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We have been challenging to cure intractable hematological disorders such as leukemia and lymphoma mainly with the aid of hematopoietic stem cell transplantation (HSCT). No less than 30 patients per year receive allogeneic HSCT in our facilities. In recent years, unrelated cord blood has been our major stem cell source for recipients who have no suitable family donors in HSCT. Since 1998 we have performed over 100 cases of unrelated cord blood transplantation (uCBT) for adult patients, which appears a distinguished experience in the world. Recent advances in identification of signaling molecules activated in a tumor-specific manner or associated with tumor-specific genomic recombination have disclosed many candidate therapeutic targets in tumors. In the field of hematological malignancies, we have already experienced remarkable clinical efficacies of imatinib mesylate, an ABL kinase inhibitor, for CML as well as rituximab, a chimeric anti-CD20 monoclonal antibody for B cell lymphoma. We extensively apply these molecular targeted therapies for in- and out-patients and are to be involved in clinical trial of newly developed agents.

1. Pancreatic Hyperamylasemia and Hyperlipasemia in Association with Cytomegalovirus Infection following Unrelated Cord Blood Transplantation for Acute Myelogenous Leukemia.

Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Tojo A

Cytomegalovirus (CMV)-associated pancreatitis is rare after allogeneic hematopoietic stem cell transplantation (SCT). We describe a patient who developed pancreatic hyperamylasemia and hyperlipasemia in association with CMV infection after cord blood transplantation (CBT). A 31-year-old man with acute myelogenous leukemia underwent CBT. A neutrophil count consistently greater than 500/muL was achieved on day 21. Positive results for CMV antigenemia on days 35 and 67 prompted 2 courses of preemp-

tive therapy with ganciclovir or foscarnet. The CMV antigenemia value again became positive on day 134. On day 141, serum amylase and lipase activities markedly increased to 1221 IU/L and 894 IU/L, respectively. The patient had no abdominal symptoms. Ultrasonography and computed tomography results showed no abnormalities of the pancreas. A diagnosis of possible pancreatitis was made. After the initiation of foscarnet therapy, the CMV antigenemia results soon became negative, and serum amylase and lipase activities returned to normal. Therefore, CMV infection was considered to play a major role in the development of pancreatic hyperamylasemia and hyperlipasemia in our patient. The present report indicates that CMV infection should be included in the differential diagnosis for patients with pancreatic hyperamylasemia after SCT.

2. Posttransplantation engraftment and safety of cord blood transplantation with grafts containing relatively low cell doses in adults.

Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Tojo A

The cell dose of a graft is a critical determinant of hematopoietic recovery and survival following unrelated cord blood transplantation. Most studies have found that the minimum acceptable nucleated cell dose should be between 1.5×10^7 and 2.0×10^7 nucleated cells per kilogram of body weight to reduce the time to myeloid recovery and increase the probability of engraftment. For some patients who have indications for hematopoietic cell transplants and for whom no other graft source except cord blood is available, it is difficult to decide whether they can receive cord blood grafts containing lower cell doses. In our study, patients who received cord blood grafts containing 1.0×10^7 to 2.0×10^7 cells/kg (n=7) exhibited slower neutrophil and platelet recoveries compared with patients who received grafts containing total nucleated cell doses of 2.0×10^7 cells/kg and above (n=93); however, 4 of those low-cell-dose recipients survived with a longer follow-up. Based on these preliminary results, cord blood grafts containing less than 2.0×10^7 cells/kg may be useful for cases where no grafts with higher cell doses or other stem cell sources are available.

3. Hemorrhagic cystitis in adults after unrelated cord blood transplantation in Japan - single institution experiences-

Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Ohno N, Uchimaru K, Tojo A

Hemorrhagic cystitis (HC) is the main complication after hematopoietic stem cell transplantation (SCT). Adenovirus (AdV) is the leading cause of late-onset HC after SCT in Japan. The incidence and outcome of HC were studied in 77 adults who underwent unrelated cord blood transplantation (CBT). Thirty-two patients developed HC in a median of 19 days (range, 11-170 days) after CBT. The cumulative incidence of HC was 41.8% at 1 year. Ten patients developed gross hematuria. The cumulative incidence of moderate-to-severe HC was 13.2% at 1 year. Only 1 patient developed severe HC; AdV was detected in a urine sample from that patient. AdV was also detected in a urine sample from another patient with moderate HC after CBT. AdV in both patients was identified as AdV type 11. The cumulative incidence of AdV-induced HC was 2.8% at 1 year. The incidence of AdV-induced severe HC after CBT may be relatively low among Japanese adults. The role of other viruses, including BK virus, in the pathogenesis of HC after CBT needs to be examined.

4. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem cell transplantation from related donors in adult patients with hematological malignancies after myeloablative conditioning regimen.

Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Tojo A

We studied the clinical outcomes of 171 adults with hematological malignancies who received unrelated cord blood transplantation (CBT) as a primary unrelated stem cell source (n=100), or bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT) from related donors (n=71, 55 BMT and 16 PBSCT). All patients received myeloablative regimens including 12 Gy of total body irradiation. We analyzed the hematological recovery, risks of graft-versus-host disease (GVHD), transplant-related mortality (TRM) and relapse, and disease-free survival (DFS) using Cox proportional hazards models. Significant delays in engraftment occurred after CBT; however, overall engraftment rates were almost the same for both grafts. The cumulative incidences of grades III to IV acute and extensive type chronic GVHDs among CBT recipients were significantly lower than those among BMT/PBSCT recipients. Multivariate analysis demonstrated no

apparent differences in TRM (9% in CBT and 13 % in BMT/PBSCT), relapse (17% in CBT and 26 % in BMT/PBSCT) and DFS (70% in CBT and 60% in BMT/PBSCT) between both groups. These data suggest that unrelated cord blood could be as safe and effective a stem cell source as related bone marrow or mobilized peripheral blood for adult patients when it is used as a primary unrelated stem cell source.

5. Varicella-zoster virus encephalitis in a patient undergoing unrelated cord blood transplantation for myelodysplastic syndrome-overt leukemia.

Fukuno K, Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Tojo A

Varicella-zoster virus (VZV) infection of the central nervous system (CNS) is rare after hematopoietic stem cell transplantation (SCT). Here, we describe the first patient who developed VZV encephalitis after cord blood transplantation (CBT). A 35-year-old man with myelodysplastic syndrome-overt leukemia underwent CBT. On day +23, a neutrophil count consistently greater than 0.5×10^9/l was achieved. On day +42, 1 mg/kg/day of prednisolone therapy was initiated for grade III acute graft-versus-host disease (GVHD). Then, the dose of prednisolone was slowly reduced. For exacerbation of chronic GVHD, the dose of prednisolone was again increased to 1 mg/kg/day on day +231. On day +265, localized cutaneous zoster in the left thoracic region occurred, but soon resolved after acyclovir therapy. On day +309, he suddenly developed diplopia. Subsequently, right facial palsy and hearing impairment occurred. No skin rash was observed. Magnetic resonance imaging (MRI) scans revealed multifocal abnormal high-signal intensity in the CNS. A high level of VZV DNA was detected in a cerebrospinal fluid specimen. He was diagnosed with VZV encephalitis. Acyclovir was given intravenously for 40 days. Four months after the onset, the neurologic symptoms had incompletely resolved. MRI scans showed substantial resolution but with mild residual lesions. The present report indicates that VZV should be considered as a possible causative agent in patients who develop multifocal neurologic symptoms of the CNS after SCT.

6. Early-onset thyrotoxicosis after unrelated cord blood transplantation for acute myelogenous leukemia.

Konuma T, Tomonari A, Takahashi S, Ooi J, Tsukada N, Tojo A

Thyroid dysfunction is a common complication after allogeneic hematopoietic stem cell transplantation (SCT). However, thyrotoxicosis as defined by elevated serum free thyroxine (FT 4) or free triiodothyronine (FT3) levels together with low thyroid-stimulating hormone (TSH) levels is rare after SCT. Here we described two patients who developed thyrotoxicosis within the first 50 days after unrelated cord blood transplantation (CBT). Patient 1 is a 32-year-old woman with acute myelogenous leukemia (AML)-M5a who underwent CBT. On day 41, she developed tachycardia. On day 48, FT4 increased to 2.2 ng/dl and TSH was suppressed to less than 0.1 IU/ml. Anti-thyroid peroxidase antibody was positive. On day 83, FT4 spontaneously decreased to 1.4 ng/dl. Patient 2 is a 42-year-old man with AML-M4 who underwent CBT. On day 42, he developed tachycardia. On day 48, FT3 increased to 4.75 pg/ml and TSH was suppressed to 0.02 IU/ml. Anti-thyroid peroxidase antibody was positive. Eight months after CBT, his thyroid function spontaneously returned to normal. The presence of anti-thyroid peroxidase antibody suggested that immune-mediated reactions might be associated with the development of thyrotoxicosis after CBT in our patients. The present study shows that thyrotoxicosis can occur during very early periods after CBT.

7. Cord blood transplantation for acute myelogenous leukemia using a conditioning regimen consisting of granulocyte colony-stimulating factor-combined high-dose cytarabine, fludarabine, and total body irradiation.

Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Tojo A

The cytotoxic effect of cytarabine (Ara-C) on myeloid leukemic cells is enhanced by concomitant use of granulocyte colony-stimulating factor (G-CSF) *in vitro*. The feasibility of a conditioning regimen consisting of G-CSF-combined 24 g/m^2 Ara-C, 90 mg/m^2 fludarabine, and 12 Gy total body irradiation was studied for five patients with acute myelogenous leukemia in cord blood transplantation (CBT). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate. After the conditioning regimen, 2.48×10^7/kg (2.28 to 3.53) of cord blood nucleated cells was infused. Neutrophil counts consistently greater than 0.5×10^9/l was achieved 24 days (22 to 32) after CBT. Grade I stomatitis and gastrointestinal toxicities occurred in all patients. Grades I and II acute GVHD occurred in one and four patients, respectively, which re-

solved without steroid therapy. Sepsis and aspergillosis occurred in two and one patients, respectively. All patients were alive without leukemia relapse at a follow-up of 15 months (12 to 43) after CBT. This conditioning regimen could avoid the toxicities of high-dose cyclophosphamide but might enhance the cytotoxic effect of Ara-C. Large-scale studies will be needed to determine the efficacy and safety of the conditioning regimen in CBT.

Publications

1. Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M, Fukuno K, Uchiyama M, Takasugi K, Iseki T, Tojo A, Yamaguchi T, Asano S. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem cell transplantation from related donors in adult patients with hematological malignancies after myeloablative conditioning regimen. Blood. 2006 Oct 12; [Epub ahead of print]
2. Tomonari A, Takahashi S, Takasugi K, Ooi J, Tsukada N, Konuma T, Iseki T, Tojo A, Asano S. Pancreatic Hyperamylasemia and Hyperlipasemia in Association with Cytomegalovirus Infection following Unrelated Cord Blood Transplantation for Acute Myelogenous Leukemia. Int J Hematol. 2006; 84 (5): 438-40.
3. Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Tojo A, Yamaguchi T, Asano S. Posttransplantation engraftment and safety of cord blood transplantation with grafts containing relatively low cell doses in adults. Int J Hematol. 2006; 84 (4): 359-62.
4. Tomonari A, Takahashi S, Ooi J, Fukuno K, Takasugi K, Tsukada N, Konuma T, Ohno N, Uchimaru K, Iseki T, Tojo A, Asano S. Hemorrhagic cystitis in adults after unrelated cord blood transplantation: a single-institution experience in Japan. Int J Hematol. 2006; 84 (3): 268-71.
5. Fukuno K, Tomonari A, Tsukada N, Takahashi S, Ooi J, Konuma T, Uchiyama M, Fujii T, Endo T, Iwamoto A, Oyaizu N, Nakata K, Moriwaki H, Tojo A, Asano S. Successful cord blood transplantation for myelodysplastic syndrome resulting in resolution of pulmonary alveolar proteinosis. Bone Marrow Transplant. 2006; 38 (8): 581-2.
6. Fukuno K, Tomonari A, Takahashi S, Ooi J, Takasugi K, Tsukada N, Konuma T, Iseki T, Moriwaki H, Tojo A, Asano S. Varicella-zoster virus encephalitis in a patient undergoing unrelated cord blood transplantation for myelodysplastic syndrome-overt leukemia. Int J Hematol. 2006; 84 (1): 79-82.
7. Konuma T, Tomonari A, Takahashi S, Ooi J, Tsukada N, Yamada T, Sato H, Nagayama H, Iseki T, Tojo A, Asano S. Early-onset thyrotoxicosis after unrelated cord blood transplantation for acute myelogenous leukemia. Int J Hematol. 2006; 83 (4): 348-50.
8. Tomonari A, Takahashi S, Ooi J, Nakaoka T, Takasugi K, Uchiyama M, Tsukada N, Konuma T, Iseki T, Tojo A, Asano S. Cord blood transplantation for acute myelogenous leukemia using a conditioning regimen consisting of granulocyte colony-stimulating factor-combined high-dose cytarabine, fludarabine, and total body irradiation. Eur J Haematol. 2006; 77 (1): 46-50.
9. Ooi J. The efficacy of unrelated cord blood transplantation for adult myelodysplastic syndrome. Leuk Lymphoma. 2006; 47 (4): 599-602.

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Founded in 1981, Department of Infectious Diseases and Applied Immunology (DIDAI) started HIV clinic in 1986. In 2006, 41 new patients with HIV infection have visited or admitted our hospital and, in total, 313 patients are currently under our clinical management. The total number of in-patients during 2006 was 46, and 5-7 beds for HIV-infected patients in infectious disease ward have been occupied. Since the number of the staff members of DIDAI is too small to care both out-patients and in-patients, members of the Division of Infectious Diseases (DID) join the clinic. IMSUT hospital provides the most up-to-date medical treatment to HIV-infected patients in Japan. DIDAI is also a treatment center for international infectious diseases such as malaria and typhoid fever.

1. Treatment of and clinical research on HIV-infection and related diseases

Tetsuya Nakamura, Takashi Odawara, Takeshi Fujii[1], Tokiomi Endoh, Jun Takeda[1], Takeshi Matsumura[1], Mieko Goto[1], Tomohiko Koibuchi[1], Mutsuo Iga[2], Takuya Maeda[2], and Aikichi Iwamoto[1,2]: [1]Department of Infectious Diseases, [2]International Research Center for Infectious Diseases

a. Treatment of HIV infection in IMSUT hospital: Statistical characteristics of HIV-infected patients in IMSUT hospital this year

As of the end of 2006, 41 new patients with HIV-1 infection visited our hospital this year (from January 1 to December 20), and 313 patients in total are under medical management in our outpatient clinic. As shown in the figure 1, the number of total patients declined in 1997 because a part of patients as well as medical stuffs moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again after 1998 in accordance with Japa-

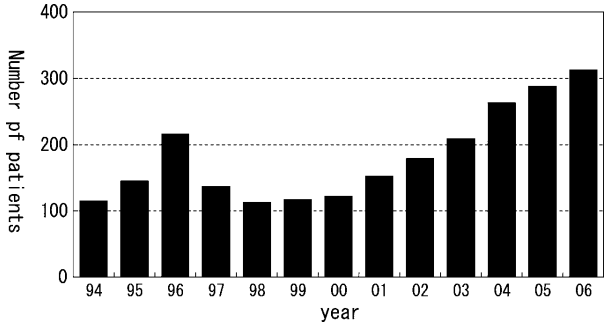

Figure. 1. Number of HIV-infected patients in IMSUT Hospital

nese statistics of HIV-infected patients. In contrast, the number of admission has decreased since 1997 because of the introduction of highly active anti-retroviral therapy (HAART) which effectively suppresses the replication of HIV. After one year of HAART, the viral loads become undetectable in more than 90% of patients, and their CD4 counts increase by approximately 200 /microL in average. Consequently, the clinical management of HIV-infected patients changed from how to treat opportunistic infections into how to control patients with HAART.

b. AIDS-related cerebral toxoplasmosis

Most toxoplasmic encephalitis is opportunistic infection complicated with the acquired immunodeficiency syndrome (AIDS) and immunosuppressive conditions. The diagnosis of this disease is difficult because of the incompetence of the serological examination for the immunocompromised patients. Although the direct detection method for the pathogen by polymerase chain reaction (PCR) using the cerebrospinal fluid (CSF) has high specificity, the sensitivity of this method is insufficient for the definitive diagnosis. We, therefore, have to synthetically diagnose with clinical symptoms, signs, laboratory data neuroradiological images and the response to anti-toxoplasmosis therapy.

This year, we reported our experience of an unique MR imaging finding of toxoplasmic encephalitis in an AIDS patient and emphasized the hyperintense foci on T1-weighted MR images can be one of the pathognomonic MR images of this disease (2).

‹Case Report› A 44-year-old man with disturbance of consciousness and respiratory insufficiency admitted to our hospital in April 2005. His consciousness had been rapidly deteriorated and he developed coma 2 to 3 days before hospitalization. Serological tests of HIV antibodies and *Toxoplasma gondii* IgG antibody were positive, but the *T. gondii* IgM antibody was not detected. The concentration of HIV RNA in plasma was 120,000 copies/ml and the CD 4 cell count was 8/mm^3. The chest X-ray showed bilateral ground glass shadow and *Pneumocystis jiroveci* (*carinii*) was detected from bronchoalveolar lavage (BAL) fluid. CSF showed mild elevated protein level of 65 mg/dl and pleocytosis, and the opening pressure was over 300mmH_2O. No malignant cells or microorganisms were detected. *T. gondii* B1-gene fragment was detected by PCR using CSF, therefore, the diagnosis of an AIDS case with toxoplasmic encephalitis was made. MRI of the brain showed multiple high intensity lesions on T2-weighted image and the corresponding T1-wighted image showed low intensity lesions. Contrast enhanced T1-weighted images showed multiple nodular and ring enhancement lesions. The chemotherapy with Trimethoprim/Sulfamethoxazole (TMP/SMX) was very effective and the patient's consciousness level was improved gradually. *P. jiroveci* pneumonia was also cured. MR imaging after 4 weeks of treatment demonstrated that the multiple nodular lesions on T1 and T2-weighted images had significantly been reduced. After 8 weeks of treatment, the contrast enhanced T1-weighted images showed only residual small lesions without contrast enhancement. Interestingly, the hypersignal intensity foci appeared at bilateral basal ganglia obviously after 2 weeks treatment on the non-enhanced T1-weighted images. Corresponding computed tomography (CT) image didn't show hemorrhagic or calcified densities. These T1 hypersignal intensity foci regressed gradually along with anti-toxoplasmic chemotherapy in proportion to other mass lesions. The T2*(star)-weighted image, which can detect the hemosiderin deposition as hypointensity lesion, operated after 12 weeks of treatment showed no hypointensity at corresponding T1 hypersignal intensity foci on basal ganglia. We concluded that the toxoplasmic encephalitis showed the hypersignal intensity foci on T1-weighted MR imaging without hemorrhage or calcification.

Toxoplasmic encephalitis of this case was diagnosed with the highly specific PCR and confirmed by the response to anti-toxoplasmosis therapy. Brain-MRI revealed unusual findings, T 1 hypersignal intensity foci, accompanied typical multiple high intense lesions on T2-weighted image during the treatment. These unique MR-findings have been reported on only a few cases of non HIV/AIDS-related toxoplasmic encephalitis. Terada and colleagues reported a case of toxoplasmic encephalitis after stem cell transplantation with T1 hypersignal intensity foci. Autopsy revealed the disseminated toxoplasmosis, and coagulative necrosis without hemorrhage or calcification was revealed at corresponding T1 hypersignal intensity foci by neuropathological study. In another post bone marrow transplantation case, inflammatory and vascular changes without hemorrhage appeared to be the cause of iso or hypersignal intensity rings by the stereotactic biopsy of T1 hypersignal intensity foci. On the other hand, Navia and colleagues demonstrated that the T1 hypersignal intensity foci were cased by coagulative necrosis with lipid-laden macrophages. The pathophysiological and neuroradiological mechanisms to create these MRI findings are far from clear yet. The reason why the T1 hypersignal intensity foci tend to localize in the basal ganglia is not

clear either.

CNS lymphoma, which is important for distinction from toxoplasmic encephalitis, shows T1 hypo-isosignal intensity foci and never shows T 1 hypersignal intensity foci except subacute hemorrhage with hypervascular CNS lymphoma. However, the CT imaging and T2* (star)-weighted MR imaging can simply distinguish it from the toxoplasmic T1 hypersignal intensity foci without hemorrhage or calcification. We reported here the unique MRI finings, T1 hypersignal intensity foci, without hemorrhage or calcification on HIV/AIDS-related toxoplasmic encephalitis. It will be helpful for the diagnosis of toxoplasmic encephalitis and may be a pathognomonic finding.

2. Treatments and Clinical Research of Tropical Diseases

Tetsuya Nakamura, Takashi Odawara, Takeshi Fujii[1], Tokiomi Endoh, Jun Takeda[1], Takeshi Matsumura[1], Mieko Goto[1], Tomohiko Koibuchi[1], Mutsuo Iga[2], Takuya Maeda[2], and Aikichi Iwamoto[1,2]: [1]Department of Infectious Diseases, [2]International Research Center for Infectious Diseases

a. Treatment of Tropical Diseases in IMSUT hospital: Statistical characteristics of HIV-infected patients in IMSUT hospital this year

This year, 89 travellers visited our clinic for consultation or treatment of tropical diseases. Forty-two of 89 visited clinic before travel for prescription of malaria prophylaxis (24 trevallers), vaccination (14 trevallers) and other general consultation (4 travellers). Forty-seven travellers visited clinic after travel because of sickness, and we diagnosed and treated 6 cases of traveller's diarrhea, 9 post-exposure prophylaxis of rabies, 4 malaria, 1 amoebiac enteritis, and 1 dengue fever.

b. Efficacy and safety of atovaquone-proguanil compared with mefloquine in the treatment of nonimmune patients with uncomplicatde *P. falciparum* malaria in Japan.

Atovaquone-proguanil is a fixed-dose combination drug of atovaquone and proguanil hydrochloride developed against drug-resistant *P. falciparum* malaria, and has been commercially available around the world since the late 1990s. Previous studies conducted on semi-immune individuals with uncomplicated *P. falciparum* malaria in endemic areas such as Thailand, Philippines, Brazil, and sub-Saharan Africa showed its excellent efficacy and safety. Recent studies carried out on non-immune travelers also showed promising results.

The Research Group on Chemotherapy of Tropical Diseases, Japan, introduced mefloquine before it was nationally licensed, and treated a number of patients. Later, in 1999, the research group also introduced atovaquone-proguanil, and so far has used it to treat at least 20 non-immune adults and 3 non-immune children with uncomplicated *P. falciparum* malaria. Here, we evaluated the efficacy and safety of atovaquone-proguanil as compared with mefloquine using data obtained from the research group. Although this study was not a formal clinical trial and thus the results may have limitations for interpretation, it could contribute in clarifying the role of atovaquone-proguanil for treatment of imported uncomplicated *P. falciparum* malaria in Japan.

All (100%) of the 20 atovaquone-proguanil-treated and 49 (98%) of the 50 mefloquine-treated adults were cured. The mean FCT and PCT in the atovaquone-proguanil group (3.7 and 3.3 days) appeared longer than those of the mefloquine group (2.9 and 2.8 days); however, the differences were not statistically significant. One patient, who fell ill after visiting Ghana, was not cured by treatment with 15 mg/kg mefloquine divided into two doses. Although the parasite count declined on day 2 of treatment, it increased significantly on day 3, with some visible *P. falciparum* ring forms showing intact morphology. This prompted the physician to judge the case a treatment failure and switch to sulfadoxine-pyrimethamine, which ultimately led to a complete cure. All the 3 children were also cured in due course with a mean FCT of 1.8 days and PCT of 3.7 days.

AEs were compared between 20 and 50 adults treated with atovaquone-proguanil and mefloquine, respectively. AEs were reported in 3 (15 %) of the 20 patients in the atovaquone-proguanil group; all of these were transient elevations of liver enzymes. Two patients showed an increase in AST and ALT levels (¯100 IU/L), but not in the total bilirubin level. These abnormal values subsequently subsided within 2-4 weeks. The third patient showed a slightly greater increase in AST (¯160) and ALT (¯190) with the total bilirubin level of 2.0 mg/dL (direct, 1.4). However, these values became normalized within 2-3 weeks without any specific treatment. In the mefloquine group, AEs were reported in 19 (38%) of the 50 patients, with some having more than one AE. The AEs included dizziness in 8 (16%), nausea/vomiting in 7 (14%), diarrhea, vivid dreams, itching/urti-

caria, or elevated liver enzymes in 2 (4%) patients for each. No AEs were reported in the 3 children.

Despite the limitations of this study, atovaquone-proguanil seemed at least equal to or even better than mefloquine for treatment of uncomplicated *P. falciparum* malaria in non-immune patients including children. Its marketing in Japan could be beneficial in offering an alternative therapeutic option. However, vigilance should be maintained on the possible occurrence of rare but severe AEs, and also of the possible spread of drug resistance, as its use will become widespread.

Publications

1. Shinoe, T., Wanaka, A., Nikaido, T., Kakuta, Y., Masunaga, A., Shimizu, J., Duyckaerts, C., Imaizumi, K., Iwamoto, A., and Kanazawa, I. The pro-apoptotic human BH3-only peptide harakiri is expressed in cryptococcus-infected perivascular macrophages in HIV-1 encephalitis patients. Neuroscience Letters 393: 102-107, 2006.
2. Maeda, T., Fujii, T., Matsumura, T., Endo, T., Odawara, T., Itoh, D., Inoue, Y., Ohkubo, T., Iwamoto, A., and Nakamura, T. AIDS-related cerebral toxoplasmosis with hyperintense foci on T1-weighted MR images: A case report. Journal of Infection 53: e167-e 170, 2006.
3. Ide, F., Nakamura, T., Tomizawa, M., Kawana-Tachikawa, A., Odawara, T., Hosoya, N., Iwamoto, A. Peptide-loaded dendritic-cell vaccination followed by treatment interruption for chronic HIV-1 infection: A phase 1 trial. J. Med. Virol. 78: 711-718, 2006.
4. Hishima, T., Oyaizu, N., Fujii, T., Tachikawa, N., Ajisawa, A., Negishi. M., Nakamura, T., Iwamoto, A., Hayashi, Y., Matsubara, D., Sasao, Y., Kimura, S., Kikuchi, Y., Teruya, K., Yasuoka, A., Oka, S., Saito, K., Mori, S., Funata, N., Sata, T., Katano, H. Decrease in Epstein-Barr virus-positive AIDS-related lymphoma in the era of highly active antiretroviral therapy. Microbes Infect. 8: 1301-7, 2006.
5. Koga, I., Odawara, T., Matsuda, M., Goto, M., Nakamura, T., and Iwamoto, A. Analysis of HIV-1 sequences before and after co-infecting syphilis. In press. Microbes and Infection, 2006.
6. Hoshino, S., Sun, B., Konishi, M., Shimura, M., Segawa, T. Hagiwara, Y., Koyanagi, Y., Iwamoto, A., Mimaya, J., Terunuma, H., Kano, S., and Ishizaka, Y. Vpr in plasma of HIV-1-positive patients is correlated with the HIV-1 RNA titers. In press. AIDS Research and Human Retroviruses.
7. Takahashi, H., Yotsuyanaggi, H., Yasuda, K., Koibuchi,T., Suzuki, M., Kato, T., Nakamura, T., Iwamoto, A., Nishioka, K., Iino, S., Koike, K., and Itoh, F. Molecular epidemiology of hepatitis A virus in the Metropolitan Area in Japan. J. Gastroenterology In press.
8. Kiryu, A., Okubo, T., Takeuchi, K., Inoue, Y., Endo, T., Odawara T., Nakamura, T., Aoki, A., Ohtomo, K. Magnetic Resonance Imaging and Diffusion Tensor Analysis of Lymphomatoid Granulomatosis of the Brain. Acta Radiologica 47: 509-513, 2006.
9. Hitani A, Nakamura T, Ohtomo N, Nawa Y and Kimura M. Efficacy and safety of atovaquone-proguanil compared with mefloquine in the treatment of nonimmune patients with uncomplicatde *P. falciparum* malaria in Japan. J. Infect. Chemother. 12: 277-282, 2006.
10. Maeda, T., Oyaizu, N., Endo, T., Odawara, T., Nakamura, T., Iwamoto, A., Fujii, T. Pneumocystis jiroveci pneumonia in an AIDS patient: Unusual manifestation of multiple nodules with multiloculated cavities. European Journal of Radiology. In Press.
11. Fujii, T, Nakamura T, Iwamoto A. Pneumocystis pneumonia in patients with HIV infection - Clinical manifestations, laboratory findings and radiological features -. J Infect Chemother. 2006, In press
12. Sugiyama K, Mukae H, Ishii H, Kakugawa T, Ishimoto H, Nakayama S, Shirai R, Fujii T, Mizuta Y, Kohno S. Elevated levels of interferon gamma-inducible protein-10 and epithelial neutrophil-activating peptide-78 in patients with pulmonary sarcoidosis. Respirology. 11, 708-14, 2006.
13. Fukuno K, Tomonari A, Tsukada N, Takahashi S, Ooi J, Konuma T, Uchiyama M, Fujii T, Endo T, Iwamoto A, Oyaizu N, Nakata K, Moriwaki H, Tojo A, Asano S. Successful cord blood transplantation for myelodysplastic syndrome resulting in resolution of pulmonary alveolar proteinosis. Bone Marrow Transplant. 38, 581-2, 2006.

Research Hospital

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Our major goal is to cure children suffering from a variety of life-threatening hematological disorders. Attempting to achieve it, we continue the commitment to treatment and follow-up care of such children, and to clinical and laboratory research that ultimately will help us devise better therapeutic approaches to the diseases. Currently efforts are directed toward establishment of novel therapies including hematopoietic stem cell transplantation and regenerative medicine, and analysis of pathogenesis of hematopoietic disorders.

1. Hematopoietic stem cell transplantation for children with high-risk leukemia

Yasuhiro Ebihara, Kohichiro Tsuji

Although a standard regimen in hematopoietic stem cell transplantation (SCT) has been available for children with acute lymphocytic leukemia (ALL) and acute myelocytic leukemia (AML), it has not been standardized for those with rare diseases including congenital bone marrow failure syndrome (CBMFS) and natural killer (NK) cell leukemia. A multi-institutional trial using regimens with a rationale should be proposed in a prospective manner. For CBMFS, we conducted *in vitro* and *in vivo* assays to assess the sensitivity of granulocyte colony-stimulating factor (G-CSF), and transplanted the patients whose leukemic cells had a high sensitivity to G-CSF using a regime including G-CSF. Thus, we could avoid intensive chemotherapy before SCT for patients with a vulnerable normal bone marrow reserve. For patients with Fanconi anemia, in particular, we employed a regimen containing fludarabine to reduce the dose of alkylating agents and irradiation to avoid the toxicity, which was otherwise likely to occur in those patients. For patients with NK cell disease, we used a regimen combining alkylating agents (cyclophosphamide and thiotepa) and total body irradiation based on the results that NK leukemic cells strongly expressed multidrug-registant genes. Now we plan to extend our experience in nationwide collaborative studies.

2. Cooperative clinical trial for pediatric myelodysplastic syndrome

Kohichiro Tsuji, Yasuhiro Ebihara, Atsushi Manabe[1], Yuji Zaike[2]: [1]St. Luke's International Hospital, [2]Department of Laboratory Medicine, Research Hospital

Pediatric myelodysplastic syndrome (MDS) is a rare disease, and only 50-100 children under the age of 16 suffer from the disease annually. The diagnosis and treatment have not been standardized and it should be determined in a nationwide manner. On behalf of the MDS committee of the Japanese Society of Pediatric Hematology, we began the pathologic central review in 1999 and reviewed all samples of patients suspected of having MDS. At present, over 500 patients have been enrolled, and standard diagnostic criteria have been proposed for

juvenile myelomonocytic leukemia (JMML), a subset of MDS. We also tested *in vitro* cell growth for patients with JMML using diagnostic samples. The results showed that spontaneous growth and hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) were observed in most children with JMML. We proposed a cooperative trial to establish the treatment for MDS (MDS99) and have enrolled over 50 patients from the whole country.

3. Molecular pathogenesis of pediatric myelodysplastic syndrome and myeloproliferative diseases

Daisuke Hasegawa[1], Yasuhiro Ebihara, Atsushi Manabe[1], Kohichiro Tsuji

Pediatric MDS and myeloproliferative diseases (MPD) are very rare disorders. The diseases are commonly seen in elderly patients. It suggests that the pathogenesis of the diseases in children may be of germline origins rather than of acquired process. In fact, germline mutations have been elucidated in a large proportion of pediatric MDS and MPD: GATA1 mutations in patients with MDS and Down syndrome; FANC mutations in those with MDS and Fanconi anemia; PTPN11 mutations or NF1 mutations in those with JMML.

We also tested the epigenetic abnormalities. Aberrant DNA methylation is frequently observed in adults with MDS, and is recognized as a critical event in the disease's pathogenesis and progression. The frequency of *p15* hypermethylation in pediatric MDS was 78%, which was comparable to that in adult MDS. In contrast, *p 15* hypermethylation in JMML was a rare event. In JMML, clinical and laboratory characteristics including *PTPN11* mutations and aberrant colony formation were not different between the patients with hypermethylated *p15* and the others. Aberrant methylation of *p16* was not detected in children with either MDS or JMML. Since *p15* and *p16* genes were unmethylated in children with JMML, in whom the disease had progressed with an increase in the number of blasts, a condition referred to as blastic crisis, we infer that the aberrant methylation of these genes is not responsible for the progression of JMML. The result suggests that demethylating agents may be effective in most children with MDS and a few patients with JMML.

4. Novel approach to therapy in juvenile myelomonocytic leukemia

Yasuhiro Ebihara, Yoshitoshi Ohtsuka[3], Atsushi Manabe[1], Yuji Zaike[3], Kohichiro Tsuji:

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JMML is a clonal myeloproliferative/myelodysplastic disorder of early childhood with poor prognosis. JMML cells are characterized by hypersensitivity to GM-CSF caused by continuously activated GM-CSF receptor-RAS signal transduction pathway through various molecular mechanisms, resulting in spontaneous colony formation *in vitro*. Bisphosphonate zoledronic acid (ZOL), a RAS-blocking compound, suppressed colony formation from bone marrow (BM) cells of JMML patients and normal volunteers without and with GM-CSF, respectively, in a dose-dependent manner in clonal culture. At 10 μM of ZOL, however, spontaneous colony formation decreased, but formation of granulocyte (G) colonies containing only granulocytes, but no macrophages was enhanced in culture of JMML BM cells, while granulocyte-macrophage (GM) colonies containing both granulocytes and macrophages retained and G colony formation was not affected in culture of normal BM cells with GM-CSF. In suspension culture, 10 μM of ZOL also inhibited spontaneous proliferation and differentiation along monocyte/macrophage lineage of JMML BM cells, but not development of normal BM cells by GM-CSF assessed in cytochemical and flow cytometric analyses. The inhibitory effect of ZOL on JMML cells was confirmed at a single-clone level, and observed even at 3 μM. The current result offers a novel approach to therapy in JMML.

5. Production of hematopoietic stem cells and functional blood cells from human embryonic stem cells

Feng Ma[4], Yasuhiro Ebihara, Sachiyo Hanada, Kazuo Ogami[5], Tokiko Nagamura-Inoue[5], Kohichiro Tsuji: [4]Laboratory of Stem Cell Therapy, Center for Experimental Medicine, [5]Department of Transfusion, Research Hospital

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of preimplantation embryos. Since ES cells have the ability to be maintained in culture indefinitely as undifferentiated cells, yet they are capable of forming more differentiated cell types, human ES cells recently established are expected as a novel source of human transplantable cells. We then planed to produce hematopoietic stem cells (HSC) for SCT and functional blood cells for transfusion medicine from human ES cells. This study was started on December 20, 2003 with the permission by the ethical committee of the Japanese Government.

On beginning this study, we thought that *in vitro* reconstitution of the circumstance surrounding embryonic hematopoietic cells is important to induce the differentiation of human ES cells into HSC and functional blood cells. To achieve this, we determined to use stromal cells from murine embryonic hematopoietic tissues to coculture human ES cells with them, since some mouse-derived stromal cells have been reported to be able to act on human hematopoietic cells. We then established stromal cells from embryonic hematopoietic tissues, such as aorta-gonad-mesonephros (AGM) region at 10 to 11 days post coitus (dpc) and fetal liver (FL) at 14 to 15 dpc of mouse embryos, because long term-repopulating HSC are first generated in AGM region at 10 dpc, and shift to FL in which hematopoiesis dramatically develops. As expected, hematopoietic cells and mature blood cells, including β globin-expressing erythrocytes and tryptase and chymase-double positive mast cells, were generated from human ES cells in the coculture with the mouse embryo-derived stromal cells.

We are now evaluating the function of the hematopoietic and mature blood cells differentiated from human ES cells, and searching the molecules contributing to the capability of the stromal cells to induce the differentiation of human ES cells into hematopoietic cells. Furthermore, the establishment of culture system without animal serum and cells is aimed to apply human ES cell-derived hematopoietic and blood cells to clinical situation.

6. Hematopoietic origin of fibroblasts (mesenchamal stem cells)

Yasuhiro Ebihara, Makio Ogawa[6], Kohichiro Tsuji: [6]Department of Medicine, Medical University of South Carolina, USA

Using transplantation of a clonal population of cells derived from a single HSC of transgenic enhanced green fluorescent protein (EGFP) mice, we have documented the hematopoietic origin of myofibroblasts. Since myofibroblasts are thought to be an activated form of fibroblasts, we tested the hypothesis that fibroblasts are derived from HSC.

Clones of cells derived from single lineage-negative (Lin^-), c-kit^+, Sca-1^+, $CD34^-$ cells of EGFP Ly-5.2 C57Bl/6 mice were transplanted into lethally irradiated Ly-5.1 mice. Using BM and peripheral blood (PB) cells from mice showing high-level multilineage hematopoietic reconstitution, we induced growth of fibroblasts *in vitro*. Culture of $EGFP^+$ BM cells from clonally engrafted mice revealed adherent cells with the typical morphology of fibroblasts. Flow cytometric analysis revealed that the majority of these cells are $CD45^-$ and express collagen-I and the collagen receptor, discoidin domain receptor 2 (DDR2). RT-PCR analysis of the cultured cells demonstrated expression of procollagen 1-a1, DDR2, fibronectin and vimentin mRNA. Fibroblast colonies consisting of $EGFP^+$ cells were observed in cultures of BM cells from clonally engrafted mice indicating an HSC origin of fibroblast colony-forming units (CFU-F). Culture of PB nucleated cells from clonally engrafted mice revealed $EGFP^+$ cells expressing collagen-I and DDR2, indicating that fibrocytes are also derived from HSC. We then concluded from these results that a population of fibroblasts and their precursors are derived from HSC.

7. Novel potentials of hematopoietic stem cells

Yasuhiro Ebihara, Makio Ogawa[6], Kohichiro Tsuji

BM-derived stem cells have shown plasticity with a capacity to differentiate into a variety of specialized cells. To examine their novel potentials, we transplanted either isolated whole BM cells or clonally expanded HSC prepared from BM cells of EGFP mice into lethally irradiated congenic non-EGFP mice.

Quantification of $EGFP^+$ cells was performed 3-20 months after transplant. $EGFP^+$ cells were found in the inner ear with all transplant conditions. They were most abundant within the spiral ligament but were also found in other locations normally occupied by fibrocytes and mesenchymal cells. Dual immunofluorescence assays demonstrated that most of the $EGFP^+$ cells were negative for CD45, a macrophage and hematopoietic cell marker. A portion of the $EGFP^+$ cells in the spiral ligament expressed immunoreactive Na, K-ATPase, or the Na-K-Cl transporter (NKCC), proteins used as markers for specialized ion transport fibrocytes. In addition, histological analyses of valve tissue from clonally engrafted recipient mice revealed the presence of numerous $EGFP^+$ cells within host valves. A subpopulation of these cells exhibited synthetic properties characteristic of fibroblasts, as evidenced by their expression of mRNA for procollagen 11. These results demonstrate that HSC contribution to mesenchymal cells, including fibrocytes in the adult inner ear and valve.

8. Development of B cell potential in the mouse embryo

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Experiments using chimeric embryos in non-mammalian vertebrates demonstrated that mesodermally derived ventral compartments (YS or its analog) and dorsal compartments (intraembryonic region) contribute to hematopoiesis in a different manner. On the other hand, the ontogenic source of mammalian definitive hematopoiesis has remained controversial, because the *in utero* development of mammals excludes embryo grafting experiments. Results of earlier mouse studies led to the general acceptance of a model that murine definitive hematopoiesis begins in YS, shifts to FL, and finally resides in BM, in contrast to the conclusion derived from nonmammal vertebrates. However, recent studies have shown that early development of murine hematopoiesis is more complex than heretofore considered.

To investigate the the ontogenic source of mammalian definitive hematopoiesis, we made mouse chimeras by grafting YS onto the YS of the host embryos before the establishment of circulation between YS and embryo proper, and cultured the whole embryo for 66 hours. Donor YS were taken from C57BL/6 Ly-5.1 and EGFP-transgenic mouse embryos, and recipient embryos from C57BL/6 Ly-5.2. Almost half of the grafts in YS-YS chimeras survived and had obvious blood flow, and the graft-derived cells achieved $12.7 \pm 0.9\%$ of the blood cells in the circulation. These graft-derived blood cells consisted mainly of erythroid cells, some myeloid cells and a few blastic cells. Interestingly, CD19-positive B cells were generated from the graft-derived cells taken out from AGM regions of the YS-YS chimeras but the frequency of the YS-derived B cells was low ($1.0 \pm 0.6\%$) when co-cultured with OP9 stromal cells, demonstrating that B cell potential exists in YS before the circulation and that YS may also contribute to definitive lymphopoiesis *in vitro* in mice, while the major source for B cell in intra-embryonic AGM region.

Publications

Sugiyama, D., Tsuji, K. Definitive hematopoiesis from endothelial cells in mouse embryo; A simple guidance. Trends. Cardiovas. Med. 16: 45-49, 2006.

Yabe H, Inoue H, Matsumoto M, Hamanoue S, Koike T, Ishiguro H, Kike H, Suzuki K, Kato S, Kojima S, Tsuchida M, Mori T, Adachi S, Tsuji K, Koike K, Morimoto A, Sako M, Yabe M. Allogeneic hematopoietic cell transplantation from alternative donors with a conditioning regimen of low-dose irradiation, fludarabine and cyclophosphamide in Fanconi anemia. Br. J. Haematol. 134; 208-212, 2006.

Ebihara, Y., Masuya, M., LaRue, A.C., Fleming, P.A., Visconti, R.P., Minamiguchi, H., Drake, C.J. and Ogawa, M. Hematopoietic origins of fibroblasts: II. In vitro studies of fibroblasts, CFU-F and fibrocytes. Exp. Hematol. 34: 219-229, 2006.

Lang, H, Ebihara, Y., Schmiedt, R.A., Minamiguchi, H., Zhou, D., Smythe, N., Liu, L., Ogawa, M. and Schulte, B.A. Contribution of bone marrow hematopoietic stem cells to adult mouse inner ear: mesenchymal cells and fibrocytes. J. Comp. Neurol. 496: 187-201, 2006.

Visconti RP, Ebihara Y, LaRue AC, Fleming PA, McQuinn TC, Masuya M, Minamiguchi H, Markwald RR, Ogawa M, Drake CJ. An in vivo analysis of hematopoietic stem cell potential: hematopoietic origin of cardiac valve interstitial cells. Circ Res. 98: 690-696, 2006.

LaRue, A.C., Masuya, M., Ebihara, Y., Fleming, P.A., Visconti, R.P., Minamiguchi, H., Ogawa, M., Drake, C.J. Hematopoietic origins of fibroblasts: I. In vivo studies of fibroblasts associated with solid tumors. Exp. Hematol. 34: 208-218, 2006.

Sugiyama D, Ogawa M, Nakao K, Ma F, Osumi N, Nishikawa S, Nishikawa S, Katsuki M, Asano S, Arai K, Nakahata T, Tsuji K. B cell can be obtained from pre-circulatory yolk sac, but with low frequency. Dev. Biol., in press.

Ma F, Wang D, Hanada S, Ebihara Y, Kawasaki H, Zaike Y, Heike T, Nakahata T, Tsuji K. Novel method for efficient production of multipotential hematopoietic progenitors from human embryonic stem cells. Int J Haematol, in press.

Shinoda G, Umeda K, Heike T, Arai M, Niwa A, Ma F, Suemori H, Luo HY, Chui DHK, Torii R, Shibuya M, Nakatsuji T, Nakahata T. 4-integrin^{+} endothelium derived from primate embryonic stem cells generates both primitive and definitive hematopoietic cells. Blood, in press.

Umeda K, Heike T, Nakata-Hizume M, Niwa A, Arai M, Shinoda G, Ma F, Suemori H, Luo HY, Chui DHK, Torii R, Shibuya M, Nakatsuji N, Nakahata T. Sequential analysis of α- and β-globin gene expression during erythropoietic differentiation from primate embryonic stem cells. Stem Cells, in press.

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We participate in cutting edge science of autoimmune, rheumatic and allergic disease and novel treatments for patients with these disorders. In addition to conventional drug studies aimed to improve the efficacy and safety of current therapies, we are going to carry out experimental protocols of particular interest for patients not responding to conventional therapy and to perform the translational research.

I. Study on CD26 molecule in normal immune response and in patients with immune-mediated diseases

Osamu Hosono, Kei Ohnuma, Noritada Yoshikawa, Hirotoshi Tanaka, Chikao Morimoto., Department of Rheumatology and Allergy.

CD26 is a T cell costimulatory molecule as well as an activation antigen with dipeptidyl peptidase IV (DPPIV) enzyme activity in its extracellular region that is preferentially expressed on memory T cells. The soluble form of CD26 (sCD26) is present in serum and recombinant soluble CD26 can enhance peripheral blood T cell proliferation induced by the recall antigen. We demonstrated that CD26 binds Caveolin-1 on antigen presenting cells, and that following CD26-caveolin-1 interaction on recall antigen-loaded monocytes, caveolin-1 is phosphorylated, with linkage to NF-κB activation, followed by upregulation of CD86. In addition, reduced caveolin-1 expression on monocytes inhibits CD 26-mediated CD86 upregulation and abrogates CD26 effect on recall antigen-induced T cell proliferation, and immunohistochemical studies revealed an infiltration of CD26+ T cells in the sublining region of rheumatoid synovium and high expression of caveolin-1 in the increased vasculature and synoviocytes of the rheumatoid synovium. Taken together, these results strongly suggest that CD26-caveolin-1 interaction plays a role in the upregulation of CD86 on recall antigen-loaded monocytes and subsequent engagement with CD28 on T cells, leading to antigen-specific T cell activation such as the T-cell-mediated antigen-specific response in rheumatoid arthritis (RA).

Serum levels of sCD26 and its specific DPPIV activity were significantly decreased in SLE and

were inversely correlated with SLE disease activity index score, but not with clinical variables or clinical subsets of SLE. Serum levels of sCD26 may be involved in the pathophysiology of SLE, and appear to be useful as a new disease activity measure for SLE. We have also measured sCD26/DPPIV levels in sera from patients with RA and found significant decrease of sCD26 and its specific DPPIV activity. We plan to examine the effect of anti-TNF therapy (anti-TNFα antibody and soluble TNF-R) therapy on serum levels of sCD26/DPPIV in patients with RA.

a. Caveolin-1 triggers T-cell activation via CD 26 in association with CARMA1

CD26 is a widely distributed 110-kDa cell surface glycoprotein, having an important role in T-cell costimulation. We previously demonstrated that CD26 binds to caveolin-1 in antigen-presenting cells (APC), and that following exogenous CD26 stimulation, Tollip and IRAK-1 disengage from caveolin-1 in APC. IRAK-1 is then subsequently phosphorylated to upregulate CD86 expression, resulting in subsequent T cell proliferation. However, it is unclear whether caveolin-1 is a costimulatory ligand for CD26 in T-cell. Using soluble caveolin-1-Fc fusion protein, we now showed that caveolin-1 is the costimulatory ligand for CD26, and that ligation of CD26 by caveolin-1 induces T-cell proliferation and NF-κB activation in a TCR/CD3-dependent manner. We also demonstrated that the cytoplasmic tail of CD26 interacts with CARMA1 in T-cells, resulting in signaling events that lead to NF-κB activation. Ligation of CD26 by caveolin-1 recruits a complex consisting of CD26, CARMA1, Bcl10, and IKKβ to lipid rafts. Taken together, our findings hence provide novel insights into the regulation of T-cell costimulation via the CD26 molecule.

b. Role of T-cell activation via CD26 and caveolin-1 in rheumatoid synovium.

Kei Ohnuma, Osamu Hosono, Chikao Morimoto., Department of Rheumatology and Allergy

CD26 is a T-cell costimulatory molecule with dipeptidyl peptidase IV (DPPIV) activity in its extracellular region. We previously reported that recombinant soluble CD26 enhances peripheral blood T-cell proliferation induced by the recall antigen tetanus toxoid (TT). Recently, we demonstrated that CD26 binds caveolin-1 on antigen-presenting cell (APC), and that residues 201-211 of CD26 along with the serine catalytic site at residue 630, which constitute a pocket structure of CD26/DPPIV, contribute to binding to caveolin-1 scaffolding domain. In addition, following CD26-caveolin-1 interaction on TT-loaded monocytes, caveolin-1 is phosphorylated, with linkage to NF-kappaB activation, followed by upregulation of CD86. Finally, reduced caveolin-1 expression on APC inhibits CD26-mediated CD86 upregulation and abrogates CD 26 effect on TT-induced T-cell proliferation, and immunohistochemical studies revealed an infiltration of CD26+ T cells in the sub-lining region of rheumatoid synovium and high expression of caveolin-1 in the increased vasculature and synoviocytes of the rheumatoid synovium. Taken together, these results strongly suggest that CD26-caveolin-1 interaction plays a role in the upregulation of CD86 on TT-loaded APC and subsequent engagement with CD28 on T cells, leading to antigen-specific T-cell activation such as the T-cell-mediated antigen-specific response in rheumatoid arthritis.

c. Anti-CD26 monoclonal antibody-mediated G1-S arrest of human renal clear cell carcinoma caki-2 Is associated with retinoblastoma substrate dephosphorylation, cyclin-dependent kinase 2 reduction, p27kip1 enhancement, and disruption of binding to the extracellular matrix

CD26 is a 110-kDa cell surface glycoprotein with a role in tumor development through its association with key intracellular proteins. In this report, we show that binding of soluble anti-CD26 monoclonal antibody (mAb) inhibits the growth of the human renal carcinoma cells in both in vitro and in vivo experiments.

Growth inhibition by anti-CD26 mAb was assessed using proliferation assay and cell cycle analysis. Anti-CD26 mAb, chemical inhibitors, dominant-negative, or constitutively active forms of specific signaling molecules were used to evaluate CD26-associated pathways. The in vivo growth-inhibitory effect of anti-CD26 mAb was also assessed in a human renal carcinoma mouse xenograft model.

In vitro experiments show that anti-CD26 mAb induces G1-S cell cycle arrest associated with enhanced p27kip1 expression, down-regulation of cyclin-dependent kinase 2, and dephosphorylation of retinoblastoma substrate. Moreover, our data show that enhanced p27kip1 expression is dependent on the attenuation of Akt activity. Anti-CD26 mAb also internalizes cell surface CD26, leading to decreased binding to collagen and fibronectin. Experiments with a mouse xenograft model involving human renal carcinoma cells show that anti-CD26 mAb treatment drastically inhibits tumor growth in

tumor-bearing mice, resulting in enhanced survival.

Taken together, our data strongly suggest that anti-CD26 mAb treatment may have potential clinical use for CD26-positive renal cell carcinomas.

II. Therapeutically targetting transcription factors

Hirotoshi Tanaka, Noritada Yoshikawa (Department of Rheumatology and Allergy), Noriaki Shimizu, Yuichi Makino, Chikao Morimoto (Division of Clinical Immunology), Hiroshi Handa (Tokyo Institute of Technology), Motoaki Sano, Keiichi Fukuda (Keio University School of Medicine)

We are interested in the mechanism of eukaryotic gene expression and development of novel therapy and/or drugs which target transcriptional machineries. For this purpose, our recent work is mainly focused on research for conditional regulation of transcription factors including the glucocorticoid receptor and hypoxia-inducible factor-1α.

a. Glucocorticoid receptor project

Glucocorticoid hormones are effective in controlling inflammation and immunity, but underlying mechanisms are largely unknown. It has been shown that both positive and negative regulation of gene expression are necessary for this process. The genes whose activity is negatively modulated in the anti-inflammatory process code for several cytokines, adhesion molecules. Most of them do not carry a classical binding site for regulation by the glucocorticoid receptor (GR), but have instead regulatory sequences for transcription factors such as AP-1 or NF-κB. Considering various severe side effects of glucocorticoids, it may be pharmacologically important to dissociate these negative regulatory function of the GR from induction of genes for metabolic enzymes, expression of which have been shown to be positively regulated by the GR. We propose that a certain class of compounds (surprisingly, some of them are non-steroidal chemicals) may dissociate transactivation and transrepression function of the GR and offer opportunities for the design of such compounds that could function more effectively as antiinflammatory drugs. In this line, we are developing novel therapeutic strategy.

(i) Redox regulation of the glucocorticoid receptor

Redox regulation is currently considered as a mode of signal transduction for coordinated regulation of a variety of cellular processes. Transcriptional regulation of gene expression is also influenced by cellular redox state, most possibly through the oxido-reductive modification of transcription factors. The glucocorticoid receptor belongs to a nuclear receptor superfamily and acts as a ligand-dependent transcription factor. We demonstrate that the glucocorticoid receptor function is regulated via redox-dependent mechanisms at multiple levels. Moreover, it is suggested that redox regulation of the receptor function is one of dynamic cellular responses to environmental stimuli and plays an important role in orchestrated crosstalk between central and peripheral stress responses.

(ii) Development of dissociating ligand for the glucocorticoid receptor

The GR function could be differencially regulated by ligands. We have recently shown that not only synthetic glucocorticoids but also certain bile acids could differentially modulate GR function. Moreover, the effects of those compounds are indicated to be ascrived to the ligand binding domain of the receptor. In this line, we are going to isolate the dissociating ligand that preferentially promotes transrepression function of the GR. Recently we have demonstrated that certain ligands can modulate interdomain communication of the GR, which will eventually contribute to isolation of novel category of ligands.

On the other hand, receptor specificity is another important aspect of novel GR regulator. In this line, we have shown that cortivazol is extremely specific for GR and does not bind to MR. We are studying the molecular basis for this receptor specificity of the ligand using cortivazol as a model. Recent microarray analyses revealed that GR and MR have distinct effects on the repertoire of target genes in rat cardiomyocyts. We will clarify novel mechanism for regulation of gene expression by GR and MR.

(iii) Molecular biology of small nuclear RNA binding protein HEXIM1

Expression of HEXIM1 is induced by treatment of vascular smooth muscle cells with a differentiation inducer hexamethylane bisacetamide. It is shown that HEXIM1 binds 7SK snRNA and inhibits P-TEFb-mediated transcriptional elongation process. On the other hand, we have found that HEXIM1 directly associates with the GR in the absence of 7SK and represses GR-mediated transcription. We are currently

working on regulation of HEXIM1 expression, physiological role of HEXIM1 in GR action.

b. Hypoxia-inducible factor (HIF)-1α project

HIF-1α is essential for not only angiogenesis but also development of certain organs. In this line, molecular biology of HIF-1α will provide us possible advantage to characterize and manipulate such processes.

Peripheral T cells encounter rapid decrease in oxygen tension as they are activated by antigen recognition and migrate into inflammatory sites or tumors. Activated T cells, therefore, are thought to have such machineries that enable them to adapt to hypoxic conditions and execute immune regulation in situ. We have recently shown that survival of CD3-engaged human peripheral blood T cells is prolonged under hypoxic conditions and HIF-1 and its target gene product adrenomedullin play a critical role for the process. It is also shown that hypoxia alone is not sufficient but TCR-mediated signal is required for accumulation of HIF-1α in human peripheral T cells. In the present study, we showed that TCR-engagement does not influence hypoxia-dependent stabilization but stimulates protein synthesis of HIF-1α, most possibly via PI3K/mTOR system, and that expression of HIF-1α and its target gene is blocked by treatment with rapamycin. Since some of those gene products, e.g., glucose transporters and phosphoglycerate kinase-1, are considered to be essential for glycolysis and energy production under hypoxic conditions and adequate immune reaction in T cells, this TCR-mediated synthesis of HIF-1α may play a pivotal role in peripheral immune response. Taken together, our results may highlight a novel aspect of downstream signal from antigen recognition by TCR with giving insight of a unique pharmacological role of rapamycin. We are currently working with the mechanism of translational regulation of HIF -1α.

c. Therapeutic intervention of signal transduction in the hypoxic cells

The hypoxia-inducible transcription factors (HIFs), key regulators of transcriptional response to hypoxic conditions, have been proven to be involved in pathogenesis of hypoxia-related diseases such as solid tumor, ischemic organ damage, and chronic inflammation, thus being a potential molecular target for therapeutic intervention of those diseases. We have been focusing on elucidation of regulatory mechanism of HIFs and establishment of a strategy to manipulate HIFs for therapeutic purpose.

1. Regulation of expression of IPAS, a dominant negative regulator of HIFs

The inhibitory PAS (Per/Arnt/Sim) domain protein, IPAS, functions as a dominant negative regulator of hypoxia-inducible transcription factors (HIFs) by forming complexes with those proteins that fail to bind to hypoxia response elements of target genes. We have previously observed that IPAS mRNA expression is upregulated in hypoxic tissues, which at least in part involves a hypoxia-dependent alternative splicing of the transcripts from IPAS/HIF-3a locus. Recently, we have identified an RNA-binding protein, designated as p75, operating in hypoxia-dependent generation of IPAS-specific splicing products. p75 binds to IPAS pre-mRNA at a 3′-splicing site for an IPAS-specific exon inclusion in a hypoxia-dependent manner and enhances the exon inclusion. Moreover, knockdown of p75 by means of siRNA introduction to the cells abrogated hypoxia-dependent generation of IPAS-type transcripts, resulting in upregulation of a HIF-1 target gene expression under hypoxic conditions. These results indicate that manipulation of IPAS expression via control of alternative splicing mechanisms would provide a novel tool for regulation of hypoxia-inducible gene expression, which may contribute to development of a novel therapeutic strategy for hypoxia-related diseases.

2. Establishment of a system for screening of the compounds to regulate HIFs.

The HIF-1 and HIF-2 are heterodimeric complexes of the basic helix-loop-helix PAS (Per, Arnt, Sim) domain proteins HIF-1α or HIF-2α, respectively, and Arnt. While Arnt is constitutively present in the nucleus, the expression of HIF-1α or HIF-2α subunit is strictly regulated by oxygen tension via protein stability control and thus responsible for the hypoxia-inducibility of this transcription factor. Since regulation of HIF-mediated signal transduction would provide a milieu for therapeutic intervention of hypoxia-related diseases, manipulation of expression level of HIF-1α or HIF-2α protein, e.g. by pharmacological approach, could be employed for potential use in the treatment of those diseases. Establishment of a system to screen or to evaluate drugs/compounds for their effect on HIF-1α or HIF-2α protein stability, therefore, has been an attractive topic over wide discipline of biological science. We have developed a stable cell line expressing fusion protein of HIF-1α and a peptide tag with high affinity to a fluorescence ligand for monitoring and visualization of the effect of the compounds

on the stability of HIF-1α protein in living cells. As a precedent, we could observe, in real-time monitoring, both stabilization and rapid decay of HIF-1-Tag fusion protein by treatment of the cells with certain types of compounds known to modify HIF-1α stability. The selected drugs/compounds by this system are potentially useful in HIF-1α-targeting therapy of the diseases; HIF-1α stabilizers for treatment of ischemic diseases including heart attack, stroke, and peripheral vascular diseases, and HIF-1α degradation-enhancer for therapy of cancer and proliferative retinopathy, and chronic inflammatory diseases.

III. Immunobiology of inflammatory cytokines and chemokines

Hiroshi Kawasaki, Chikao Morimoto

We are investigating the linkage of innate immunity and acquired immunity to fully understand the pathogenesis of various immune-mediated disorders. Chemokines are interesting molecules in that they recruit immunocytes to resume immune response and inflammatory response in local tissue.

In allergic disorders, basophils migrate from the blood stream to inflamed tissue sites. Since transbasement membrane migration is an important step for local basophil accumulation, we performed a human basophil transmigration assay using a model basement membrane, Matrigel. IL-3 in the upper chamber was critical for basophil trans-basement membrane migration over baseline levels, since none of the chemoattractants placed in the lower chambers induced migration. RANTES, IL-8, 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid (5-oxo-ETE) and platelet-activating factor (PAF) significantly up-regulated the transmigration of IL-3-treated basophils. Neutralizing experiments indicated the involvement of β_2 integrin and matrix metalloproteinase (MMP)-2/9 in basophil transmigration. Real-time quantitative PCR revealed that basophils constitutively expressed transcripts for MMP-9, and at lower levels, MMP-2, but cell-surface expression was only detected for MMP-9. MMP-9 was also detected in the cytoplasm and culture supernatant of the basophils. Treatment with IL-3 up-regulated the surface level of MMP-9 on the basophils. Our results suggest that basophils possess a unique regulatory mechanism for trans-basement membrane migration which is affected by cytokines, chemoattractants, β_2 integrin and MMPs, especially MMP-9. MMP-9 may be critically involved in the pathogenesis of local basophil influx in allergic diseases.

In the T cell activation, Cas-L molecule identified by Morimoto et al. is implicated to work as an important adaptor in the receptor- downstream signaling upon the ligation of inflammatory cytokines. Cas-L also exerts trans-activation of various surface molecules. In pararell with the various events associated with T-cell activation including IL-2 secretiom and upregulation of CD25 expression, the expression of chemokine receptor is also enhanced. We are interested in the pursuit of the possibility of Cas-L mediated trans-activation of chemokine receptor upregulation.

Publications

Ohnuma, K., Inoue, H., Uchiyama, M., Yamochi, T., Hosono, O., Dang, NH., Morimoto, C. T-cell activation via CD26 and caveolin-1 in rheumatoid synovium. Mod Rheumatol. 16: 3-13 (2006)

Inamoto, T., Yamochi, T., Ohnuma, K., Iwata, S., Kina, S., Inamoto, S., Tachibana, M., Katsuoka, Y., Dang, NH., Morimoto, C. Anti-CD26 monoclonal antibody-mediated G1-S arrest of human renal clear cell carcinoma Caki-2 is associated with retinoblastoma substrate dephosphorylation, cyclin-dependent kinase 2 reduction, p27(kip1) enhancement, and disruption of binding to the extracellular matrix. Clin Cancer Res. 12: 3470-7 (2006)

Suzukawa, M., Komiya, A., Likura, M., Nagase, H., Yoshimura-Uchiyama, C., Yamada, H., Kawasaki, H., Ohta, K., Matsushima, K., Hirai, K., Yamamoto, K., Yamaguchi, M. Transbasment membrane migration of human basophils: role of matrix metalloproteinase-9. International Immunology. 18: 1575-1583 (2006)

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Department of Advanced Medical Science was established in September 1997. We are investigating, (1) analysis of the gradient expression of genes in human colonic mucosa, (2) analysis the role of newly identified non-coding RNA for organ development, (3) analysis on the mechanisms of cardiac outflow tract development, (4) serological identification of melanoma antigens by recombinant expression cloning. We are planning and progressing several projects described below to develop new therapies for several diseases, including cancer and ischemic disorders.

Analysis of the Gradient Expression of Genes in Human Colonic Mucosa

Ohno H. et al.

Ulcerative colitis (UC) is characterized by continuous inflammation extending from rectum to oral colonic mucosa. Epidemiological data have provided incontrovertible evidence that both genetic and environmental factors are important in the disease susceptibility. We speculate that the gradient expression of genes in human colonic mucosa might be related to the disease development and progression. In this study, we compared the expression levels of genes in a normal adult human colon and made the catalogue of genes at higher level in the distal colon. First, we compared the expression levels of genes at different segments of colon by screening cDNA microarray. Next, RT-PCR analysis were conducted to confirm the expression levels of these genes. Finally, we evaluated the expression levels of these genes throughout the GI tract and in other tissues by northern blot analysis. As a result of this analysis, the three genes showed the expression gradient to increase toward the distal colon and one of them was specifically expressed in colon. We have generated rabbit polyclonal antibodies against the protein encoded by this gene. We are currently examining expressions of the protein in human colon cell lines and tissues.

Analysis the role of newly identified non-coding RNA for organ development

Watanabe T. et al.

To understand the process of organ development is of importance with respect to the possibility of medical approach for congenital diseases. Recently, Nakaoka et al. identified a novel gene named Hag2, which was unlikely to code protein and was expressed tissue-specifically in the mouse development. Hag2 gene was a single exon gene of some 8kb in length located on mouse chromosome 1. In vitro translation of the capped transcript of Hag2 produced a single

polypeptide of 17kDa with a predicted open reading frame of 156 amino acids not homologous to the other reported proteins. However, although some portions of its sequence were well conserved between mouse and human, the start codon in the sequence of human was replaced and consequently deleted indicating that this gene could encode a non-coding RNA. Hag 2 gene expression was developmentally regulated in mouse embryos; initially it was detected at embryonic day (E) 9, became most extensive at E10.5, and decreased from E11.5 thereafter. At E10.5 Hag2 expression was clearly detected in the pharyngeal arches, limb buds and somites. To elucidate the role of Hag2 for the formation of craniofacial organs, vertebrae, and extremities, we made Hag2 mutant mouse, in which β-galactosidase gene was knocked-in instead of a portion of Hag locus. The results of β-gal staining showed the activity of β-galactosidase mimicked the endogenous expression of Hag2 during development. The phenotype of Hag2 knockout mouse is now under investigation, to know the function of Hag2 gene during development.

Analysis on the mechanisms of cardiac outflow tract development

Nakaoka T. et al.

Malformations of the cardiovascular system in the human account for most of the premature deaths caused by congenital abnormalities and, most often, are linked to abnormalities in the formation of the cardiac outflow tract. During embryonic development, the outflow tract is progressively added to the distal end of the heart tube by accretion of new myocardial tissue derived from a novel heart-forming field, dubbed anterior or secondary heart field. The heart defect (hdf) mouse is a recessive lethal mutation that arose from a LacZ reporter containing a transgene insertional mutation. The most striking feature of the hdf homozygous embryo is the immature formation of the outflow tract, through which diminutive right ventricle connects directly to the aortic arches. Therefore, the hdf mouse is a good model system to investigate the formation of the cardiac outflow tract, which will shed light on the molecular mechanisms of congenital abnormalities, especially linked to the outflow tract abnormalities. Now, the project to investigate the molecular mechanisms responsible for defective outflow tract formation in the *hdf* mouse is under way in two ways.

Serological identification of melanoma antigens by recombinant expression cloning

Sharif U.A. et al.

We previously conducted dendritic cell therapy on 10 melanoma patients and remarkable tumor reductions were observed in two patients. We aimed to identify some of the unique antigens targeted by dendritic cell therapy in these patients. To identify the target antigens, we first employed two-dimensional electrophoresis combined with Western blots analysis and matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS) methods. Through this strategy, carbonic anhydrase II (CA-II) was identified as an antigen that elicited serum antibody response to dendritic cell therapy in the patient (Yoshiura K, et. al. *Clin. Cancer Res*. 11: 8201-7, 2005). To further extend this study, we then employed cDNA expression cloning which is recognized by IgG antibodies in sera from melanoma patient. We constructed cDNA libralies from the melanoma tissues of the patients and the screening is now under way.

Publications

Fujita, S., Seino, K., Sato, K., Sato, Y., Eizumi, K., Yamashita, N., Taniguchi, M., Sato, K. Regulatory dendritic cells act as regulators of acute lethal systemic inflammatory response. *Blood* 107: 3656-3664, 2006.

Iso-O,N., Noto, H., Hara, M., Togo, M., Karasawa, K., Ohashi, N., Noiri, E., Hashimoto, Y., Kadowaki, T., Kimura, S., Watanabe, T., Tsukamoto, K. Adenovirus-mediated gene transfer and lipoprotein-mediated protein delivery of plasma PAF-AH ameliorates proteinuria in rat model of glomerulosclerosis. *Molecular Therapy*. 13: 118-26, 2006.

Tomonari, A., Takahashi, S., Ooi, J., Nakaoka, T., Takasugi, K., Uchiyama, M., Tsukada, N., Konuma, T., Iseki, T., Tojo, A., Asano, S. Cord blood transplantation for acute myelogenous leukemia using a conditioning regimen consisting of granulocyte colony-stimulating factor-combined high-dose cytarabine, fludarabine, and total body irradiation. *Eur. J. Haematol*. 77: 46-50, 2006.

Nomura, Y., Inoue, Y., Yokoyama, I., Nakaoka, T., Itoh, D., Okubo, T., Ohtomo, K. Evaluation of left ventricular function with cardiac magnetic resonance imaging using Fourier fitting.

Magn Reson Imaging 24: 1333-9, 2006.

Okazaki, H., Tazoe, F., Okazaki, S., Isoo, N., Tsukamoto, K., Sekiya, M., Yahagi, N., Iizuka, Y., Ohashi, K., Kitamine, T., Tozawa, R., Inaba, T., Yagyu, H., Okazaki, M., Shimano, H., Shibata, N., Arai, H., Nagai, RZ., Kadowaki, T., Osuga, J., Ishibashi, S. Increased cholesterol biosynthesis and hypercholesterolemia in mice overexpressing squalene synthase in the liver. *J. Lipid Res*. 47: 1950-8 2006.

Hara, M., Iso-O, N., Satoh, H., Noto, H., Togo, M., Ishibashi, S., Kimura, S., Kadowaki, T., Hashimoto, Y., Tsukamoto, K. Differential effects of apolipoprotein E isoforms on lipolysis of very low-density lipoprotein triglycerides. *Metabolism*. 55: 1129-34 2006.

Yamashita, N., Tashimo, H., Matsuo, Y., Ishida, H., Yoshiura, K., Sato, K., Yamashita, N., Kakiuchi, T., and Ohta, K. Role of CCL21 and CCL19 in allergic inflammation in the ovalbumin-specific murine asthmatic model. *J. Allergy. Clin. Immunol*. 117: 1040-6, 2006.

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We have been engaged in the surgical treatment of solid tumors and the immunotherapy of various malignancies. We have also been offering diagnostic services, including upper and lower endoscopic examination and ultrasonic examination. The principal goal of our department is to develop and conduct clinical trials in the early stages (Phase I and II) on patients at Research Hospital. We have performed phase I clinical trials of melanoma vaccine using gp100 derived peptides and immunotherapy using dendritic cells in combination with local irradiation therapy. We have also initiated phase I/IIa clinical trials using epitope peptides based vaccine against gastrointestinal malignancy.

1. Summary of surgical treatment and other procedures performed in 2006

Akihiko Itoh, Takuya Takayama, Koji Yoshida, Akira Kanamoto, Shigenori Nagai, Eri Ichikawa, Hideaki Tahara.

Surgical operations have been performed in 182 cases under general anesthesia and spinal or epidural and/or local anesthesia. As shown in Table 1, major operations were performed in 100 patients with malignant diseases and with benign diseases. We especially endeavor in the treatment of the far advanced malignant tumors. In this period, the chemotherapy with imatinib against multiple liver metastasis of GIST, the resection of the peritoneal seeding and retroperitoneal metastasis around the kidney of GIST (genotyping showed the c-kit was wild type), chemo-radio therapy against esophageal cancer with multiple lymph node metastasis and invasion to the bronchus, removing the ovarian metastasis of the gastric cancer combined chemotherapy and Caudate lobectomy against repeating metastatic liver cancer were performed. In adition, minimum invasive surgery and operations without complications were tried to performed. As aresult of our efforts, the post-operation hospitalization was 17 days.

Table 1. 100 major operations performed in 2006

GIST	4	
Stomach	13	
Colo-rectum*	14	*:1 carcinoid
Liver**	9	**:6HCC and 2metastatic liver cancer
Biliary Tract***	9	***:2 bile duct cancer
Pancreas	2	
Spleen	2	
Breast	11	
Miscellaneous	36	
Total	100	

Procedures other than surgical operations performed in 2006 were as follows: gastroduodenal endoscopy (437 cases), and colorectal endoscopy (196 cases).

2. Phase I/IIa clinical trial of melanoma vaccine using gp100 derived peptides restricted to HLA-A*2402

Takuya Takayama, Marimo Sato, Akira Kanamoto, Akihiko Itoh, Kohji Yoshida, Eri Ichikawa, Shigenori Nagai, Hideaki Tahara

Epitope peptides derived from gp100, a melanoma associated antigen, are used for the cancer vaccine to treat the patients with advanced malignant melanoma. We have performed phase I clinical trial that six patients with stage IV melanoma were immunized with a vaccine consisting of HLA-A*2402-restricted epitope peptide derived from gp100 melanoma differential antigen (gp100-int4) emulsified with incomplete Freund's adjuvant (IFA). No adverse effects without grade I toxicity were observed in these patients. Patient 1 had a partial regression of multiple liver metastases and decrease of tumor maker after vaccination. In two patients (Patient 2 and 3), vitiligo was observed after vaccination.

From phase I data, phase I/IIa clinical trial of melanoma vaccine using gp100 derived peptides were performed. HLA-A*2402-restricted gp100 derived peptide (gp100-int4) was used with IFA and interleukin (IL-2) in order to augment for anti-tumor immunity. Our goals in this clinical trial are to examine these clinical efficacy, furthermore, safety and immune responses associated with the peptide vaccination. We have enrolled 14 melanoma patients during year 2003. So far, the protocols were well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted. Patient 9 had a stable disease of multiple lung metastases for 24 months since the first vaccination. In two patients (Patient 8 and 9), vitiligo was observed after vaccination. Immunological monitoring was performed to determine IFN-g production and analyze A24/gp100 tetramer staining using PBMC stimulated with gp100-int4 peptide. PBMC from Patient 9 was determined significant amount of IFN-g production and specific reactivity of IFN-g production to gp100-int4 peptide after vaccination. By A24/gp100 tetramer analysis, A24/gp100 tetramer and CD8 double positive subset was detected after vaccination in PBMC from Patient 9. Furthermore, melanoma-specific CTLs were established from CD8 and A24/gp100 tetramer double positive subset in this patient. Importantly, these CTLs were able to lyse 888mel (HLA-A24 positive and naturally expressing gp 100), but not to lyse 397mel (HLA-A24 negative and naturally expressing gp100) and HT29 (HLA-A24 positive and gp100 negative). It might be of significance that not only HLA-tetramer but also IFN-g production were necessary to evaluate immunological response induced by peptide-based vaccine. In patients 9, now she has been enrolled the modified protocol to vaccinate the peptide during 4 weeks.

3. Phase I clinical trial of epitope peptides based vaccine with novel tumor associate antigen, RNF43 and URLC10, found by genome-wide exploration using cDNA Microarray Profiling (GET-MAP) against colorectal cancer and esophageal cancer patients.

Takuya Takayama, Marimo Sato, Akira Kanamoto, Akihiko Itoh, Kohji Yoshida, Eri Ichikawa, Shigenori Nagai, Hideaki Tahara

We have performed genome-wide exploration using cDNA Microarray Profiling, and successfully identified a new tumor-associated antigen (TAA) which can induce potent cytotoxic T-cells (CTLs) specific to tumor cells. In our preceding study, we identified multiple new genes using gene expression profiling with a genome-wild cDNA microarray containing 23040 genes. Among them, we selected RNF43 (Ring Finger Protein 43) as a promising candidate for a TAA expressed by colon cancer. We examined whether the RNF43 protein contains antigenic epitope peptides restricted to HLA-A*0201 or HLA-A*2402. The CTL clones were successfully induced with stimulation using the peptides binding to HLA-A*0201 and HLA-A*2402, and these CTL clones showed the cytotoxic activity specific to not only the peptide-pulsed targets but also the tumor cells expressing RNF43 and respective HLAs. These results strongly suggest that RNF43 is a new TAA of colon cancer. Furthermore, we also selected URLC10 (up-regulated lung cancer 10) as a promising candidate for a TAA expressed by esophageal cancer. As the same above, we determined the URLC10 specific epitope peptide restricted by HLA-A* 2402. These results also suggest that our strategy might be a promising one to efficiently discover clinically useful TAAs.

From these basic results, phase I clinical trial has been performed to evaluate safety, immunological response and clinical response against advanced colorectal cancer and esophageal cancer patients. Epitope peptides derived from RNF 43 are used for the cancer vaccine to treat the patients with advanced colorectal cancer. Patients with HLA-A*0201 were treated with RNF 43 derived peptide. Patients with HLA-A*2402, were treated with RNF43 derived peptide. Epitope peptides derived from URLC10 are used for the cancer vaccine to treat the patients with advanced esophageal cancer. Patients with

HLA-A*2402, were treated with URLC10 derived peptide. All of the peptides were used with IFA in order to augment for anti-tumor immunity. In colorectal cancer, six patients with HLA-A*2402 have been enrolled, and two patients with HLA-A*0201 has been enrolled so far. We perform to analyze the immunological response using HLA tetramer and the specific INF-g production.

4. Phase I clinical trial of epitope peptides based vaccine targeting tumor vascular endothelial cell.

Takuya Takayama, Marimo Sato, Akira Kanamoto, Akihiko Itoh, Kohji Yoshida, Eri Ichikawa, Shigenori Nagai, Hideaki Tahara

Angiogenesis has been shown to be a critical mechanism for tumor progression. Multiple studies have suggested that tumor growth can be suppressed if tumor angiogenesis can be inhibited using various types of anti-angiogenic agents. Recent studies in mouse systems have shown tumor-angiogenesis can also be inhibited if cellular immune response could be induced against vascular endothelial growth factor receptor2 (VEGFR2), which has been shown to be one of the key factors in tumor angiogenesis. We first identified the epitope peptides of VEGFR2 and showed that stimulation using these peptides induces CTLs with potent cytotoxicity in the HLA class I restricted fashion against not only peptide-pulsed target cells but also endothelial cells endogenously expressing VEGFR2. In A2/Kb transgenic mice which express α1 and α2 domain of human HLA-A*0201, vaccination using these epitope peptides *in vivo* was associated with significant suppression of the tumor growth and prolongation of the animal survival without any adverse effects. In anti-angiogenesis assay, tumor-induced angiogenesis was significantly suppressed with vaccination using these epitope peptides. Furthermore, CTLs specific to the epitope peptides were successfully induced in cancer patients, and the specificities of the CTLs were confirmed using functional and HLA -tetramer analysis. These results *in vitro* and *in vivo* strongly suggest that the epitope peptides derived from VEGFR2 could be used as the agents for anti-angiogenic immune-therapy against cancer in clinical settings.

From these basic results, phase I clinical trial has been performed to evaluate safety, immunological response and clinical response against advanced gastric cancer and breast cancer patients and patients with gastrointestinal stromal cells. Epitope peptides derived from VEGFR2 are used for the cancer vaccine to treat the patients with advanced cancer patinets. Patients with HLA-A*0201 were treated with VEGFR2 derived peptide. Patients with HLA-A*2402, were treated with VEGFR2 derived peptide. All of the peptides were used with IFA in order to augment for CTL activity.

5. Phase I clinical trial of melanoma vaccine using gp100 derived peptides restricted to HLA-A*2402 with fully matured dendritic cells to induce Th1 type immune responses

Takuya Takayama, Marimo Sato, Akira Kanamoto, Akihiko Itoh, Koji Yoshida, Eri Ichikawa, Shigenori Nagai, Takuya Tsunoda, Hideaki Tahara

We have performed phase I/IIa clinical trial against stage IV malignant melanoma patients were immunized with a vaccine consisting of HLA-A*2402-restricted epitope peptide derived from gp100 melanoma differential antigen (gp 100-int4) with incomplete Freund's adjuvant (IFA) and interleukin (IL-2). We have enrolled 14 melanoma patients at the present time. The protocols were well tolerated and have not seen any severe adverse events. One of fourteen patients has showed a stable disease of multiple lung metastases for 24 months since the first vaccination. PBMC stimulated with gp100-int4 peptide in this patient revealed the specific reactivity of IFN-gamma production to gp100-int4 peptide and the existence of this peptide specific CD8 T cells after vaccinations by immunomonitoring analysis including A24/gp100 tetramer staining.

Dendritic cells (DC) administration appears to be very promising approach for immunotherapy against cancer. To further magnify the immune responses and obtain the clinical benefits, we have focused on the gp100-int4 peptide loaded DC vaccination. However, what we found from the results of phase I clinical trial using DC in our institute were the dysfunction of immature DC derived from cancer patients. Thus, we developed a new culture method to obtain the fully matured DC that is capable of T helper type 1 (Th1) polarization. From these backgrounds, we are going to utilize this fully matured DC to the phase I clinical trial of peptide vaccinations.

The goals in this clinical trial using our propagated DC are to examine the safety and immune responses, furthermore, the clinical efficacy associated with the peptide loaded fully matured DC vaccinations.

Publications

Nakamura Y, Futamura M, Kamino H, Yoshida K, Nakamura Y, Arakawa H. Identification of p53-46F as a super p53 with an enhanced ability to induce p53-dependent apoptosis. Cancer Sci. 97(7), 633-641, 2006.

Nishioka K, Doki Y, Miyata H, Tamura S, Yasuda T, Kimura Y, Kishi K, Yoshida K, Fujiwara Y, Yano M, Monden M. Bile acid promotes the proliferation of squamous cell carcinoma of the esophagus, independent of its inducing COX-2 expression. J Surg Res. 132(1), 130-135, 2006.

伊藤精彦，左葉切除(S２＋S３切除)，肝臓外科の要点と盲点　第２版，幕内雅敏監修・編集，文光堂，275−277，2006

金本　彰，肝離断法の比較，肝臓外科の要点と盲点　第２版，幕内雅敏監修・編集，文光堂，167，2006

金本　彰，高山忠利，肝癌の免疫治療，臨床消化器内科，1037−1042，2006

伊藤精彦，腹腔鏡下胆嚢摘出術，実写とイラストで学ぶ　外科手術手技図譜，幕内雅敏監修，永井書店，192−200，2006

伊藤精彦，胆道再建を要する肝切除術，実写とイラストで学ぶ　外科手術手技図譜，幕内雅敏監修，永井書店，201−211，2006

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Clinical radiology includes diagnostic radiology, nuclear medicine, and radiation oncology. Diagnostic radiology plays a crucial role in evaluating various neoplastic and infectious diseases in clinical practice. In nuclear medicine, we develop analytic methods to estimate in vivo physiology, as well as studying the tracer kinetics and physical characteristics of detectors. Total body irradiation prior to bone marrow transplantation is a major role of our division of radiation oncology. Moreover, imaging technologies can aid in evaluating disease process and therapeutic efficacy in small animal models.

Monitoring of disease progression by bioluminescence imaging and magnetic resonance imaging in an animal model of hematological malignancy

Yusuke Inoue, Kiyoko Izawa[1], Yoshitaka Masutani[2], and Arinobu Tojo[1]: [1]Division of Molecular Therapy, Advanced Clinical Research Center, [2]Department of Radiology, Graduate School of Medicine, University of Tokyo.

Imaging technologies are increasingly used for animal experiments of tumor models. We evaluated disease progression in a mouse model of a hematological malignancy using a multimodality approach that included bioluminescence imaging (BLI) and magnetic resonance imaging (MRI). Mice were inoculated intravenously with Ba/F3 cells transduced with firefly luciferase and p190 BCR-ABL genes. Disease progression in a given mouse was observed longitudinally by in vivo BLI and MRI. Imaging studies, including in vivo BLI and MRI of living mice and ex vivo BLI of excised organs, were also performed at various time points. Longitudinal studies allowed the assessment of disease progression for each mouse. Light emission detected by in vivo BLI was extensive, and the whole-body signal increased with time after inoculation. Spontaneous death for all inoculated mice occurred at similar signal levels. MRI demonstrated progressive hepatosplenomegaly and growth of hepatic nodules. Ex vivo BLI demonstrated proliferation of the implanted cells in various organs, and the signal for each organ increased with time and as the whole-body signal, observed by in vivo BLI, increased. MRI measurements of liver and spleen volumes closely correlated with the wet weights of these organs, and volume increases significantly correlated with the BLI organ signal. These results indicate that BLI and MRI allow repeated assessment of disease progression in a mouse model of a hematological malignancy and provide quantitative markers of disease severity. BLI and MRI measurements reveal different details of disease progression and may play complementary roles in comprehensive assessment. As a next step, we are studying BLI/MRI fusion imaging, which requires appropriate fixation devices, correction for image distortion, and software for registration and display.

Imaging of living mice using a 1-T compact magnetic resonance imaging system

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We investigated appropriate techniques to image living mice with a 1-T compact magnetic resonance (MR) imaging system installed in the animal facility of the Institute of Medical Science. Images of the entire trunks of living mice were obtained on the system using a T1-weighted 3D FLASH sequence, and image quality was evaluated in relation to imaging techniques. Restraint of respiratory motion improved the image quality. Decreasing the slice thickness reduced artificial inhomogeneity in signal intensity. Substantial effects of TR and FA on image quality were also demonstrated. With the determined techniques, images covering the entire trunk with a voxel size of $0.26 \times 0.26 \times 0.52$ mm were acquired in an acquisition time of 5 min 28 sec and a total experiment time of shorter than 20 min, and various organs and subcutaneous tumors were clearly visualized. The compact MR imaging system provides images of living mice with acceptable quality in a reasonable time. Considering its convenience, it appears to be suitable for use in routine mouse experiments. We are now attempting to shorten the TE for further improvement of image quality and to simplify the operation for enhancement of convenience.

Technical considerations of bioluminescence imaging

Yusuke Inoue, Kiyoko Izawa[1] and Arinobu Tojo[1]

In vivo bioluminescence imaging (BLI) allows high-throughput, sensitive detection of luciferase expression in living mice. However, the identification of organs bearing luciferase activity is often difficult using in vivo BLI because of its projectional nature and low spatial resolution, and ex vivo imaging of excised organs plays a complementary role. We investigated the importance of exposure to the atmosphere in ex vivo BLI. Mice were inoculated with Ba/F3 cells transduced with firefly luciferase and p190 BCR-ABL. They were killed following in vivo BLI, and whole-body imaging was done after death and then after intraperitoneal air injection. In addition, the right knee was exposed and imaged before and after cutting the adjacent bones. Extensive light signals were seen on in vivo imaging. The luminescence disappeared after the animal was killed, and air injection restored the light emission from the abdomen only, suggesting a critical role of atmospheric oxygen in luminescence after death. Although no substantial light signal at the right knee was seen before bone cutting, light emission was evident after cutting. These results indicate that light emission requires exposure to the atmosphere in ex vivo BLI and that bone destruction is required to demonstrate luciferase activity in the bone marrow after death. Now, we are characterizing background signals in in vivo BLI. The background signals are very low but sometimes confusing in detecting a low level of luciferase activity. We aim at enhancing the ability of in vivo BLI to define weak luciferase expression.

Application of contrast agents for magnetic resonance imaging of the mouse

Yusuke Inoue and Kohki Yoshikawa[3]

Contrast agents for magnetic resonance (MR) imaging alter signal intensity according to its concentration, and its use can improve image quality and increase information about morphology and function. We showed better delineation of the kidney in living mice when Gd-DTPA, a nonspecific extracellular agent, was administered intravenously or subcutaneously. We investigated the characteristics and utility of gadobenate dimeglumine (Gd-BOPTA), a hepatocyte-specific agent, for MR imaging of the mouse liver. Mice were imaged under isoflurane anesthesia using a T1-weighted, three-dimensional fast low-angle shot (3D FLASH) sequence before and after intravenous or subcutaneous injection of Gd-BOPTA, and the time course of the contrast effect was examined. The appropriate dose for subcutaneous injection was determined visually, and the inter- and intra-observer reproducibilities in liver volumetry were evaluated with and without contrast injection. When mice were imaged sequentially before and after Gd-BOPTA injection and isoflurane anesthesia was maintained throughout the experiment, a long-lasting contrast effect was noted in the liver. Subcutaneous injection caused delayed, but favorable, enhancement. Washout from the liver was definitely accelerated in conscious mice in comparison with anesthetized mice. Visual evaluation indicated that a dose of 0.1 mmol/kg was appropriate for clear delineation of the entire liver margin, and the application of Gd-BOPTA significantly improved the inter- and intra-observer reproducibilities of liver volumetry. These results indicate that the intravenous or subcutane-

ous injection of Gd-BOPTA has a favorable contrast effect for the mouse liver, resulting in clear visualization of the liver border and improved reproducibility of liver volumetry. The possible influence of anesthesia on the pharmacokinetics of a contrast agent should be considered in determining the optimal scan timing. Next, we will compare gadoxetate disodium (Gd-EOB-DTPA) with Gd-BOPTA in mouse liver imaging.

In vivo fluorescence imaging of the reticuloendothelial system using quantum dots: combination with bioluminescence tumor monitoring

Yusuke Inoue, Kiyoko Izawa[1], Kohki Yoshikawa[3], and Arinobu Tojo[1]

The reticuloendothelial system of a living mouse can be visualized noninvasively by in vivo fluorescence imaging (FLI) using quantum dots (QDs). We are investigating the characteristics of the imaging technique. The liver, spleen, bone marrow, and superficial lymphnodes was delineated one hour after the intravenous injection of QDs. Washout of QDs were slow, and long-term, repetitive assessments were feasible without additional administration. Ex vivo imaging proved selective accumulation of QDs in the reticuloendothelial system, which facilitated detection of deep lymphnodes. Next, we assessed the feasibility of combined bioluminescence and fluorescence imaging (BLI/FLI). Mice were inoculated with Ba/F3 transduced with firefly luciferase and p190 BCR-ABL. One week after intravenous inoculation with Ba/F3 cells transduced with firefly luciferase and p190 BCR-ABL, they were injected with QDs and imaged with a CCD camera system. The fusion images of BLI/FLI could be easily obtained and aided the identification of organs bearing the luciferase-expressing cells. Injection of QDs did not affect BLI signals soon after injection or long-term proliferation of inoculated cells. In vivo FLI of the reticuloendothelial system would aid in assessing morphology, searching for lymphnodes, and defining the sources of BLI signals.

Quantitative MR image study in neuropsychiatric disorders: voxel-based analysis of diffusion tensor data sets.

Haruyasu Yamada, Osamu Abe[1], Shigeki Aoki[1], Kiyoto Kasai[5], Hidenori Yamasue[5]: [5]Department of Psychiatry, Graduate School of Medicine, University of Tokyo.

Magnetic resonance (MR) diffusion tensor imaging (DTI) has been reported to be useful in evaluation of the normal appearing brain in neuropsychiatric disorders. DTI is a unique and relatively new technique to visualize and evaluate cerebral white matter. The orientation of white matter tracts can be analyzed and tracked by the methods named as diffusion tensor tractography (DTT) or fiber tracking. Quantitative diffusion indices such as apparent diffusion coefficient (ADC) and fractional anisotropy (FA) have been used for the evaluation of normal appearing white matter in various diseases. Disruptions in connectivity may explain some of the symptoms in neuropsychiatric disorders such as schizophrenia. The purpose of our study was to investigate diffusion anisotropy in neuropsychiatric patients' brain by voxel-based analysis of DTI and voxel-based morphometry (VBM), using statistical parametric mapping (SPM). We studied patients with schizophrenia diagnosed by DSM-IV criteria and age-matched controls. The data were obtained with a 1.5-T MRI system. We used single-shot spin-echo echo-planar sequences and measured diffusion properties along 6 non-collinear directions. The ADC and FA maps were generated on a voxel-by-voxel basis and converted into Analyze format, and also normalized into the Montreal Neurological Institute (MNI) space. The normalized ADC and FA maps were analyzed using SPM employing the framework of the General Linear Model. The significant FA decrease in the patient group was found in the parahippocampal white matter (WM) of the left limbic lobe, the parahippocampal WM of the right limbic lobe, middle frontal WM of the right frontal lobe, and so on (corrected $p < 0.05$). Limbic system such as the hippocampus is considered to have relation with schizophrenia. The parahippocampal gyrus, which is known to be disturbed in schizophrenia with other medial temporal lobe regions, plays a role in the higher cognitive processes such as learning and memory. Our result may reflect reduced diffusion anisotropy of the white matter pathway of the limbic system as decreased FA indices. Voxel-based analysis of the diffusion tensor data set allows a voxel-wise comparison encompassing the whole brain without operational bias or hypothesis. This study suggests that the voxel-based diffusion tensor analysis may be robust enough to perceive changes in diffusion anisotropy in patients with schizophrenia.

Publications

Inoue, Y., Tojo, A., Sekine, R., Soda, Y., Kobayashi, S., Nomura, A., Izawa, K., Kitamura, T., Okubo, T., and Ohtomo, K. In vitro validation of bioluminescent monitoring of disease progression and therapeutic response in leukaemia model animals. *Eur. J. Nucl. Med. Mol. Imaging*. 33: 557-565, 2006.

Inoue, Y., Izawa, K., Tojo, A., Sekine, R., Okubo, T., and Ohtomo, K. Light emission requires exposure to the atmosphere in ex vivo bioluminescence imaging. *Mol. Imaging*. 5: 53-56, 2006.

Inoue, Y., Nomura, Y., Haishi, T., Yoshikawa, K., Seki, T., Tsukiyama-Kohara, K., Kai, C., Okubo, T., and Ohtomo, K. Imaging of Living Mice Using a 1-T Compact Magnetic Resonance Imaging System. *J. Magn. Reson. Imaging*. 24: 901-907, 2006.

Tsukamoto, T., Kotani, Y., Ohgami, Y., Omura, K., Inoue, Y., and Aihara, Y. Activation of insular cortex and subcortical regions related to feedback stimuli in a time estimation task: an fMRI study. *Neurosci. Lett*. 399: 39-44, 2006.

Yokoyama, I., Inoue, Y., Moritan, T., Ohtomo, K., and Nagai, R. Myocardial glucose utilization in type II diabetes mellitus patients treated with sulfonylurea drugs. *Eur. J. Nucl. Med. Mol. Imaging*. 33: 703-708, 2006.

Maeda T, Fujii T, Matsumura T, Endo T, Odawara T, Itoh D, Inoue Y, Okubo T, Iwamoto A, Nakamura TAIDS-related cerebral toxoplasmosis with hyperintense foci on T1-weighted MR images: A case report. *J. Infect*. 53: e167-70, 2006.

Kiryu, S., Okubo, T., Takeuchi, K., Inoue, Y., Endou, T., Odawara T., Nakamura T., Aoki, S., and Ohtomo, K.Magnetic resonance imaging and diffusion tensor analysis of lymphomatoid granulomatosis of the brain. *Acta. Radiol*. 47: 509-513, 2006.

Ohgami, Y., Kotani, Y., Tsukamoto, T., Omura, K., Inoue, Y., Aihara, Y., and Nakayama, M. Effects of monetary reward and punishment on stimulus-preceding negativity. *Psychophysiology*. 43: 227-236, 2006.

Nomura, Y., Inoue, Y., Yokoyama, I., Nakaoka, T., Itoh, D., Okubo, T., and Ohtomo, K. Evaluation of left ventricular function from cardiac magnetic resonance imaging using Fourier fitting. *Magn. Reson. Imaging*. 24: 1333-1339, 2006.

Abe, O., Yamasue, H., Kasai, K., Yamada, H., Aoki, S., Iwanami, A., Ohtani, T., Masutani, Y., Kato, N., and Ohtomo, K. Voxel-based diffusion tensor analysis reveals aberrant anterior cingulum integrity in posttraumatic stress disorder due to terrorism. *Psychiatry. Res*. 146: 231-242, 2006.

Kudo, N., Kasai, K., Itoh, K., Koshida, I., Yumoto, M., Kato, M., Kamio, S., Araki, T., Nakagome, K., Fukuda, M., Yamasue, H., Yamada, H., Abe, O., Kato, N., and Iwanami, A. Comparison between mismatch negativity amplitude and magnetic mismatch field strength in normal adults. Biol. Psychol. 71: 54-62, 2006.

Inoue, Y., Izawa, K., Tojo, A., Nomura, Y., Sekine, R., Oyaizu, N., and Ohtomo, K. Monitoring of disease progression by bioluminescence imaging and magnetic resonance imaging in an animal model of hematological malignancy. *Exp. Hematol*. In press.

Inoue, Y., Yoshikawa, K., Nomura, Y., Izawa, K., Shimada, M., Tojo, A., and Ohtomo, K. Gadobenate dimeglumine as a contrast agent for magnetic resonance imaging of the mouse liver. *NMR. Biomed*. In press.

Kuwabara, K, Nishishita, T., Morishita, M., Oyaizu, N., Yamashita, S., Kanematsu, T., Obara, T., Mimura, Y., Inoue, Y., Kaminishi, M., Kaga, K., Amino, N., Kitaoka, M., Ito, K., Miyauchi, A., Noguchi, S., Uchimaru, K., Akagawa, E., Watanabe, N., Takahashi, TA, Sato, K., Inazawa, T., Nakaoka, T., Yamashita, N. Results of a phase I clinical study using dendritic cell vaccinations for thyroid cancer. *Thyroid*. In press.

Abe, O., Yamasue, H., Aoki, S., Suga, M., Yamada, H., Kasai, K., Masutani, Y., Kato, N., Kato, N., and Ohtomo, K. Aging in the CNS: comparison of gray/white matter volume and diffusion tensor data. *Neurobiol. Aging*. In press.

井上優介，大友　邦．分子イメージング：インビボ生体発光イメージング．BIO Clinica. 21：55–60，2006.

Research Hospital

Department of Laboratory Medicine
検査部

Associate Professor Naoki Oyaizu, M.D, Ph.D.

助教授(部長) 医学博士 小柳津 直 樹

Our department consists of seven subdivisions of clinical physiology, hematology, biochemistry, serology, bacteriology, molecular diagnosis and pathology, and engages in laboratory analysis and diagnosis of clinical materials submitted from the hospital. This year, we newly developed a new subdivision; a division of flow cytometrical analysis. Along with ongoing practices of translational research projects in the research hospital, our department also functions as an integrated diagnosis & monitoring laboratory to evaluate the safety and effectiveness of experimental therapeutic approaches.

General Scheme

Our basic research strategy is to characterize molecular mechanisms underlying pathology, develop a way to measure this in the clinical materials, and to evaluate the effectiveness of molecular-targeted therapy on its endopoints. In particular , combining and integrating molecular-/biochemical-based laboratory assays on the solid background of morphological/ pathological examinations, hence enables to evaluate the effectiveness of experimental clinical trials. We believe that such an approach is indispensable to direct experimental therapeutic approaches in a correct way as well as to promote translational research . Developing molecular-based assays in clinical materials requires expertise in pathology and molecular biology; we are thus focusing our specialty on achieving this goal.

1. Pathological evaluation of cancer immunotherapy

We have initiated to analyze the surgical specimen obtained from the patients under cancer immuno-therapy conducted in the research hospital. By applying sophisticated immunohistochemical techniques, we are now intensively analyzing materials from cases including GM-CSF-based gene therapy for renal cell carcinoma and dendritic cell-based or peptide-pulsed anti-melanoma immuno-therapy. Our goal is to evaluate the effectiveness and to elucidate the mechanisms of anti-tumor immune response elicited by the therapy *in situ*.

2. Elucidation of immunopathological mechanisms of autoimmune-based hematological disorders

We found the presence of characteristic pathological findings in bone marrow specimen from some patients with MDS-RA, aplastic anemia, or pure red cell aplasia, which implicate that common immunopathological mechanism may be operative in these hematological abnormalities; that is destruction of erythroid precursors by immune-based mechanisms in the bone marrow. In collaboration with the Department of Hematology, we are going to elucidate molecular mechanisms on the ground of pathology, thereby establish new disease entity and develop new therapeutic interventions.

3. Analysis of the chimeric gene expression of hematological disorder

We have initiated to analyze bcr-abl gene expression in specimen from patients with CML and Ph1+ve ALL by real-time PCR and nested RT-PCR techniques. In addition, we sequenced the amplified products to provide information for the molecular resistance to STI571 treatment. We are now expanding target molecules, which includes PMA/RARa and TEL/AML1.

4. Developing quick & inclusive diagnosis system for infections disease

Since the introduction of new therapeutic maneuver, host-pathogen interactions altered drastically and came into aspects. This results in altered recognition and molecular interaction of infected cells with immune cells, which leads to atypical pathological as well as clinical manifestations. To distinguish infectious disease and immunological disorder is a critical issue, however as a result of modified manifestations, it is difficult to achieve this in some occasions. To circumvent this, we are pursuing to establish a quick and inclusive diagnosis system of infectious disease.

5. Introducing flow cytometrical (FCM) analysis for immunological disorder

FCM is a powerful tool for a diagnosis and a monitoring disease status for immunological disorders including HIV infection. We had spent several years to build infrastructure and cultivate specialized operators before finally starting FCM analysis this year. Currently, we provide lymphocyte-subset analysis on a routine basis. We are planning to expand FCM analysis for hematological disorders in the near future.

Publications

1. Fukuno K, Tomonari A, Tsukada N, Takahashi S, Ooi J, Konuma T, Uchiyama M, Fujii T, Endo T, Iwamoto A, Oyaizu N, Nakata K, Moriwaki H, Tojo A, Asano S. Successful cord blood transplantation for myelodysplastic syndrome resulting in resolution of pulmonary alveolar proteinosis. Bone Marrow Transplant. 38: 581-582, 2006
2. Hishima T, Oyaizu N, Fujii T, Tachikawa N, Ajisawa A, Negishi M, Nakamura T, Iwamoto A, Hayashi Y, Matsubara D, Sasao Y, Kimura S, Kikuchi Y, Teruya K, Yasuoka A, Oka S, Saito K, Mori S, Funata N, Sata T, Katano H. Decrease in Epstein-Barr virus-positive AIDS-related lymphoma in the era of highly active antiretroviral therapy. Microbes Infect. 8: 1301-7. 2006

Research Hospital

Department of Applied Genomics
ゲノム診療部

Associate Professor Noriharu Sato, M.D., D.M.Sc.
Clinical Associate Naoyuki Takahashi, M.D., D.M.Sc.

助教授 医学博士 佐藤典治
助 手 医学博士 高橋直之

Our department was established to support the translational researches of our hospital. We are studying whether expression profiles of CD4＋, CD8＋, CD 14＋ and CD56＋ cells, respectively, in cord-blood transplanted patients could be useful to predict GVHD severity. We are also participating in the clinical study to predict the sensitivity either to Imatinib or Gefitinib in patients suffering from CML or adenocarcinoma of the lung, respectively. We have opened the out-patient clinic for genetic counseling in collaboration with the divisions of pediatrics, internal medicine, nursing and the laboratory of molecular medicine.

1. Analysis of gene expression profile of inflammatory cells in GVHD

Naoyuki Takahashi and Noriharu Sato

In hematopoietic stem cell transplantation, we sometimes observe severe GVHD (graft-versus-host disease) in HLA-matched transplants. In order to find the factors affecting the severity of GVHD, we analyzed expression profile of about 600 selected transcripts in CD4＋, CD8＋, CD 14＋, and CD56＋ peripheral blood cells at the peak of GVHD as well as in controlled phase by using custom-made oligonucleotide array. The analysis of the gene expression profile for 4 cases of GVHD showed increased expression of several immunoregulatory factors in addition to many proinflammatory factors at the peak of the disease. Among those factors were CEBPD and SOCS3 in CD4＋ T cell subset, and CEBPD and CTLA4 in CD8＋ T cell subset. In addition, the expression of transcription factors including several members of Kruppel like factor family was changed. There is possibility that some of these factors become drug targets.

2. Analysis of the role of Kruppel like family transcription factors in the inflammatory response

Naoyuki Takahashi and Noriharu Sato

The control of inflammatory cell activation is important in the treatment for chronic inflammation, including autoimmune disease and atheroscrerosis. The molecular mediators regulating the balance of proinflammatory and anti-inflammatory factors remain incompletely understood. Recently, it has been reported that Kruppel-like transcription factors play key roles in inflammatory response. We identified several Kruppel-like factors including KLF4 as being markedly induced in monocytic cell lines (U937, THP-1, and HL-60) in response to proinflammatory cytokines such as interferon-γ or tumor necrosis factor-α, and decreased in response to all-trans retinoic acid. These findings suggested an important role of Kruppel-like factors as regulators of macrophage activation.

3. Genetic counseling and related activities

Naoyuki Takahashi, Noriharu Sato, Reiko Sada[1], Momoyo Ohki[2], Kohichiro Tsuji[3], Koichiro Yuji[4], Kisako Sato[5], Masae Ono[6], Shiro Ikegawa[7], Toshihiro Tanaka[7], Mayumi Tamari[7], Tsuyoshi Sakamoto[8], Yoichi Furukawa[9] and Yusuke Nakamura[10]: [1]Division of Bioengineering, [2]Bunkyo University, [3]Department of Pediatric Hematology-Oncology, [4]Department of Hematology/Oncology, [5]Department of Nursing, [6]Department of Pediatrics, Tokyo Teishin Hospital, [7]Riken SNP Research Center, [8]Department of Neurology, Jikei Medical University, [9]Promotion of Genome-Based Medicine Project, [10]Laboratory of Molecular Medicine.

At our genetic counseling clinic, we have seen clients who are suffering or who have family members suffering from genetic diseases. Genetic diseases and related problems seen at our clinic this year include Werner's syndrome, familial amyotrophic lateral sclerosis, consanguineous marriage, familial adenomatous polyposis, familial tumor, hepatic epithelioid hemangioendotheliom, a client of suspected balanced chromosomal translocation and so on.

As an initial step to perform individualized medicine, Human Genome Center has started microarray analyses of leukemic cells and lung cancer to predict drug sensitivity. We have also participated in this project concerning eligibility of the patients, informed consents, and notification of the test results.

Publications

Matuzaki, E., Takahashi-Yanaga, F., Miwa, Y., Hirata, M., Watanabe, Y., Sato, N., Morimoto, S., Hirofuji, T., Maeda, K. and Sasaguri, T. Differentiation-inducing factor-1 alters canonical Wnt signaling and suppresses alkaline phosphatase expression in osteoblast-like cell lines. J. Bone. Miner. Res. 21: 1307-1316, 2006.

Research Hospital

Division of Clinical Trial Safety Management
医療安全管理部

COE Lecturer Fumitaka Nagamura, M.D., D.M.Sc.
COE Clinical Associate Seiichiro Kobayashi, M.D., D.M.Sc.

特任講師 医学博士 長 村 文 孝
特任助手 医学博士 小 林 誠一郎

Division of Clinical Trial Safety Management was established in 2001. Our missions are divided into two areas. One is the risk management of the Research Hospital (RH), and the other is the support/monitoring of clinical studies conducting at RH. The latter work is the collaboration between the staffs of DCTSM and Translational Research Coordinators (TRC), which are organized by co-medical staffs, including research nurse, pharmacist, dietician, psychologist and clinical laboratory technologist. The aim of DCTSM is to carry out the safe and ethically-protected clinical trials in the Research Hospital in addition to the data management. In order to accomplish them we are doing the following activities.

Risk management of Research Hospital

Fumitaka Nagamura, Seiichirou Kobayashi, Reiko Mori

Prevention and management of medical accidents are the critical problems at RH. The demand to avoid and to reduce the accidents has been increasing year by year. The main purpose of the RH is to promote the translational researches, and the reliance to RH is indispensable to promote them. Staffs of DCTSM engage in the risk management at the Research Hospital. Medical accidents and incidents are reported to DCTSM by written forms (by direct communication in case of severe accident). When the urgent action is required, the meeting (Iryoujiko Kinkyu Taisaku Kaigi) is immediately held to discuss the tentative provisions, to protect the involved patient, and to promote the clinical circumstances. This meeting was held for three times in this year, however no severe medical accident to be reported and/or to be announced happened. Medical accidents and the responses of DCTM are reported in the Council of Risk Management in the RH, which is held monthly.

Educational seminars on risk management are required by the regulation to avoid the medical accidents. DCTSM took place two seminars and one lecture meeting this year. Through these educations, consciousness for risk management will be tightened.

Advise/Review of clinical study protocols before the discussion at the Institutional Review Board (IRB: Chiken-Sinsa-Iinkai)

Fumitaka Nagamura.

One of the roles of DCTSM is to keep the quality of protocols as well as studies themselves. To perform this task, we discuss and advise on the protocols with principal investigators, and made it a rule to submit a protocol and written consent documents to DCTSM before submitting to the Institutional Review Board.

From January 2006 to December 2006, we received three entirely protocols and numerous questions from RH. All the protocols were either Phase I or Phase I/IIa studies belonging to Translational Rsearch. Pre-review of these proto-

cols were finished within two to three weeks from the receipt. The format of pre-review is based on the style of applied in the U.S. Food and Drug Administration. Our opinions are summarized into three sections: safety issue (most concern); major problem; and minor problems/suggestions. These opinions are not obligations which posses enforcement, but those to improve the safety and quality of clinical studies. Final decision should be made at the Institutional Review Boards. Furthermore, we performed these activities for other institutes. We received many requirements from other institutes.

To assist the planning of clinical studies and writing protocols, we have disclosed "Guideline". Recently many regulations and guidelines were announced. To clear these and to match the Institute's organization, we have been engaging in the revisions of the rules of out institute and in reconstitution of the organization through Working Group.

Education on clinical studies for workers at Research Hospital

Fumitaka Nagamura.

The major missions of Translational Research Coordinator are to keep patients' right, to conduct translational research more ethically, and to perform translational research scientifically. The role of TRC is not the same as that of Clinical Research Coordinator (CRC) in terms of the aggressive intervention to keep studies ethically conducted. The problem of education for research coordinators including CRC is the new but the critical problem in Japan. To educate workers of the hospital, as well as coordinators, the division took place the educational course on clinical studies. This meeting consists of 10 sections, and participants were required to resister for preparing the course materials. This year, ten workers applied for this course. The content of this course consists of the basic knowledge of clinical studies, regulations, laws, coordinating skills, environment of clinical studies, and the system of the Research Hospital in terms of the conduction of Translational Research.

Comparison of the basis for approval and pivotal studies on hematological malignancies between the US and Japan

Fumitaka Nagamura

Clinical trials are designed based on certain hypotheses and to meet the requirements for approval from regulatory agencies. Drugs approved for hematological malignancies from January 1985 to December 2005 in both the US and Japan were selected. Of these candidates, only indications common to both countries were considered. Supportive care drugs, immunomodulators, biochemical modulators, and off-label use were excluded. Package inserts, reviews and analyses by regulatory agencies, and publications on clinical trials were examined. Results: Ten drugs were met the criteria. "Line or type of therapy" was specified for all drugs in the US and three in Japan. Limitations on age were indicated in the package inserts of 5 drugs in the US and 1 in Japan. In Japan, no description on phase was seen in 2 drugs, and 1 comparative study was applied. The number of efficacy parameters examined in each pivotal study (range, mean, and median) were 1-11, 4.7, and 3 in the US and 1-5, 2.3, and 2 in Japan, respectively. Survival was used as an efficacy parameter in 7 drugs in the US, but none in Japan (PFS: 1 drug). Differences in efficacy parameters between the two countries have decreased, and inappropriate uses of efficacy parameters have disappeared recently in Japan. Conclusion: The high ratio of RCT and the large number of patients enrolled into clinical trials are characteristics of the US. The approved indications in Japan are broad-based, however, the description, number of patients, and the use of efficacy parameters were inferior. The basic principle for approval of regulatory agencies in Japan had been response rate, however, the policy has become changing. So, the criticism of Japanese clinical trials, such as poor design, insufficient information, and less utilization for approval by other countries may be resolved. The high ratio of RCT and the large number of patients enrolled into clinical trials are characteristics of the US. The approved indication in Japan are broad-based, however, the description, number of patients, and the use of efficacy parameters were inferior. Although the improvement has begun, the criticism of Japanese clinical trials, such as poor design, insufficient information, and less utilization by other countries may be a result of the standards of approval by the regulatory agencies in Japan.

Publications

1. 長村文孝　東京大学医科学研究所附属病院臨床試験管理推進室―探索型臨床研究(トランスレーショナル・リサーチ)推進のために―癌と化学療法33(5)697-699，2006
2. 長村文孝　トランスレーショナルリサーチ　臨床試験の進め方　51-62，2006
3. 長村文孝　トランスレーショナルリサーチにおける医学と心理学の連携　心理学ワールド(印刷中)
4. 長村文孝　IND申請資料作成のポイント　新薬承認申請／早期申請を成功させるメディカルライティングのノウハウ　技術情報協会(印刷中)
5. Fumitaka Nagamura, Tokiko Nagamura-Inoue, Arinobu Tojo. Comparison of the Basis for Approval and Pivotal Studies on Hematological Malignancies between the US and Japan. Proc Am Soc Hematol 108 (11), 2006
6. Inoue Y, Tojo A, Sekine R, Soda Y, Kobayashi S, Nomura A, Izawa K, Kitamura T, Ohkubo K. In vitro validation of bioluminescent monitoring of disease progression and therapeutic response in leukaemia model animals. Eur J Nucl Med Mol Imaging. 2006; 33: 557-565

Research Hospital

Department of Cell Processing and Transfusion
セルプロセッシング・輸血部

Professor Arinobu Tojo, M.D., D.M.Sc.
Lecturer Tokiko Nagamura-Inoue, M.D., D.M.Sc.

教 授 医学博士 東 條 有 伸
講 師 医学博士 長村(井上)登紀子

Our department is divided into two functional parts. One is the transfusion medicine including transfusion-related-examinations and another is cell processing part. In cell processing part, we have the supportive function for cell therapy including hematopoietic stem cell Transplantation (HSCT), immunotherapy (Dendritic cell therapy) and the management for Room for Clinical Cellular Technology (RCCT) (4). Our on-going researches are (1) Analysis of immunological characteristics of post-stem cell transplantation patients and hematopoietic malignancies, (2) Expansion of T, NK and NKT cells for GVL/T effect and expansion of regulatory T cells for prevention of GVHD after HSCT and (3) Mesehchymal stem cells derived from umbilical cord.

(1) Analysis of immunological characteristics of post-stem cell transplantation patients and hematopoietic malignancies

Shin Nakayama[1], Tokiko Nagamura-Inoue, Satoshi Takahashi[1], Arinobu Tojo: [1]Department of Hematology/Oncology, Advanced Clinical Research Centre, [2]Division of Cell Processing, The Institute of Medical Science, The University of Tokyo

The study of immune reconstitution following unrelated cord blood transplantation (UCBT) in adult patients is of great concern because of immaturity of cord blood immunological cells. This year we studied the effect of the regulatory T cell and $CD26^{high}$ positive T cells in patients with hematopoietic malignancies, especially chronic graft-versus host disease (cGVHD) and the patients with chronic myelogenous leukemia patients CML under the different treatment. In cGVHD patients we found increase of monocyte and CD8+ T cell % and decrease of CD8+CD 26+T cell compared to normal control. In CML patients, we discovered the several interesting differences between immune cells of the patients treated with interferon alpha and imatinib (STI).

(2) Expansion of T, NK and NKT cells for GVL/T effect and expansion of regulatory T cells for prevention of GVHD after HSCT.

Tokiko Nagamura-Inoue, Kazuo Ogami, Shin Nakayama[1], Arinobu Tojo: [1]Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo

Cord blood transplantation (CBT) is rapidly increasing in number. However, the graft failure, viral infections such as CMV and the relapse after CBT remain to be resolved. In CBT, we can not obtain the donor-derived lymphocyte for donor lymphocyte infusion (DLI), because the donor is the unrelated baby. Instead of DLI, we are now creating new system of T and NK cell expansion derived from donor-origin blood using coated anti-CD3 and CD28 antibodies with the cytokine for the prevention of infection and relapse (GVL/T). This expansion might

also be useful for the acceleration of engraftment. On the other hand, GVL/T effects may also induce GVHD. For the prevention and/or control of GVHD, we are now exploring the CD 25+CD4+T (rT) expansion using the patient peripheral blood after CBT and succeeded the FOXP3+rT cells in our system.

(3) Mesenchymal Stem cells derived from umbilical cord

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Mesenchymal stem cells (MSCs) are multipotent cells defined by multilineage potential, and immunosuppressive ability, thus holding promise for tissue engineering, gene therapy, and immunotherapy. Recently we found the Wharton's jelly and vessels of the umbilical cord contains MSCs, expressing MSC markers (SH2, SH3). We are now studying the ability of differentiation and the supportive ability as immunosuppressant. The final purposes of the study are to establish the source of regenerative medicine and engraftment support of cord blood stem cells, gene delivery system and banking together with cord blood.

(4) Current project in Room for Clinical Cellular Technology (RCCT)

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Cell processing room for cell therapy is one of the critical factors to implement the projects including stem cell transplantation, immunotherapy and gene therapy. It is also mandatory to separate and manipulate cells under quality-controlled sterilized circumstances that could meet with GMP approval. Room for Clinical Cellular Technology (RCCT) consisted of clinical clean room and P3 biohazard room has been established since 1997 and utilized for cell processing. Cell processing of cord blood cells for cord blood cell banking (Tokyo Cord Blood Bank) and transplantation, regenerative medicine for the osteoblast-like cells derived from bone marrow mesenchymal cell are in execution.

Publications

1. Hikaru Okada, Tokiko Nagamura-Inoue, Yuka Mori, Tsuneo A. Takahashi. Expansion of Valpha24Vbeta11 NKT cells from cord blood mononuclear cells using Interleukin-15, IL-7 and Flt3-L depends on monocytes. European Journal of Immunology, 36, 236-244, 2006
2. Tokiko Nagamura-Inoue, Shunro Kai, Minoko Takahashi, Koji Kato, Kei-Ichi Isoyama, Masatoshi Jinzen, Hiroshi Azuma, Masao Nakabayashi, and Shunichi Kato. Japan Cord Blood Bank Network. Unrelated Cord blood transplants for the patients with CML, a Japan Cord Blood Bank Network Analysis, Blood, 108, p888a, 2006
3. Tokiko Nagamura-Inoue, Hideki Kodo, Tsuneo A. Takahashi, Hideo Mugishima, Arinobu Tojo, Shigetaka Asano, Tokyo Cord Blood Bank. Four cases of donor cell-derived acute myeloid leukemia following unrelated cord blood transplantation for adult patients; Tokyo cord blood bank experiences, Blood, 108, 893a. 2006

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Our clinical practice and clinical as well as experimental studies have been focused on (1) anesthetic management in patients undergoing major surgery, (2) management of intraoperative and postoperative pain, and (3) management of chronic intractable pain. We have published several works on these subjects last year.

1. Safety in anesthetic management, especially focusing on cerebral circulation during anesthesia and surgery

The Bispectral Index (BIS) is a recently developed derivative of processed electroencephalogram that has been proven to closely correlate with the level of consciousness during natural sleep and general anesthesia. It has been widely used in the area of anesthesia to evaluate sedative/hypnotic state in patients undergoing surgery under general anesthesia.

We have also found that BIS is also useful to detect cerebral ischemia during pediatric and adult cardiac surgery especially when used in combination with the near-infrared spectroscopy (NIRS) to measure oxygen saturation of the brain. Simultaneous monitoring with BIS and NIRS revealed that in children, especially in infants, cerebral ischemia occurred frequently during cardiac surgery presumably due to immaturity of the cerebral vascular autoregulation. We also reported successful anesthetic management of patients with compromised circulation.

2. Management of intraoperative and postoperative pain

We have published several works on management of intraoperative and postoperative pain. We have developed a rabbit model of surgical anesthesia/analgesia, which allows for repeated and quantitative evaluation of depth of surgical anesthesia/analgesia provided by a variety of anesthetics/analgesics. We also published several review articles on how to manage postoperative pain, and original articles comparing various modalities of postoperative pain management.

3. Management of chronic intractable pain

We published several works on new treatment modalities for chronic intractable pain syndrome with various drugs including ketamine and ATP, after application of drug tests to differentiate the mechanisms underlying the pain. We also reviewed usefulness of epiduroscopy in pain management in patients with chronic intractable low back pain.

We will continue to research on these subjects and publish several additional reports this year.

Publications

1. Xu H, Arita H, Hayashida M, Zhang L, Sekiyama H, Hanaoka K. Pain-relieving effects of processed Aconiti tuber in CCI-neuropathic rats. Journal of Ethnopharmacology. 103(3): 392-397, 2006
2. Shu H, Arita H, Hayashida M, Sekiyama H, Hanaoka K. Effects of processed Aconiti tuber and its ingredient alkaloids on the development of antinociceptive tolerance to morphine. Journal of Ethnopharmacology. 103(3): 398-405, 2006
3. Shu H, Arita H, Hayashida M, Chiba S, Sekiyama H, Hanaoka K. Inhibition of morphine tolerance by processed Aconiti tuber is mediated through activation of kappa opioid receptors. Journal of Ethnopharmacology. 106 (2): 263-71, 2006.
4. Li C, Sekiyama H, Hayashida M, Sumida T, Arita H, Hanaoka K. Effect of repeated topical application of clonidine cream in a rat model of postoperative pain. PAIN RESEARCH 21(1): 25-32, 2006.
5. Hayashida M, Fukunaga A, Fukuda K, Sakurai S, Mamiya H, Ichinohe T, Kaneko Y, Hanaoka K. The characteristics of intravenous adenosine-induced antinoception in a rabbit model of acute nociceptive pain: a comparative study with remifentanil. Anesthesia and Analgesia 103(4): 1004-1010, 2006
6. 林田眞和，今村佐知子，花岡一雄．術後痛の成因と生体に及ぼす影響．Anesthesia 21 Century 8：1368–1373，2006
7. 花岡一雄，山本華甫里，長瀬真幸，有田英子，林田眞和．痛みの診断―ドラッグチャレンジテスト．日本医師会雑誌 135：86–87，2006
8. 林田眞和，今村佐知子．手術後の痛み対策 社会復帰を早める患者主体の鎮痛法．科学 76(7)：716–720，2006.
9. 林田眞和，今村佐知子，池田和隆，花岡一雄．術後痛対処における現況と展望．日本医師会雑誌 135(4)：803–805，2006
10. 林田眞和，今村佐知子．ディスポーザブル持続注入ポンプ．麻酔 55(9)：1118–1127，2006
11. 林田眞和，藤本弘幸，花岡一雄．術後痛の成因．花岡一雄編，術後痛 改訂第2版．克誠堂出版．東京，2006，pp 2–18.

Research Hospital

Core Facility for Therapeutic Vectors
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The primary function of the Core Facility for Therapeutic Vectors (CFTV) is to support clinical trials that require the genetic modification and/or ex vivo manipulation of patients' tissue under current Good Manufacturing Practice (cGMP) conditions.

1. Preparation of Standard Operating Procedures (SOPs)

The cGMP compliance is maintained using written SOPs which codify all aspects of laboratory activities including facility design and operations of the personnel. Identify areas for improvement and develop SOPs for each operation.

2. Adoption of ISO

Quality management system of the CFTV has been assessed and found to be in accordance with the requirements of the quality standards detailed ISO9001: 2000; in the scope of development and manufacture of cell and gene therapy products.

3. Validation of CFTV

The CFTV is organized with two distinct units; 1) Vector Unit, the primary viral vector production suite which may also function as ex-vivo transduction suite; 2) Cell Unit, cell processing suite capable of generaling dendritic cells for immunotherapy and gene therapy. There are two self-contained vector production suites in the Vector Unit and two self-contained tissue culture suites in the Cell Unit. These suites are kept Class 10,000. There are many features incorporated into the design of this CFTV to minimize the risk of cross-contamination between products; i.e., unidirectional traffic flow, individual airlocks to each production suite, single-pass HEPA filtered supply air, 100 percent exhaust from the biological safety cabinets through dedicate ducts, among others. Periodical validation has been performed on the facility and the equipments in CFTV to ensure cGMP compliance.

4. Production of Master Virus Seed Stock (MVSS)

We are preparing the cGMP compliant MVSS which contains a replication-defective recombinant adenoviral vector encoding human interleukin-12 driven by a CA promoter (CMV-IE enhancer with the chicken β-actin promoter). The vector backbone is based on the E1-and E3-deleted serotype 5 adenovirus with a modified fiber , harboring an integrin-binding CDCRGDCDC-motif within the HI-loop of its

knob protein.

5. Establishment of Master Cell Bank (MCB)

We are supporting establishment of master and working cell banks for producing genetically engineered herpes simplex viruses and manufacture of a viral vector for using in clinical gene therapy trials. The MCB is preparing from the WHO Vero 10-87 Cell Seed.

6. Preparation of Peptide-Loaded Dendritic Cells (DCs)

We are supporting phase I clinical trials of melanoma vaccine using gp100 derived peptides restricted to HLA-A 2402 with fully matured DCs to induce Th1 type immune responses. We are preparing the fully matured and peptide-loaded DC in the Cell Unit of the CFTV.

7. Acceptance of projects

(1) Cancer gene therapy using IL-12 transduced dendritic cells
Department of Surgery and Bioengineering, Advanced Clinical Research Center, IMUST
(2) Vaccine therapy with peptide-loaded dendritic cells for advanced melanoma
Department of Surgery and Bioengineering Advanced Clinical Research Center, IMUST
(3) Oncolytic viral therapy using genetically engineered herpes simplex viruses for malignant brain tumors.
Department of Neurosurgery, Graduate School of Medicine, The University of Tokyo

8. Support

This CFTV is supported in part by the 21st Century COE Program from Japan Society for the Promotion of Science.