in CNS.[7] Cervical lymph nodes have been shown to play an important role as the major draining lymph node for DC in CNS.[8,9]

Immunosuppressive factors have been found in the environment associated with brain tumor. These include soluble factors such as TGF-β1, -2 and -3,[10-12] PGE2,[13-15] IL-10[16-18] and gangliosides.[19-21] Interactions between cell surface molecules such as Fas-FasL,[22,23] PD-1-PD-L1,[24,25] receptor-binding cancer antigen expressed on Sico cells,[26] and CD70[27,28] has been suggested to play a role in the suppression of immunological reaction against brain tumor. These factors may play an important role for brain tumor in their evasion from immunosurveillance and may be attractive targets for the manipulation for effective immunotherapy.

In summary, recent works have explored the characteristics of CNS as an immune-specialized rather than immune-privileged site. Many of the fundamental mechanisms shown in non-CNS models seem to work also in CNS in general. Future work will segregate the generality and specificity of immune reaction in CNS more precisely and will help the development of effective immunotherapy of brain tumor.

DEVELOPMENT OF SPECIFIC CANCER IMMUNOTHERAPY

Immune system has been considered to protect host by eliminating exogenous agents as nonself while keeping self-tissues intact, known as immunological tolerance. Therefore, whether tumor cells that developed from normal tissues can be recognized by immune system or not had been a warm debate. In 1953, Foley et al[29] showed the existence of tumor specific antigens in 3-methylcholanthrene-induced mouse tumors by demonstrating the development of specific immunity to individual tumor in mice immunized with different tumor lines. Burnet and Thomas[30,31] formally introduced the notion that the immune system could protect the host from neoplastic disease as the cancer immunosurveillance hypothesis. However, following studies indicated the low immunogenisity of the naturally occurring spontaneous tumors compared to the carcinogen-induced tumors and suggested the difficulty of immunotherapy of human malignancies. In 1980s, technology to establish tumor-reactive cytotoxic T cells from peripheral blood mononuclear cells or tumor infiltration lymphocytes (TIL) has emerged. In 1991, Boon and his colleagues[32] identified MAGE-1/MAGE-A1 as a tumor antigen of human melanoma recognized by a cytotoxic T-cell line established from a patient with malignant melanoma. Since this memorial milestone, numerous tumor antigens and their epitopes recognized by $CD8^+$ or $CD4^+$ T cells have been identified.[33] Recent studies confirmed the high incidence of tumor formation in a variety of immunodeficient mice clearly indicating the existence of immunosurveillance of cancer.[34]

Identification of the tumor antigens in human tumors made possible to develop the therapeutic approaches to enhance the specific immunity against tumors. Specific immunotherapy of tumor consists of two major approaches. In one approach, identified tumor antigen in many kinds of form are directly administrated into hosts as cancer vaccine to develop specific immune response in patients, known as active immunotherapy. Another approach use technologies to establish tumor-reacting immune cells in vitro by culturing patients lymphocytes in order to react to tumor antigens, followed by the administration of established immune cells into patients, known as passive immunotherapy. Recent progress in the gene-therapy technology provided the means to endow T cells with defined antigen specificity as well as increased functional properties.

ACTIVE IMMUNOTHERAPY OF CANCER

Initially, tumor cells and their lysates have been tested for their potential as vaccine against tumor. Recent progress in the identification of tumor-associated antigens made possible to utilize the synthetic peptides of antigen epitopes for T-cell recognition, recombinant or synthetic proteins that contain multiple T-cell epitopes, or nucleic acids that encode the antigens. In 2010, Provenge® was approved by FDA for the treatment of patients with prostate cancer as the first drug of therapeutic vaccine against tumor in USA. This vaccine consists of in vitro cultured patients' immune cells including DC that incorporate fusion protein of PAP and GM-CSF. Oncophage® utilizes the heat shock protein manufactured from patient's own tumor tissue. This heat shock protein is considered as a molecular chaperone that contains antigenic peptides derived from the tumor. Oncophage® was approved in 2008 in the Russian Federation and subsequently in EU for the treatment of patients with renal cancer. DCVax-Brain® is a DC vaccine utilizing patient's DC pulsed with patient's own tumor lysate and was approved in 2007 in Switzerland as a drug to treat brain cancer.

ACTIVE IMMUNOTHERAPY OF BRAIN TUMOR

A variety of mouse models have demonstrated that peripheral vaccinations against intracranial tumor can be effective despite the existence of BBB and immunosuppressive characteristics of tumor.[1] Clinical trials of cancer vaccines for patients with brain tumors, however, are in the early stages and await precise evaluation of their effectiveness in randomized studies, although encouraging results indicated some objective clinical responses and potential improvements of patient's survival.

Besides DCVax-Brain®, there have been a substantial number of studies utilizing brain tumor cell-based vaccine approaches that have basically demonstrated safety and preliminary efficacy.[35-54] These studies utilize either irradiated or fixed tumor cells, tumor cell lysates, DC pulsed with tumor/tumor lysates/peptides eluted from tumor, or DC-tumor fusion cells. The whole tumor cell-based approach has benefits as to: (1) it does not require the identification of antigens; (2) it may contain multiple antigens to be recognized by a wide variety of immune cell types including both $CD8^+$ and $CD4^+$ T cells. However, the whole tumor cell-based medicinal product may have its limitation and shortcomings as to: (1) because of the component of self antigens it may induce immunological tolerance mediated by regulatory mechanisms such as regulatory T cells, (2) normal brain components may otherwise induce autoimmune encephalitis, and (3) high costs, troublesome procedures, and complex quality control issues associated with large-scale culture of autologous tumor cells may hamper feasibility and widespread application.

Taking advantage of the identification of brain tumor-associated antigens, peptide-based vaccine strategies including DC vaccines pulsed with antigenic peptides have been evaluated. It has been difficult to find a tumor antigen that is widely expressed in brain tumors but completely absent in normal tissues. Nevertheless, a variety of molecules are known to be expressed preferentially in brain tumors and epitope peptides to elicit T-cell response were identified (Table 1). Among these, some peptides are in the process of clinical evaluation as therapeutic vaccines. Yajima et al[55] reported a Phase I study of personalized peptide-based vaccine in patients with recurrent malignant gliomas. In this trial, each patient was tested for their humoral immune response against a panel of antigens prior to the enrollment. The personalized combination of peptides was decided according to the positive immune reaction to the peptides because the authors consider that it can be a measure of pre-existence of sensitized T-cell population. The treatment was well tolerated and resulted in an 89-week median survival of the treated patients. Izumoto et al[56] reported a Phase II clinical trial utilizing a Wilm's Tumor (WT) 1-derived peptide. In this study, median progression-free survival was 20 weeks and possible association between the WT1 expression level and clinical responses was reported. However, the overexpression of WT1 antigen in solid tumors including brain tumors is controversial, and therefore the rationale for WT1-based immunotherapy for brain tumor awaits further evaluation. Recently Okada et al[57] reported a Phase I/II trial of a vaccination with α-type 1 polarized DC (αDC1) loaded with 4 glioma-associated antigen epitope synthetic peptides ($EphA2_{883\text{-}891}$, IL-13 $R\alpha2_{345\text{-}353:1A9V}$, $YKL\text{-}40_{201\text{-}210}$, $GP100_{209\text{-}217:M2}$) and administration of polyinosinic-polycytidylic acid [poly(IC)] stabilized by lysine and carboxymethylcellulose (polyICLC) in patients with recurrent malignant gliomas. They reported that the regimen was well tolerated. The vaccine induced the upregulation of type 1 cytokines including IFNα and CXCL10. Of 22 patients enrolled, 9 achieved progression free status lasting at least 12 months. Two patients experienced objective clinical tumor regression. Of these two patients, one demonstrated sustained complete response.

Table 1. CTL epitopes of glioma

Antigens	HLA Restriction	Expression in Normal Cells	References
EphA2	A*0201	Site of cell-to-cell contact	87-89
IL-13R	A*0201, A*2402	Testis	90-93
gp100	16 HLA class I epitopes	Melanocytic cells	94
YKL-40	A*0201	Macrophages, Neutrophils, Serum, Blood vessels, Extracellular matrix, Astrocytes	57,95,96
SOX2	A*0201	Testis, Neutal stem cells, Fetal brain	97-99
SOX11	A*0201	Fetal brain	98
HER2/neu	13 HLA class I epitopes	Ubiquitously expressed	94
EGFRvIII	A*0201	No	100
MAGE-1	11 HLA class I epitopes	Testis, Placenta	94
TRP-2	A*0201	Melanocytis cells	101
AIM-2	A1	Testis, Liver	94
Survivin	A*0201, A*2402, A1, A3	Undetected in most differentiated normal adult tissues	102-105
SART-1	A*2402, A*2601	Testis	106,107
WT1	A*0201, A*2402, A1	Kidney, Bone marrow, Pleura, Peritonium, Testis, Ovary, Hematopoietic stem cells	108-112

PASSIVE IMMUNOTHERAPY OF CANCER

Passive immunotherapy of cancer includes the administration of various immune effector cells and effector molecules such as mAbs, cytokines, or receptor ligands. The approach to administrate effector molecule is very important in the scope of manipulation of immunological balance in the tumor-bearing hosts. However, it rather belongs to the molecular targeted therapy. This review solely focuses on the administration of effector T cells.

All of adoptive T-cell therapies remain as experimental therapies at present. However, the strategy to administrate a large number of tumor-reactive T cells is an attractive approach to the treatment for patients with malignancy. Indeed, adoptive immunotherapy with in vitro expanded lymphocytes derived from patient's TIL for the treatment of malignant melanoma has demonstrated objective clinical response by RECIST criteria in around 50% of enrolled patients in recent early phase trials.[58] Initial trials to administrate tumor-reactive T-cell line/clone were unsuccessful in both clinical response and persistence of infused cells.[59-61] Recent advances in several areas of human T-cell biology suggested that these disappointed results might be related to two major obstacles. One obstacle comes from immunosuppressive environment of tumor-bearing hosts.[62] The second comes from the reduced quality of T cells generated by long term in vitro culture for their expansion.[63] To overcome these obstacles, recent protocols incorporate the pretreatment of patients with lymphodepleting chemotherapy and/or irradiation. The reason for the effectiveness of these pretreatment is not fully understood but is suggested to depend on (1) depletion of immunosuppressive cell populations such as regulatory T cells, (2) ablation of cytokine

competition between infused and pre-existing lymphocytes, known as cytokine sinks, (3) supply of spaces for infused cell to expand, known as homeostatic expansion, and (4) improvement of APC function and availability.[58] Recent protocols also tend to use lymphocytes cultured in relatively short period in vitro in order to preserve T-cell quality to maintain in vivo survival. Combination of these maneuvers with high dose IL-2 administration has reported a significant improvement of adoptive therapy with TIL for patients with progressive malignant melanoma as up to 72% of patients demonstrated objective clinical response of CR or PR in RECIST criteria.[64] Although the combination of conditioning of patients has shown to be effective, if every kinds of adoptive T-cell therapy requires such intensive pretreatment and/or IL-2 administration for in vivo maintenance of infused cells as well as successful clinical response needs further evaluation.

ANTIGEN-RECEPTOR GENE-MODIFIED T CELLS FOR TREATMENT OF CANCER

In addition to the advances in the control of T-cell quality and fate, ex vivo genetic manipulation has been developed to extend the availability of adoptive T-cell therapy. Adoptive T-cell therapy has been almost exclusively applied to patients with malignant melanoma with very limited exceptions. It is because the isolation and expansion of tumor-reacting lymphocytes that pre-exist in patients has been difficult in patients with other solid tumors. Moreover, the T cells with T-cell receptor (TCR) of sufficiently high affinity are generally in very low incidence because majority of human tumor-associated antigens are of self-antigens to some extent and are poorly immunogenic. To overcome this problem, genetic engineering of polyclonal patients' lymphocytes by retrovius or lentivirus vector encoding tumor-reactive TCR has been developed.[65,66] In this technique, large amount of polyclonal lymphocytes can be redirected their specificity by in vitro culture in relatively short period to a tumor-associated antigen with considerably high affinity because the TCR is derived from a preselected T-cell clone reactive to tumor (Figs. 1 and 3). The adoptive transfer of lymphocytes engineered to express MART-1 specific TCR into patients with metastatic melanoma demonstrated long-lasting maintenance in 2 out of 15 patients enrolled, both demonstrated objective tumor regression.[66] Subsequent trial with higher avidity of TCR demonstrated objective clinical response in up to 30% of patients.[67] Recent report on the usage of artificially modified high avidity TCR reacting to NY-ESO-1 antigen demonstrated objective clinical responses in four (60%) of six patients with synovial cell sarcomas and five (45%) of 11 patients with melanoma.[68]

The existence of endogenous TCR in T cells has been reported to be associated with the inefficient expression of transduced TCRs in T-lymphocytes. It is because endogenous TCR competes with introduced TCR for CD3 molecules. In addition, the introduced TCR α and β chains form mispaired TCRs with endogenous TCR subunits, which not only further decrease the expression level of transduced TCR pairs but also cause the generation of T cells with unexpected specificities including self-reactivity.[69] To improve the efficacy of TCR engineering, we developed novel retroviral vectors encoding both siRNA that down-regulate the endogenous TCR and a siRNA-resistant TCR specific for tumor-associated antigens such as MAGE-A4 or WT1 (Fig. 2). These vectors efficiently suppressed the endogenous TCR and enhanced the expression of transduced tumor-associated antigen-specific TCR resulting in the enhanced tumor cytotoxicity.[70]

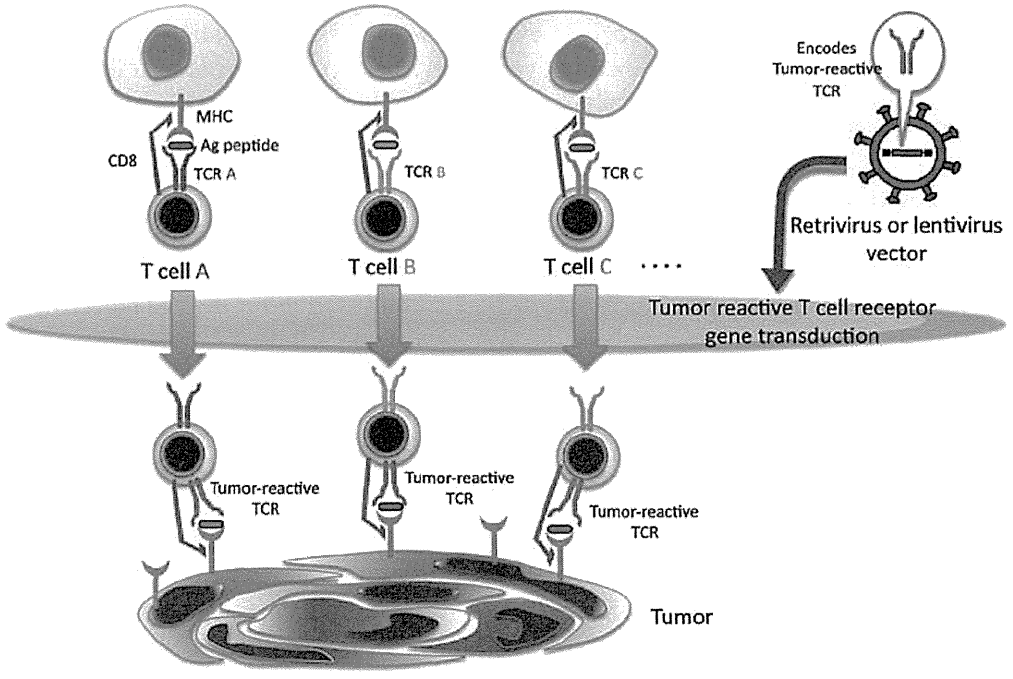

Figure 1. Antigen recognition of TCR-engineered CD8+ T cells. Polyclonal T cells are redirected their antigen specificity by retroviral or lentiviral transfer of tumor-reactive TCR gene. A large amount of tumor-reactive T cells are generated by relatively short period of in vitro culture.

Another unique attempt to provide tumor-reacting capacity to polyclonal lymphocytes by genetic engineering is to engineer lymphocytes to express a chimeric antigen receptor (CAR) that consists of antigen-binding region of mAb fused with the signal-transduction domain of CD3ζ or FcεRIγ (Fig. 3).[71] Theoretical advantages of this method are (1) independence of MHC class I expression of tumor, (2) capability to engineer not only CD8+ T cells but many of other cell types including CD4+ T cells, (3) avoidance of the influence of endogenous TCR that is one major obstacles in TCR transduction, and (4) better persistence and penetration into tumor site compared to mAb drug. Initial clinical trials (a trial targeting α-Folate receptor to treat ovarian cancer,[72] a trial targeting carbonic anhydrase IX to treat renal cell carcinoma,[73] a trial targeting CD20 to treat lymphoma[74]) demonstrated very limited persistence of transferred cells without clear clinical responses. The absence of appropriate costimulatory signal was considered to be responsible at least in part for these results. To overcome this obstacle, next generation of CARs that includes signal transduction domains of CD28 and some other costimulatory receptors such as OX40, 4-1BB have been developed.[71] Recently, the improved persistence of lymphocytes engineered to express anti-CD19 CAR incorporating CD28 signaling fragment in patients with lymphoma has been demonstrated.[75]

One alternative approach for prolonged in vivo persistence of CAR engineered lymphocytes was demonstrated utilizing Epstain-Barr virus (EBV)-specific T cells engineered to co-express CAR specific for GD2.[76] In this trial, CAR engineered EBV-specific cytotoxic T cells persisted longer than CAR engineered polyclonal T cells in patients with neuroblastoma. The authors reasoned that EBV-specific engineered T

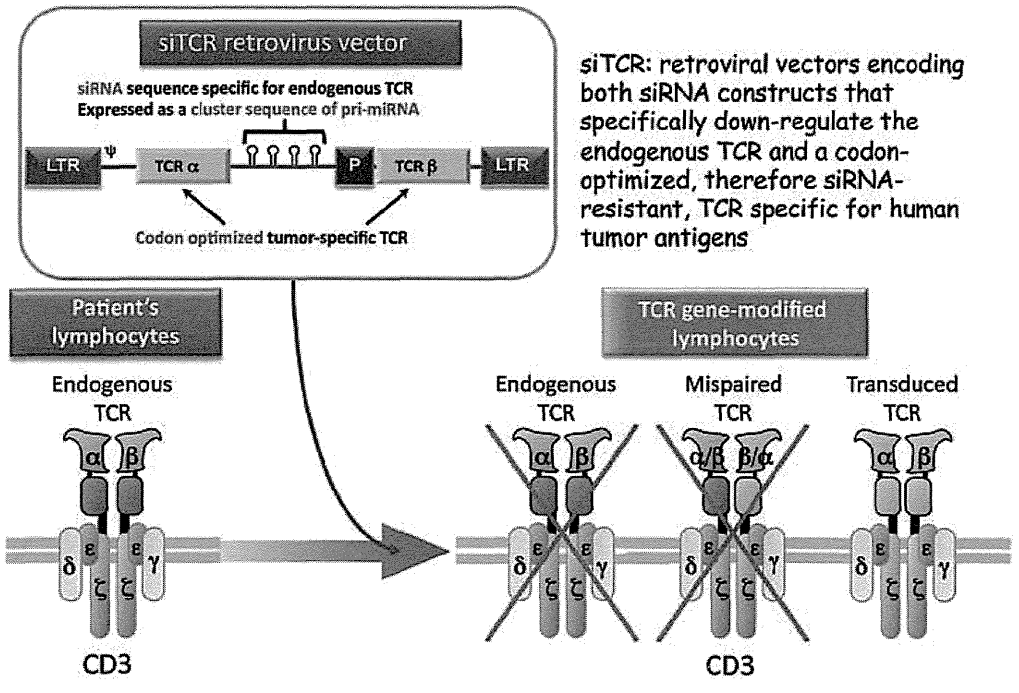

Figure 2. Retrovirus vectors to down-regulate endogenous TCR by siRNA. Novel retrovirus vectors that encode both siRNA to down-regulate endogenous TCR and codon-optimized TCR specific for tumor-associated antigens were created. These vectors achieved high expression of induced TCR with low proviral copy number in the transduced lymphocytes.

cells could receive optimal costimulation by physiologic condition using EBV-specific TCR, enhancing survival and anti-tumor activity.

On-target adverse events, however, have been reported for TCR gene therapies targeting melanocyte-differentiating antigens especially when high-avidity TCRs were used.[67] The patients in the trial showed severe histological destruction in normal tissues where melanocytic cells exist, such as skin, eyes, and inner ears. T cells engineered to express TCR specific to carcinoembryonic antigen also induced a severe transient inflammatory colitis.[77] Case reports exploring the severe adverse events seen in the patients receiving T cell with CAR targeting CD19[78] or HER2/neu[79] highlighted the potential risk in the usage of receptor genes reactive not only to tumor cells but also a subset of normal cells. These observations showed the potential power of T-cell therapy to overcome the immunological tolerance in cancer patients as well as the need of careful approach in clinical trials. Interestingly, lymphocytes engineered to express TCR specific to a cancer/testis antigen, NY-ESO-1, did not demonstrate adverse events despite the fact that this TCR was modified to be very high affinity,[68] suggesting the importance of the selection of target antigen. Incorporation of suicide gene might also be one of the promising ways to solve the risk of on-target toxicity.

In vitro experiments and mouse models have shown the strategy of genetic engineering of lymphocytes to enhance their functions as well as resistance to tumor-mediated immunosuppression through the addition of genes encoding homeostatic or pro-inflamatory cytokines,[80,81] chemokine receptors,[82] anti-apoptotic molecules,[83] and costimulatory molecules[84,85] as well as the silencing of coinhibitory molecules,[86] although these modifications await the validations of their concepts in clinic.

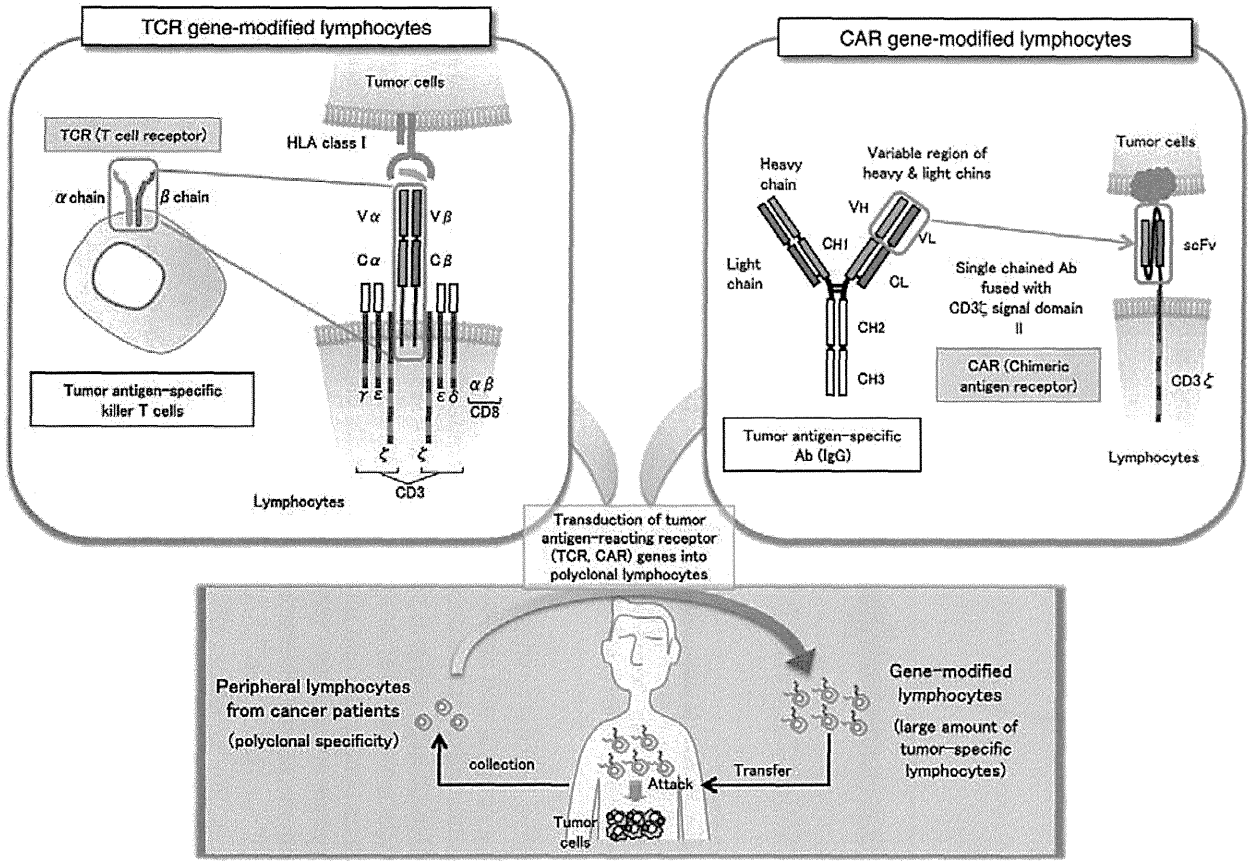

Figure 3. Adoptive cell therapy with antigen-receptor gene-modified lymphocytes. TCR genes derived from tumor-reactive cytotoxic T cells are transduced into patients' lymphocytes. Alternatively, single chained VH domain and VL domain derived from antibody reactive to tumor-associated antigen are fused with signal transduction domain of CD3ζ to create a chimeric antigen receptor (CAR) gene. CAR gene is transduced into patient's lymphocytes to generate tumor-reactive T cells (T-body). These lymphocytes genetically engineered to become tumor-reactive are adoptively transferred into patients with tumor. Modified from Figure 6-2; Naoko Imai et al. In: Masabumi Shibata, ed. Cancer Biology. Yodosha, 2011:260-9.

PASSIVE IMMUNOTHERAPY OF BRAIN TUMOR

Based on the above discussion on the understanding that CNS is an immune-specialized rather than immune-privileged site, we envisage that adoptive T-cell therapy with tumor antigen-specific T cells can be applied to the treatment of patients with brain tumors. Several clinical trials using adoptive T-cell therapy for patients with brain tumor are currently active according to the NIH clinical trial database (www.clinicaltrials.gov). These include the usage of CMV-specific T cells, T cells genetically engineered to express IL-13-Zetakine, and CAR targeting HER2. As mentioned previously, CAR targeting GD2 expressed in EBV-specific T cells showed promising outcome in early phase trial,[76] encouraging the extensive evaluation of this strategy into trials with later phases. Since the list of tumor-associated antigens for brain tumor consistently grow (Table 1), the evaluation of adoptive T-cell therapy against new targets will also prove useful for the development of effective and safe therapeutic protocols for patients with brain tumor.

CONCLUSION

Passing more than a half of a century after Frank Macfarlane Burnet proposed the concept of cancer immunosurveillance, we are facing a stage that immunotherapy is emerging as a realistic and useful modality in the treatment for patients with cancer. This is also unmistakably true in the challenge to fight with brain tumor. Among the immunotherapies that are currently in development, adoptive T-cell therapy with lymphocytes genetically engineered to express tumor antigen-specific receptor is certainly a promising strategy to treat patients with glioma. To further overcome the multiple layers of immuno-suppression/evasion mechanisms of tumor, the progress in basic science in immunobiology and oncology harmonized with extensive effort in clinical studies is indispensable. Combination of active and passive immunotherapy with manipulation of immunologic balance in cancer patients will open a new gate for effective immunotherapy of cancer.

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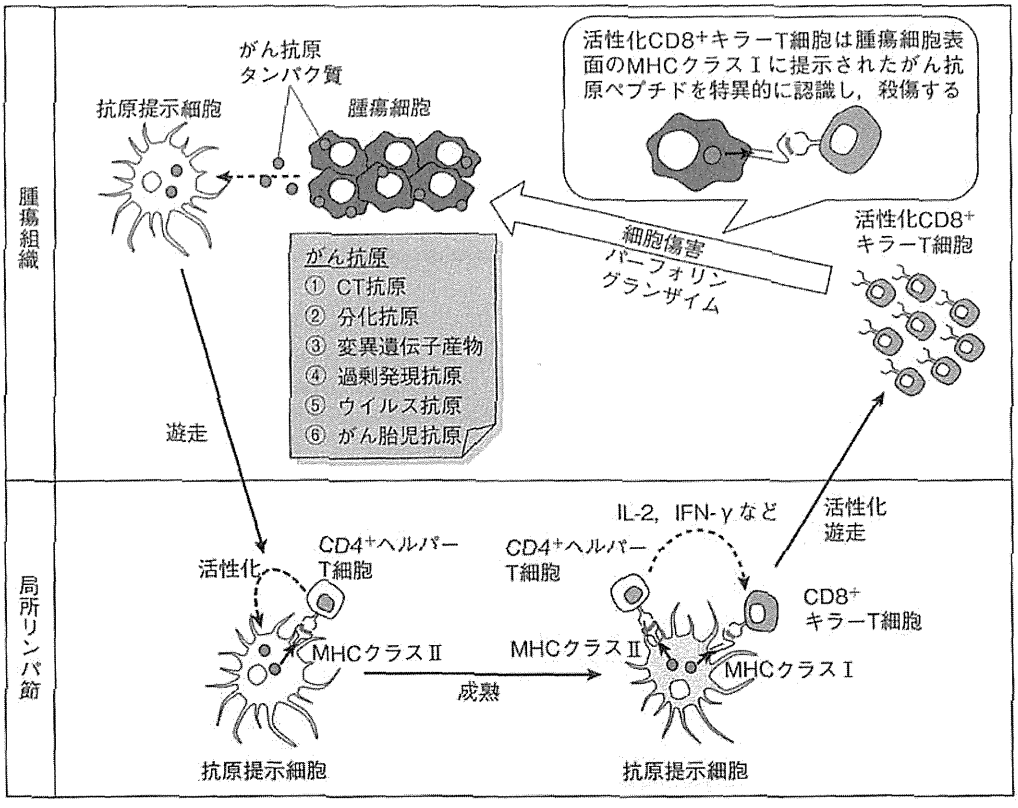
Chapter 6

1 がん抗原

免疫学の進歩により，腫瘍を直接的に殺傷する能力をもつCD8⁺キラーT細胞（細胞傷害性T細胞，cytotoxic T lymphocytes：CTL），その働きを調節するCD4⁺ヘルパーT細胞，両者に抗原を提示して活性化させる抗原提示細胞などのさまざまな免疫担当細胞の役割が解明されてきた．また，T細胞が認識する抗原は，腫瘍細胞表面上のMHC分子に結合した腫瘍細胞自身が産生するタンパク質に由来する8～十数個のアミノ酸からなるペプチドであることが明らかとなり，多くのがん抗原とそのエピトープが同定された．近年，これらの抗原を標的とした特異的免疫応答を高める治療法の開発が行われており，医療現場に登場し始めた．

概念図

T細胞によるがん抗原の認識と抗腫瘍免疫応答



がん抗原を標的とした特異的な細胞破壊機構の主役はCD8⁺キラーT細胞である．キラーT細胞が腫瘍細胞に細胞傷害を呈するまでのメカニズムを示す．腫瘍局所でがん抗原を取り込んだ抗原提示細胞は局所リンパ節へ遊走し，CD4⁺ヘルパーT細胞に抗原を提示し，これを活性化する．CD4⁺ヘルパーT細胞との接触は樹状細胞を成熟させ，CD4⁺ヘルパーT細胞から産生されるIL-2，IFN-γなどにより樹状細胞のさらなる成熟，CD8⁺キラーT細胞の活性化が誘導される．CD8⁺キラーT細胞は腫瘍局所へ遊走し，腫瘍細胞表面のMHCクラスⅠ分子に提示されたがん抗原を特異的に認識し，殺傷する．このように，T細胞による抗腫瘍免疫応答が効率よく誘導されるには，CD4⁺ヘルパーT細胞とCD8⁺キラーT細胞の両方が抗原提示細胞により提示された同一のがん抗原を認識し，感作される必要がある．さらに，抗原提示細胞による抗原の効率よい提示，T細胞の活性化，がん局所への遊走などには自然免疫系にかかわる細胞，サイトカイン，抑制性細胞の阻害など多くのファクターが関与している

Memo

《MHC（Major histocompatibility complex：主要組織結合遺伝子複合体）分子》
MHCは細胞表面に発現する膜結合型糖タンパク質で，細胞内で抗原が分解されてできた8～十数個のアミノ酸からなるペプチドを分子の先端にある溝に結合して細胞表面に発現する．$CD8^+$キラーT細胞はMHCクラスⅠ分子に提示されるペプチドを，$CD4^+$ヘルパーT細胞はMHCクラスⅡ分子に提示されペプチドを認識する．ヒトのMHCをHLA（Human leukocyte antigens：ヒト組織結合抗原）と呼ぶ．

Memo

《エピトープ：epitope（抗原決定基：antigenic determinant）》
抗体やT細胞受容体の抗原結合部位に接合する抗原の一部分．抗体は抗原タンパク質の立体構造を認識することが多く，一次構造上不連続な場所にあることが多い．これに対し，T細胞のエピトープは，MHC分子に結合した抗原タンパク質由来のペプチドである．$CD8^+$キラーT細胞（＝CTL）が認識するエピトープをCTLエピトープ，$CD4^+$ヘルパーT細胞が認識するエピトープをヘルパーエピトープと呼ぶ．がん抗原タンパク質は複数のCTLエピトープとヘルパーエピトープを含んでいると考えられる．

1 がん抗原の発見の歴史

免疫系は外来異物（非自己）を排除するが，自己細胞を攻撃しない（免疫寛容）ため，自己細胞に由来するがん細胞が免疫系に認識されうるのか，ということは長らく疑問であった．1953年，E. J. Foleyは化学発がん剤で誘発した腫瘍をマウスに移植し，ある程度発育してから摘除すると，このマウスは同一腫瘍の再移植を拒絶することを示し，がん抗原の存在を証明した．しかし，1976年に自然発生腫瘍は発がん剤誘発腫瘍と比較して免疫原性はほとんどない，またはあっても非常に低いことが報告されると，ヒトがんの免疫療法の開発は難しいのではないかと考えられるようになる．1980年代後半になり悪性黒色腫患者の末梢血や腫瘍浸潤リンパ球から自己腫瘍を特異的に認識する細胞傷害性T細胞が培養できるようになり，1991年，T. Boonらは悪性黒色腫の患者から樹立した腫瘍特異的T細胞が認識する抗原としてMAGE-1（後のMAGE-A1）を単離した[1]．以来，数多くの$CD8^+$キラーT細胞および$CD4^+$ヘルパーT細胞が認識することのできるがん抗原とそのエピトープの同定がされてきた[2) 3)]．2001年以降は，免疫不全マウスにおける高い発がん率が確認され，免疫によるがんのコントロールが再認識されている[4)]．

2 がん抗原同定法

がん抗原の同定方法には，腫瘍を認識するCTLを用いたcDNAクローニング法，腫瘍細胞からペプチドを抽出する直接抽出法，患者抗体が認識する抗原を解析するSEREX（serological identification of antigens by recombinant cloning）法，DNAマイクロアレイやRDAなどのDNAサブトラクション法を用いたがんと正常組織の比較による方法，がん抗原候補タンパク質からエピトープを予測して確認する reverse-immunology法などがある[5)]．主なものの特徴を表6－1に示す．

表6－1　がん抗原の同定方法

cDNAクローニング法	腫瘍細胞とこれを認識するCTLクローンを用いたcDNAの発現クローニング法である．まず，腫瘍細胞からcDNAライブラリーを作製し，標的がん抗原陰性の細胞株に遺伝子を導入する．これをCTLクローンの反応性によりスクリーニングして抗原をコードする遺伝子を同定する．CTL株と標的となる培養がん細胞株を樹立する必要があるため，適用は限られているが，悪性黒色腫からMAGE，gp100などが同定された
直接抽出法	腫瘍細胞表面からMHC分子に結合するペプチドを酸処理によって抽出する方法である．細胞を直接酸処理するかあるいはMHC分子をまず精製し，これからペプチドを回収しアミノ酸配列を決定する．この方法を行うためには，ペプチドの量にして数十pM程度，細胞数にして10^{11}個前後の大量の材料が必要である
SEREX法	1995年，Pfreundschuhらが開発した方法で，がん細胞から調整したcDNAファージライブラリーを大腸菌で発現させ，患者血清でスクリーニングしてIgG抗体が認識する抗原を同定する方法である．SEREX法にはCTL株，培養がん細胞株ともに必要なく，がん組織と患者血清があればよい．また，同定された抗原をコードする遺伝子がすでにcDNAの形でファージにクローニングされているため，その塩基配列を調べるだけで遺伝子の同定が可能である．がん抗原の大規模スクリーニングが可能となり，多くのがんに適用されてきた．なお，この方法で同定された抗原はヘルパーT細胞には認識されるが，必ずしもCTLに認識されるとは限らないため，同定されたタンパク質を用いて動物を免疫することによりT細胞を誘導し，そのT細胞が元の腫瘍細胞に反応することを確認する作業が必要である．NY-ESO-1，SSX，XAGEなどがこの方法で同定された
reverse immunology法	DNAマイクロアレイなどの網羅的解析で腫瘍細胞内で強発現している分子や変異がん遺伝子など，先にがん抗原タンパク質を仮定し，アンカーモチーフなどからMHC拘束性のエピトープペプチドを予測・合成して腫瘍特異的細胞傷害性T細胞株を誘導することで腫瘍抗原であることを証明する方法．HER-2/neu，CEAなどがこの方法で同定された

3 がん抗原の分類

免疫療法の標的として理想的ながん抗原の特徴として，がん幹細胞を含むすべてのがん細胞に安定して高発現するが正常細胞では発現しないもの，多くのがん種に高い割合で発現するもの，免疫原性が強いもの，がん細胞の増殖生存にかかわるために抗原消失が起こりにくいもの，などがあげられる．2009年，米国国立癌研究所（NCI）は橋渡し研究を加速させるためのパイロットプロジェクトとして，免疫療法の応用に適しているがん抗原の順位付けを発表した．臨床試験に用いられている抗原を中心に75個のがん抗原について，①治療への反応性，②免疫原性，③腫瘍原性，④発現の特異性（がん細胞のみに発現されるものが良好），⑤発現レベルと陽性率（ともに高い方がよい），⑥がん幹細胞における発現，⑦抗原陽性腫瘍の患者数，⑧ペプチドの長さ（長く，複数のエピトープを含むものがよい），⑨細胞内局在と発現（細胞表面に発現し，循環血液中の発現はないものがよい），の項目についてスコア化し，ランク付けしたものである．WT1，MUC1，LMP2，HPV E6 E7，EGFRvⅢ，HER-2/neu，idiotype，MAGE-A3，p53 nonmutant，NY-ESO-1などが上位にランクインしている[6]．がん抗原の分類法はいくつか報告されているが，その成因と特徴から以下のように分類できる[2]．

1）がん/精巣抗原

がん/精巣抗原（cancer-testis antigens：CT抗原）とは正常組織では精巣（および胎盤）のみにしか発現が認められないが，腫瘍組織では，悪性黒色腫，膀胱がん，肺がんなどさまざまな種類のがん細胞に発現するもの．精巣にはHLAクラスⅠの発現がなくCTLの標的とはならないので，実質的にはがん細胞のみに発現している抗原であり，免疫原性が高いものが多く，免疫療法の標的抗原として大きく期待されている[7]．1991年T. Boonらにより MAGEが発見されて以来，BAGE，NY-ESO-1など次々に抗原が同定され，2011年1月までに，100個以上のファミリーが

同定されており，データベースが公開されている[8]．CT抗原は，その約半数がX染色体にコードされ（CT-X抗原），それ以外の抗原（non-X CT抗原）はゲノムに広く分布している．いずれも精子形成過程にかかわる遺伝子であり，CT-X抗原の多くは精原細胞に発現している．non-X CT抗原は精母細胞や精細胞など，やや分化の進んだ時期に発現するものが多く，減数分裂に関与しているものが多い．CT-X抗原は反復配列をもつことが多く，複数の遺伝子でファミリーを形成することも多い．1つの腫瘍細胞に複数のCT-X抗原が発現していることもしばしば認められる．

2）分化抗原（組織系列特異的抗原）

分化抗原（differentiation antigens）とは腫瘍細胞だけでなく，正常細胞にも発現が認められるが，特定の細胞・組織の分化に伴い発現するもの．悪性黒色腫で同定されたgp100，Melan A，チロシナーゼなどは，いずれも正常色素細胞（メラノサイト）に存在するメラノソーム内の酵素であるが，腫瘍で発現が増強している．悪性黒色腫患者ではこの抗原に対するCTLにより，皮膚の白斑が誘導されることがある．共通抗原として治療に利用しやすいが，がん細胞にとって必須ではないので抗原消失が起こりやすく，発現を共有する正常細胞に対する自己免疫反応を起こす可能性がある．

3）変異遺伝子産物

変異遺伝子産物（mutation）はがん細胞の増殖生存にかかわる遺伝子異常に由来する変異ペプチド抗原であり免疫原性が高く，腫瘍特異的で抗原消失を起こしにくい．β-カテニン，CDK4，カスパーゼ8などで突然変異が報告されているが，多くは患者ごとに固有の抗原であるため，現状では治療への応用は難しい．一方，慢性骨髄性白血病にみられるBCR-ABL，がん遺伝子ras，がん抑制遺伝子p53のように共通部位の変異を認めるものも抗原として認識されることが報告されており，治療への応用が試みられている．

4）過剰発現抗原

過剰発現抗原（overepressed antigens）とは正常組織にも発現しているが，がん細胞に過剰に発現するもの．がん細胞の増殖，生存にかかわるものが多い．乳がんや胃がんで高発現するHer-2/neuなどがあげられる．

5）ウイルス抗原

ウイルスの中には腫瘍の発生に関与するものがある．ウイルス遺伝子産物が細胞内でプロセッシングを受け，腫瘍抗原ペプチドとなっていることが多い〔ウイルス抗原（viral antigens）〕．したがって，ウイルス誘発腫瘍のがん抗原は共通抗原となることが一般的で，この点が物理・化学的に誘発された腫瘍細胞にみられる固有抗原とは異なる．

ヒトパピローマウイルス（human papillomavirus：HPV）は子宮頸がんの発症に関連しており，子宮頸がん細胞では，HPVウイルスタンパク質（E6，E7）が発現し，CTLの標的抗原となることがわかっている．また，エプスタイン・バーウイルス（Epstein Barr virus：EBV）はB細胞リンパ腫，鼻咽頭がんなどの発症に関与し，EBV-EBNA-2，-3，-4，-6はEBV-LMP2などのタンパク質が抗原となることが示されている．$CD4^+$ヘルパーT細胞に感染し，成人T細胞白血病の原因となるヒトT細胞白血病ウイルス（HTLV-1）の遺伝子産物であるTaxもCTLの標的抗原ペプチドとなる．

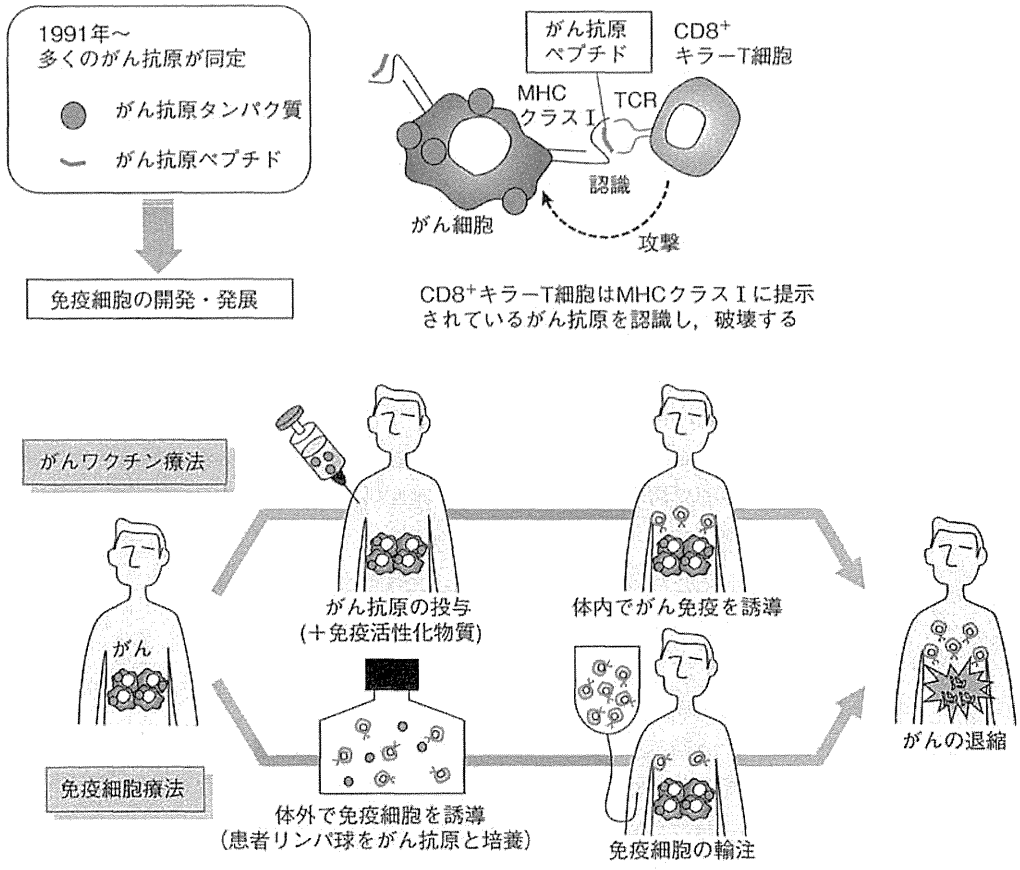


図6－1　がん抗原を標的とした特異的がん免疫療法

がん抗原が同定されて以来，抗原に対する特異的な免疫応答を高める治療法の開発が行われてきた．特異的免疫療法にはがん抗原をさまざまな形で直接患者に投与することにより患者体内で特異的免疫応答の誘導を図る「がんワクチン療法」と患者由来のリンパ球（腫瘍浸潤リンパ球や末梢血）を体外で放射線照射した患者自身のがん細胞と共培養したり，がん抗原で刺激するなどして，がん抗原特異的な免疫細胞を誘導し，患者に輸注するという「免疫細胞療法」がある

4 がん抗原を標的とした特異的免疫療法

がん抗原が同定されて以来，がん特異的なT細胞の活性化によりがんを治療する特異的免疫療法が試みられ，特に，細胞破壊機構の主役であるCD8$^+$キラーT細胞を活性化させる治療法の開発が行われてきた．特異的免疫療法は図6－1に示すように「がんワクチン療法」と「免疫細胞療法」の2つに大きく分けられる．

1）がんワクチン療法

がん抗原をさまざまな形で直接患者に投与することにより，患者体内で特異的免疫応答の誘導を図る方法である．がんワクチンとして使用できる分子には，がん抗原タンパク質そのもの，エピトープペプチド（CTLエピトープ，ヘルパーエピトープ，または両者を含むロングペプチドなど），それらをコードするmRNA，cDNAなどがある．腫瘍細胞そのものや，がん抗原を提示させた樹状細胞などの抗原提示細胞を投与することも試みられている．これまでに薬剤として承認されている治療用がんワクチンとして，脳腫瘍に対するDCVax-Brain（腫瘍細胞由来抽出物で感作

させた自己樹状細胞，米Northwest Biotherapeutics社，2007年7月にスイスで承認），転移性腎がんに対するOncophage（患者腫瘍細胞より抽出した熱ショックタンパク質-ペプチド複合体，米Antigenics社，2008年4月にロシアで承認），前立腺がんに対するProvenge〔PAP（prostatic acid phosphatase）抗原とGM-CSF融合タンパク質で感作した自己樹状細胞，Dendreon社，2010年4月に米国で承認〕がある．また，予防用がんワクチンとして，子宮頸がんに対するGardasil（HPV6，11，16，18型のL1タンパク質の非感染性ウイルス様粒子＋アジュバント（adjuvant），米Merck社，2006年6月に米で承認），Cervarix（HPV16型および18型のL1タンパク質の非感染性ウイルス様粒子＋アジュバント，英GlaxoSmith Kline社，2007年5月に豪で承認，日本でも2009年10月に承認）がすでに広く用いられている．現在，これ以外にも，EGFRvIII，MAGE-A3，NY-ESO-1などを標的とした多くの臨床試験が国内外で行われ，第II相，第III相試験にあるものも少なくない[9]．

Memo

《アジュバント》
ワクチンの反応を非特異的に増強させる分子群．各種免疫担当細胞を刺激するサイトカインやToll-like受容体の刺激分子，抗原分子の投与部位における停留時間を長くする作用をもつものなど，さまざまなものが利用されている．

2）免疫細胞療法

体外でがん抗原特異的な免疫細胞を誘導し，患者に輸注する方法である[10]．現在薬剤として承認に至っているものはなく，すべて試験的段階である．NCIのS. A. Rosenbergらのグループは悪性黒色腫の患者の腫瘍浸潤リンパ球から腫瘍特異的リンパ球を調整し，患者に輸注するという治療を行っている．最新の報告では，化学療法および全身放射線療法を用いた骨髄破壊性の強い前処置を行うことにより，RECIST基準での奏効率49～72％という良好な成績を示している[11]．材料として腫瘍浸潤リンパ球を用いるこの方法はがん抗原が分子レベルまで同定されていなくても行える．しかしながら，そもそも腫瘍から腫瘍特異的リンパ球を増殖させる技術は（特に悪性黒色腫以外では）非常に難しく，治療が適用できる患者は限定される．そこで，最近では遺伝子導入の技術を用いて，人工的に大量の腫瘍特異的リンパ球を作製する方法（遺伝子改変T細胞輸注療法）が試みられている．図6－2に示すように，がん抗原特異的なキラーT細胞クローンや抗体から抗原認識部位の遺伝子を取り出し，ウイルスベクターなどを用いて，患者リンパ球に体外で遺伝子導入するものである[12) 13)]．

i）T細胞受容体遺伝子導入T細胞療法

T細胞受容体（T cell recoptor：TCR）遺伝子導入T細胞療法とはがん抗原特異的キラーT細胞クローンから得られたTCR遺伝子を患者末梢血より得られた$CD8^+$T細胞に導入して輸注細胞を調整した遺伝子改変細胞を輸注する治療法である．S. A. Rosenbergらは，MART-1を標的とした悪性黒色腫に対する試験[14) 15)]，CEAを標的とした大腸がんに対する試験[16)]，NY-ESO-1を標的とした悪性黒色腫・滑膜細胞腫に対する試験[17)]などを行い，12～67％の奏効率を報告している．ただし，抗原陽性細胞を直接殺傷することのできるT細胞を輸注するこの治療法では正常細胞に対する副作用の報告もある（分化抗原であるMART-1を発現している皮膚，眼，内耳や，がん胎児抗原であるCEAを発現している大腸粘膜の傷害）．このことは，T細胞輸注療法を適切な条件で行うとトレランスや免疫抑制の問題を打開しうるという希望と，それゆえの副作用への配慮の必用性を示している．わが国では，三重大学とタカラバイオ株式会社が共同研究として，