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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. Human herpesvirus 6 variant B infection in adult patients after unrelated cord blood transplantation

Tomonari A, Takahashi S, Ooi J, Uchimaru K, Tojo A

Human herpesvirus 6 variant B (HHV-6B) infection in 23 adult patients undergoing cord blood transplantation (CBT) was studied. By the method of quantitative polymerase chain reaction, HHV-6B DNA was detected in sera from 15 patients (65%) at day 14 or 15 (week 2), 16 (70%) at day 21 or 22 (week 3), and three (13%) at day 28 or 29 (week 4) after CBT. HHV-6B DNAemia was not found in all 20 patients examined at day 7 or 8 (week 1). Overall incidence of HHV-6B DNAemia reached to 87% (20 out of 23). This incidence was much higher than after unrelated bone marrow transplantation (19%, *P*

<0.0001). In CBT patients, positive HHV-6B DNAemia at week 3 was significantly associated with early skin rash (88% *vs* 14%, $P<0.005$) and grade II-IV acute graft-versus-host disease (aGVHD) (69% *vs* 14%, $P<0.05$). In contrast, positive HHV-6B DNAemia at week 2 was associated with neither skin rash nor aGVHD. Prospective large-scale studies are needed to determine the role of HHV-6 infection in CBT patients.

2. Clinical Outcomes of Cord Blood Transplantation from Unrelated Donors Comparable with Marrow or Blood Transplantation from Related Donors in Adults: A Single Institute Analysis.

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

We studied the clinical outcomes of 163 adults with hematological malignancies who received unrelated CBT (n=92), or BMT or peripheral blood stem cell transplantation (PBSCT) from related donors (n=71, 55 BMT and 16 PBSCT) between January 1997 and February 2005. All patients received myeloablative regimens including 12 Gy of total body irradiation and almost the same supportive care. We analyzed the hematopoietic recovery, rates of GVHD, risks of TRM and relapse, and DFS using Cox proportional hazards models. The age, sex, cytomegalovirus serological status, time from diagnosis to transplantation, and GVHD prophylaxis regimens were not significantly different between both groups. Overall rates of high-risk patients in the CBT and in BMT/PBSCT groups were 58% and 63%, respectively. Human leukocyte antigen (HLA) was scored serologically for HLA-A and B and genetically for DRB1 alleles. There were no complete matches in CBT and 54 (76%) matched grafts in BMT/PBSCT. Median numbers of leukocytes and CD34+ progenitor cells before freezing of cord blood grafts were 2.4×10^7/kg and 0.9×10^5/kg, respectively. Median follow-up was 27 months for CBT and 50 months for BMT/PBSCT.

Significant delays in neutrophil and platelet engraftment rates occurred after CBT; however, overall myeloid engraftment rates were almost the same for both grafts (94% in CBT and 98% in BMT/PBSCT). The cumulative incidences of grades II to IV acute GVHD, of grades III and IV acute GVHD, and of requiring steroids for treating acute GVHD among CBT recipients were 58%, 8%, and 18%, respectively. Those among BMT/PBSCT recipients were 58%, 19%, and 38%, respectively. Chronic GVHD affected 68 of 75 CBT and 49 of 60 BMT/PBSCT evaluable recipients. Twenty-two CBT and 30 BMT recipients developed extensive GVHD. The 1-year cumulative incidence of TRM, the 3-year cumulative incidence of relapse, and the 3-year probability of DFS in CBT recipients were 9%, 18%, and 71%, compared with 13%, 26%, and 60% in BMT/PBSCT recipients. Multivariate analysis demonstrated no apparent difference in those outcomes between both groups.

Taken together, engraftment speed was slower and severe acute GVHD and extensive chronic GVHD tended to be lower in CBT recipients compared with BMT/PBSCT recipients; however TRM, relapse and DFS were comparable in both groups. These data suggest that cord blood from unrelated donors could be as safe and effective a stem cell source as bone marrow or mobilized peripheral blood from related donors for adults when it is used as a primary unrelated stem cell source.

3. Unrelated cord blood transplantation after myeloablative conditioning for adult patients with refractory anemia.

Ooi J, Takahashi S, Tomonari A, Soda Y, Ohno N, Uchimaru K, Tojo A.

We report the results of unrelated cord blood transplantation (CBT) after myeloablative conditioning in 3 patients with myelodysplastic syndrome-refractory anemia (MDS-RA). All patients were treated with total body irradiation, cytosine arabinoside (Ara-C), and cyclophosphamide, followed by unrelated HLA-mismatched CBT. Granulocyte colony-stimulating factor was infused continuously, starting 12 hours before Ara-C therapy and continuing until the end of Ara-C therapy. All patients received standard cyclosporine and methotrexate therapy as graft-versus-host disease prophylaxis. All patients had myeloid reconstitution, and the times to reach an absolute neutrophil count $>0.5\times10^9$/L were 23, 20, and 26 days. All patients showed full donor chimerism at the time of the first bone marrow examination (on day +42, +43, and +62) after CBT. All patients are alive and free of disease at between 17 and 39 months after CBT. These results suggest that adult MDS-RA patients without suitable related or unrelated bone marrow donors should be considered as candidates for CBT.

4. Atypical Hypersensitivity to Mosquito Bites without Natural Killer Cell Proliferative Disease in an Adult Subject

Konuma T, Uchimaru K, Tojo A,

Hypersensitivity to mosquito bites (HMB) is a

rare disorder that occurs in the first two decades of life and is considered to be associated with chronic Epstein-Barr virus (EBV) infection and natural killer (NK) cell leukemia/lymphoma. EBV-encoded small nuclear RNA (EBER)-positive NK cells infiltrate the skin lesion at the site of the mosquito bite. In this report, we present an adult patient with mantle cell lymphoma complicated by atypical HMB. The anti-EBV antibody titer of the patient indicated reactivation of chronic infection with this virus, and EBV-DNA in the peripheral blood mononuclear cells (PBMCs) was detected by quantitative PCR after chemotherapy. However, EBER-positive cells were not detected in the skin lesion at the bite site or in the lymph node using *in situ* hybridization (ISH). Peripheral NK cell lymphocytosis and EBV-associated lymphoproliferative disease did not develop. These findings suggest that some patients with chronic EBV infection may develop HMB without NK cell proliferative disease.

5. $CD34^+CD7^+$ leukemic progenitor cells may be involved in maintenance and clonal evolution of chronic myeloid leukemia.

Kosugi N, Tojo A.

We analyzed $CD34^+$ cells co-expressing CD7 in chronic myeloid leukemia (CML) in chronic or accelerated phase (CP or AP) to clarify their role in progression or regression of the disease during treatment. Enumeration of $CD34^+CD7^+$ cells was performed on bone marrow nucleated cells from normal donors and CML patients. Fluorescence *in situ* hybridization (FISH) analysis was performed on sorted $CD34^+CD7^+$ and $CD34^+CD7^-$ cells to examine the occupancy rate of each fraction by $BCR\text{-}ABL^+$ cells with or without additional cytogenetic abnormalities. The proportion of $CD34^+CD7^+$ cells was significantly affected by the treatment outcome and/or the disease status as follows: $20.5\pm10.4\%$ in normal donors ($n=22$); $18.1\pm10.2\%$ in CP with major cytogenetic response ($n=14$); $53.0\pm12.9\%$ in CP at diagnosis ($n=18$); $55.0\pm15.8\%$ in CP with no or minor cytogenetic response ($n=28$); $70.2\pm18.1\%$ in AP ($n=6$). The proportion of $CD34^+CD7^+$ cells decreased in parallel with cytogenetic improvement in individual patients. In 6 untreated CP patients, the ratio of $BCR\text{-}ABL^+$ cells was comparable between each fraction. In 3 patients with major cytogenetic response, the ratio of $BCR\text{-}ABL^+$ cells was remarkably lower in $CD34^+CD7^-$ cells than in $CD34^+CD7^+$ cells. In 3 AP patients with additional cytogenetic abnormalities, extra signals were detected at much higher rate in $CD34^+CD7^+$ cells than in $CD34^+CD7^-$ cells. Our results suggest that $CD34^+CD7^+$ cells may be involved in maintenance and clonal evolution of $BCR\text{-}ABL^+$ cells in CML.

Publications

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Department of Infectious Diseases and Applied Immunology (DIDAI) was founded in 1981. In 1986, clinic for patients with human immunodeficiency virus (HIV) infection was opened by former professor, K. Shimada. In 2005, 42 new patients with HIV infection have visited or admitted our hospital and, in total, 286 patients are currently under our clinical management. The total number of in-patients during 2005 was 31, and several 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 outpatients and in-patients, members of the Division of Infectious Diseases (DID) and the Division of Clinical Immunology of the Advanced Clinical Research Center join the clinic. Supported by clinicians of three department and divisions, basic scientists of immunology and virology in DID, and dedicated medical and paramedical stuffs, 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[1], Takeshi Fujii[1], Tokiomi Endoh[1], Jun-ichi Takeda[1], Fuyuaki Ide[1], Takeshi Matsumura[1], Hitomi Nakamura[1], Miou Sato[1], Mieko Goto[1], Mutsunori Iga[2], Takuya Maeda[2] and Aikichi Iwamoto[1,2]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Department of Infectious Disease Control, 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

Forty-two new patients with HIV-1 infection visited our hospital this year, and as of the end of this year, 286 patients in total are under medical management in our outpatient clinic. As shown in the figure, the number of total patients declined in 1997 because a part of patients as well as medical stuffs were moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again with exponential curve after 1998 in accordance with Japa-

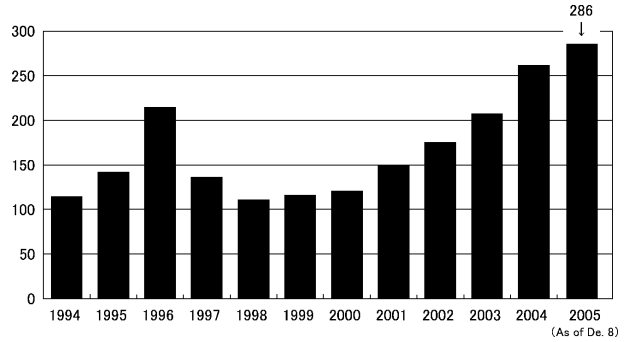

Figure 1 The number of HIV-infected patients in IMSUT hospital

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. Clinical research of HIV infection in IMSUT hospital: Specific immune therapy for human immunodeficiency virus followed by structured treatment interruption of antiretroviral therapy (Phase I study).

Although highly active antiretroviral therapy (HAART) has significantly improved prognosis of HIV-1 infection, life-long therapy is required for continuous viral suppression. Long-term toxicity of HAART is therefore of medical concern and economical cost of HAART has become one of major social problems. These issues have facilitated attempts of strategic or structured treatment interruption (STI). However, successful results were not obtained in patients who started HAART in chronic phases, because HIV-1-specific immunity was already exhausted at the moment of treatment interruption. Based on the pathogenesis of HIV-1 infection, therapeutic HIV-1 vaccine that is administered during HAART and potentiates HIV-1-specific immunity will be a theoretically feasible strategy to expect better viral control after STI. To test the hypothesis, we conducted a phase 1 clinical trial in which autologous dendritic cells (DCs) loaded with HIV-1-derived CTL epitope peptides were administered to 4 HIV-1-infected individuals and HAART was discontinued thereafter. DCs were used as highly specialized antigen-presenting cells that not only restore qualitative impairment of CTLs but also stimulate naive $CD8^+$ T cells newly provided from thymus during HAART.

As immunogens for vaccination, we used in this study HIV-1-derived peptides that were known to elicit strong CTL response and restricted to HLA-A*2402 expressed in approximately 70% of Japanese population (allele frequency of A*2402; 36.5%) [16-19]. The selected CTL epitope portions with A*2402 restriction were amino acid position from 28 to 36 of Gag (Gag28), from 296 to 306 of Gag (Gag296), from 138 to 147 of Nef (Nef138), and from 584 to 594 of Env (Env584). Whereas amino acid sequences in Gag296 epitope portion have been shown conserved, other 3 epitope portions have been reported to have various amino acid mutations. Thus, as shown in Table 2, we selected for the 3 epiope portions both wild type peptides (Gag28-wt, Nef138-wt and Env584-wt) and one of mutant peptides (Gag28-3R, Nef138-2F and Env584-4Q) together with a conserved Gag296 peptide (Gag296) in order to apply the therapeutic vaccine to a large proportion of patients with HLA-A*2402 and provide a wide breadth of immunological response. Sequence analysis of HIV-1 derived from 4 enrolled participants revealed that at least 2 out of 4 epitope portions had amino acid sequences identical to immunized peptides.

Four male patients with undetectable VL under HAART were enrolled in this study. 6.8-9.7 $\times 10^9$ of PBMCs were collected from each participant by leukopheresis, and 0.7-1.8$\times 10^7$ mature DCs were harvested for each vaccination without contamination by pathogens or reactivation of autologous HIV-1. Peptides were either loaded to DCs by mixture (participant 1 and 2) or separately (participant 3 and 4), and peptide-loaded DCs were injected subcutaneously to areas near axilla in two or three divided doses. During courses of 6 vaccinations in 4 participants, one episode of subcutaneous bleeding, erhythema at injection site and general malaise were reported as local and generalized adverse events, all of which were not serious and disappeared without specific treatment.

Serum VLs after discontinuation of HAART were examined every week after treatment interruption and became positive above detection limit of 50 copies/ml in all four participants at week 3, 3, 1, and 2, respectively. Participant 4 experienced fever at 38℃, myalgia, skin rash and neck lymph node swelling one week after interruption accompanied with mild liver dysfunction and thrombocytopenia, which mimicked acute retroviral syndrome and subsided spontaneously in two weeks. All participants met criteria to restart HAART (at week 8, 4, 5, and 3, respectively) and VLs have been suppressed to an undetectable level by 11 to 30 weeks after re-

start of their original HAART regimens. Difference between peak VLs after treatment interruption and VLs before start of HAART did not exceed 0.5 in $\log_{10}$ scale in all 4 participants. CD4 counts decreased after discontinuation of HAART in all participants to the level of approximately 200/ml. After restart of HAART, CD4 counts in participant 2 and 4 recovered gradually, but those in participant 1 and 3 fluctuated at lower level than before treatment interruption in spite of successful viral control by restarted HAART.

HIV-1-specific CTL response to immunized peptides was evaluated by ELISPOT assay to detect IFN-g-producing cells. Unseparated PBMCs were used for the assay because preliminary experiments showed that IFN-g production responding both to immunized peptides and control peptides was only seen in $CD8^+$ population (data not shown). We could observe significant response to Nef138-wt in participants 1 and 2 and weak response to Nef138-2F in participant 1 after 5th vaccination. The response in participant 2 was specifically induced by DC-based vaccine because we could not detect any response to control peptides of Gag (1-115), CMV-pp 65, and EBV-TL9. In participant 1, however, response after 5th vaccination was also observed to control peptides of Gag (1-115) and EBV-TL9, suggesting that this response to Nef138-wt and Nef138-2F included non-specific stimuli by DC injection. When HAART was discontinued and autologous virus rebounded, specific response in participant 1 and 2 was induced to Nef138-wt and Nef138-2F as well as Gag (1-115), whereas no significant response was observed to other immunized peptides.

The limited breadth of response in participant 1 and 2 to immunized peptides raised a possibility that difference in the avidity between immunized peptides and HLA-A*2402 molecules affected the results because we loaded 7 peptides by mixture to DCs for these participants. Thus, we tested avidity of seven peptides using T2-A24 stabilization assay. As shown in Fig. 3, Env584-wt, Env584-4Q, Nef138-wt and Nef138-2 F bound HLA-A*2402 with relatively high avidity, whereas Gag28-3R bound with moderate avidity and both Gag296 and Gag28-wt with low avidity. Based on this result, each peptide for participant 3 and 4 was incubated with DCs separately and used for vaccination. However, injection of separately-loaded DCs could not induce specific response to 7 immunized peptides although rebound of autologous HIV-1 after treatment interruption induced strong response to Nef138-2F, Env584 and Gag (1-115). We also conducted tetramer-binding assay using Nef138-wt -tetramer and ELISPOT assay using autologous DCs as antigen-presenting cells to amplify IFN-g production, but could not find peptide-specific population or response in participant 3 and 4.

Since one of the concerns regarding interruption of HAART is emergence of drug resistance mutations, we sequenced reverse transcriptase and protease genes of HIV-1 derived from plasma before start of HAART and 4 weeks after treatment interruption when VLs were detectable in all participants (7,100, 58,000, 24,000 and 100,000copies/ml, respectively). We found in participant 3 a nucleotide substitution from G to A at position 108 in protease regions by population sequencing, which resulted in amino acid change from methionine to isoleucine at position 36. Sequences of clones obtained from the PCR product revealed that 5 out of 5 clones had M36 I mutation. We further sequenced HIV protease genes in this participant's plasma 6 weeks after treatment interruption (29,000copies/ml), but could not find M36I mutation in all 8 clones we sequenced. We did not find nucleotide substitution of protease genes in other three participants and reverse transcriptase genes in all participants.

2. Treatments and Clinical Research of Tropical Diseases

Tetsuya Nakamura, Takashi Odawara[1], Takeshi Fujii[1], Tokiomi Endoh[1], Jun-ichi Takeda[1], Fuyuaki Ide[1], Takeshi Matsumura[1], Hitomi Nakamura[1], Miou Sato[1], Mieko Goto[1], Mutsunori Iga[2], Takuya Maeda[2] and Aikichi Iwamoto[1,2]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Department of Infectious Disease Control, 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, more than 100 travellers visited our clinic for consultation or treatment of tropical diseases, including malaria prophylaxis, pretravel vaccination, post-exposure prophylaxis of rabies and treatment of traveller's diarrhea, malaria, amoebiac enteritis, typhoid fever, dengue fever, shigellosis, fascioliasis and gnathostomiasis.

b. Clinical research of Tropical Diseases in IMSUT hospital: CLINICAL CHARACTERISTICS OF IMPORTED MALARIA IN JAPAN

We have reviewed previous experience of malaria cases in IMSUT hospital. This year, the re-

sult was published in American Journal of Travel Medicine and Hygene. This retrospective review of malaria cases at IMSUT hospital corresponds to 14.9% of the total cases in Japan over 10 years. This study represents the largest review of clinical characteristics and outcomes of patients with malaria in Japan. Falciparum malaria accounted for about 50% of total malaria cases at the IMS Hospital. The proportion of *P. falciparum* is relatively high in France (around 80%), moderate in Germany and the United Kingdom (around 60%), and low in the United States (around 40%). The proportion of falciparum malaria in Japan seems to lie between European countries and the United States. European countries are geographically close to Africa where *P. falciparum* is a dominant species, whereas the United States is closer to Central and South America where *P. vivax* is predominant. Japanese travel to both Africa and Asia/Oceania likely accounts for this mid-range proportion of *P. farciparum* in IMS Hospital.

VFR, which is one of the most common reasons for travel to malarious areas in western countries, accounted for only 1.0% of Japanese travelers. In UK, where immigrants compose a substantial percentage of populations, VFR, holidays and business accounted for 56%, 12% and 6.5%, respectively. Since Japan is racially homogeneous, VFR is not likely to be a major reason for travel to malaria-endemic countries. Business travels accounted for 70% among Japanese patients throughout the decade. In Germany where the number of immigrants from malarious countries is small like Japan, however, business travel accounted for only 18%, and 75% was holiday travels. This contrasting result might be explained by the difference in the number of sight-seeing travelers to malarious areas between Japan and Germany. Nevertheless, detailed investigation would be required to clarify it. Improving travel advices to overseas employees is likely to contribute to reduction in the number of imported malaria in Japan.

There are no national guidelines for malarial prophylaxis or treatment in Japan. At IMS Hospital, mefloquine is used to treat falciparum malaria without complications, intravenous quinine for severe falciparum malaria, and chloroquine for non-falciparum malaria. Although we have not experienced mefloquine-resistant falciparum malaria, it is well known that multi-drug resistant falciparum malaria has emerged in South East Asia, especially at the border between Thailand and Myanmar, and between Thailand and Cambodia. Since many Japanese visit Thailand, health care providers must be aware of potential resistance when treating patients returning from these areas. Fortunately, we experienced no deaths from malaria at our institution. However, we previously reported that the case fatality rate (CFR) nationally from falciparum malaria is 3.3%. This CFR is as high as that of Germany (3.6%) and much higher than that of France (1.98%), the United States (1.01%) and the United Kingdom (0.65%). A high CFR could be attributable to better mortality reporting compared to total case reporting, a high proportion of patients without immunity to malaria, or poor management of complicated malaria. These factors may explain the discrepancy of CFR between the IMS Hospital and the rest of Japan. Alternatively travelers who are aware of their malaria risk may present earlier to reference hospitals for tropical medicine. Unawareness of malarial risk will lead to delayed infectious diseases doctor consultation and result in unfavorable outcomes.

P. vivax infection generally causes non-fatal disease. Primaquine administration after treatment with schizonticides is required to eradicate the dormant form of *P. vivax* in the liver. Since primaquine is not always effective against hypnozoites, relapse is occasionally observed even after adequate primaqine therapy. Of the cases of vivax malaria that relapsed after primaquine therapy at IMS Hospital, 75% were from Oceania. Both primaquine and chloroquine resistant *P. vivax* have emerged in the same area. We have shown that the number of malaria cases from Oceania has increased from 1997-2001. And further increases are likely since Japanese travel to Papua New Guinea is increasing. We, therefore, are more likely to encounter imported cases of primaquine and/or chloroquine resistant *P. vivax*. Cases of vivax malaria from Oceania, therefore, require careful observation during treatment of the acute febrile phase, and consideration for a modified dosage or duration of primaquine (for example, a longer duration or higher dose of primaquine therapy).

Although there had been neither guidelines nor approved drugs for malaria chemoprophylaxis in Japan until 2001, travelers used to collect information and get drugs in a variety of ways. We unexpectedly found that the proportion of Japanese patients who were taking chemoprophylaxis has dropped drastically in 1997-2001. The difference may reflect the absence of chloroquine failures as this drug was used less frequently for prophylaxis. This suggests that Japanese travelers are correctly informed of chemoprophylactic regimens that successfully prevent malaria acquisition. In support of this speculation, the annual number of imported malaria cases at the IMS Hospital and nationally has remained stable in the last decade despite record high levels of travel by both Japanese people

and foreigners in 2000 (http://www.immi-moj.go.jp/toukei/index.html). However, given the substantial number of travelers who still contract malaria without chemoprophylaxis, further efforts must be made to educate travelers.

The interval between the onset of symptoms and presentation to a hospital is another concern because any delay in diagnosis can lead to increased mortality with falciparum malaria. Kain and others reported that the mean duration from onset of symptoms until first medical consultation in Canadian travelers was 3.6 days (95% CI: 2.5-4.7) in hospitals without expertise in tropical medicine and 3.8 days (95% CI: 2.3-5.3) in hospitals with a tropical medicine unit. The mean duration in our cases was 4.7 days (95%CI: 3.7-5.7). However, since the duration in our cases was not normally distributed (Figure 1), a precise comparison with our data is difficult. Nevertheless, even a median duration of 3.0 days in our cases is an unacceptable length that could cause severe malaria or death in a non-immune population. Delayed diagnosis despite early presentation to hospitals is another common problem that can increase mortality of falciparum malaria at health care facilities lacking infectious diseases unit. However, since almost all cases in the IMS Hospital were diagnosed on the first day of presentation, the data shown above also represents the duration from the onset of symptoms to diagnosis.

This retrospective study reveals clinical problems relevant to malaria imported to Japan. A high frequency of relapse of vivax malaria despite primaquine administration in patients from Oceania must be relayed to health care providers. In addition, the absence of chemoprophylaxis and the delay in initial medical consultation suggest a continued ignorance of travelers regarding malarial risk. In order to reduce morbidity and mortality due to malaria, travelers must be informed of malaria risks, the necessity of chemoprophylaxis, and the importance of immediate medical consultation if fever develops. To this end, it is important to develop travel medicine referral centers to coordinate the education of health care providers and travel companies regarding malaria.

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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

Hirohide Kawasaki, Yasuhiro Ebihara, Kohichiro Tsuji

Although a standard regimen in hematopoietic stem cell transplantation (SCT) has been available for children with acute lymphoblastic leukemia (ALL) and acute myeloid 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 Philadelphia chromosome-positive acute lymphoblastic leukemia in children

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Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ALL) continues only 3-5% of ALL in children: however, its prognosis is known to be very poor despite of contemporary multiagent intensive chemotherapy. The meta-analysis of over 300 children with Ph^+ALL demonstrated the efficacy of allogeneic hematopoie-

tic SCT from matched sibling donor. Imatinib mesylate was recently produced as a specific tyrosine kinase inhibitor for bcr-abl fusion gene product in chronic myelogeneous leukemia (CML). Because the number of children with Ph^+ ALL is small, a nationwide trial for the disease is mandatory. On behalf of the Japanese Pediatric Leukemia/Lymphoma Study Group, we proposed a trial, which employs intensive chemotherapy and a new drug, imatinib mesylate, to maintain a remission status, followed by allogeneic SCT at the 8th month after the diagnosis. This is a phase II study to evaluate the efficacy of imatinib mesylate. The efficacy will be assessed with molecular quantification techniques (qualitative and quantitative real-time polymerase chain reaction (PCR) method). The toxicity of the drug will be monitored and graded by the criteria of NCI-CTC VER2.0. The study was permitted by the ethical committee of the Japanese Society of Pediatric Hematology, and opened in 2004. At the present time, eight patients have been enrolled into this study.

3. Cooperative clinical trial for pediatric myelodysplastic syndrome

Kohichiro Tsuji, Hirohide Kawasaki, Atsushi Manabe[1], Yuji Zaike[3], Yasuhiro Ebihara; [3]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 300 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.

4. Molecular pathogenesis of pediatric myelodysplastic syndrome and myeloproliferative diseases

Daisuke Hasegawa[4], Hirohide Kawasaki, Atsushi Manabe[1], Yasuhiro Ebihara, Kohichiro Tsuji; [4]Department of Pediatrics, Tokyo Medical University

Pediatric MDS and myeloproliferative diseases (MPD) are very rare disorders. The diseases are commonly seen in elderly patients, puggesting 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,*p15* 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.

5. Novel approach to therapy in juvenile myelomonocytic leukemia

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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 me-

chanisms, 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.

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

Feng Ma, Sachiyo Hanada, Hirohide Kawasaki, Yasuhiro Ebihara, Kazuo Ogami[6], Tokiko Nagamura-Inoue[6], Kohichiro Tsuji; [6]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 permitted by the ethical committee of the Japanese Government on December 20, 2003, and started.

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 were generated from human ES cells in the coculture with the mouse embryo -derived stromal cells. We are now evaluating the function of the 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 to hematopoietic cells.

7. Hematopoietic origin of fibroblasts

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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 such as kidney mesangial cells and brain mycroglia cells. 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 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-α1, 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.

8. Definitive hematopoiesis from endothelial cells in the mouse embryo

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We previously reported erythropoiesis from acetyl (Ac) LDL incorporating endothelial cells (EC) in the mouse embryo. However, it still has been unclear whether the other types of definitive hematopoietic cells (HC) can be generated from these cells. We then tagged EC at 10 dpc mouse embryo with Ac-LDL-DiI by injection of Ac-LDL-DiI into recipient embryos, and showed that they release DiI^+ HC into circulation after 12 hours of whole embryo culture (WEC). The hematopoietic clusters in the main arteries of mouse embryo were also stained with DiI. The circulating DiI^+ HC expressed c-Kit and half of these cells displayed blastic morphology. *In vitro* culture and *in vivo* reconstitution experiments demonstrated that the circulating DiI^+ HC contained definitive multi-potent progenitors including HSC. Furthermore, the sorted DiI^+ HC were able to colonize FL when introduced back into the blood stream of a secondary recipient embryo. These results suggest that Ac-LDL incorporating EC can produce definitive HSC and HC colonizing FL in the mouse embryo.

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We participate in cutting edge science of autoimmune, rheumatic and allergic diseases and of 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. For these purposes, we have vigorously been collaborating with the Division of Clinical Immunology (Prof. Chikao Morimoto).

I. Therapeutically targetting transcription factors

Hirotoshi Tanaka[1], Noritada Yoshikawa[1], Yuichi Makino[1], Noriaki Shimizu[2], Hiroshi Nakamura[2], Tetsuya Hisada[2], Chikao Morimoto[2], Motoaki Sano[3], Keiichi Fukuda[3]: [1]Department of Rheumatology and Allergy, Division of Clinical Immunology, [2]Division of Clinical Immunology, [3]Department of Regenerative Medicine and Advanced Cardiac Therapeutics, 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 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-

steridal 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) 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 preferencially 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.

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

Transcription is a complex process compsed of preinitiation, initiation, promoter clearance, elongation, termination. Major efforts have been put on the initiation process including regulation of transcription factor function. However recent studies have clarified the modulatory role of elongation process in gene expression. Discovery of P-TEFb accelerated the understanding of molecular nature of elongation. It recently is shown that HEXIM1 binds 7SK snRNA and inhibits P-TEFb-mediated transcriptional elongation process. We have found that HEXIM1 directly associates with the GR in the absence of 7 SK and represses GR-mediated transcription. That is, HEXIM1 has a dual role in regulation of gene expression via connecting initiation and elongation. We are currently working on regulation of HEXIM1 expression, physiological role of HEXIM1 in inflammation, vascular disorders, and cardiac diseases.

b. Hypoxia-inducible Factor (HIF)-1 project

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

II. Study on soluble CD26 in normal immune response and in patients with immune-mediated diseases

Osamu Hosono[1,2], Yuichi Makino[1,2], Noritada Yoshikawa[1,2], Hirotoshi Tanaka[1,2], et al., Kei Ohnuma[2], Chikao Morimoto[2], et al.: [1]Department of Rheumatology and Allergy, and [2]Division of Clinical Immunology

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-□B 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 infliximab (anti-TNF□ monoclonal antibody) therapy on serum levels of sCD26/DPPIV in patients with RA.

III. Study on the in vitro and in vivo immune effect of roxithromycin

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Roxithromycin (RXM) is a macrolide antibiotic that is effective in the treatment of chronic lower respiratory tract diseases including diffuse panbronchiolitis and bronchial asthma. Its mechanism of action apart from its antibacterial action remains unclear. To further determine the mechanism of action of RXM, we evaluated the effect of RXM on T cell functions and the inflammatory responses in mice with collagen induced arthritis (CIA).

RXM did not affect the production of Th1-type and Th2-type cytokines, whereas it specifically inhibited production of proinflammatory cytokines such as TNF-□ and IL-6 by T cells and macrophages. RXM inhibited T cell migration. We found that RXM treatment of mice with CIA reduced the severity of arthritis and serum level of IL-6, as well as leukocyte migration into the affected joints and destruction of bones and cartilage. Our findings strongly suggest that RXM may be useful for the therapy of rheumatoid arthritis such as minocycline. Therefore, we are going to examine the therapeutic efficacy of RXM in patients with rheumatoid arthritis.

IV. Immunobiology and clinical applications of innate and acquired immune systems.

a. The Role of TRAIL in the prevention of Acute Graft-Versus-Host Dosease.

Hiroshi Kawasaki[1], and Chikao Morimoto[1]: [1]Department of Rheumatology and Allergy, and Division of Clinical Immunology (in collaboration with Katsuaki Sato, Takami Matsuyama, and Kouichi Hirai)

We report here the potential usefulness of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) for the treatment of lethal acute graft-versus-host disease (GVHD) and leukemia relapse. Dendritic cells (DCs) genetically modified to express TRAIL showed more potent cytotoxicity than soluble TRAIL against both alloreactive T cells and leukemic cells mediated through TRAIL/death receptor (DR) pathway. In addition, cell gene therapy with genetically modified DCs expressing TRAIL was more effective than in vivo gene transfer of TRAIL for the protection against acute GVHD and leukemia relapse. Thus, gene transfer of TRAIL involving DCs is useful for the treatment of acute GVHD and leukemia relapse by selective targeting of the pathogenic T cells and leukemia relapse.

b. The control amd trans-activation of Chemokine Receptor Expression by β1 Integrin down stream signaling

Hiroshi Kawasaki[1], Satoshi Iwata[1], and Chikao Morimoto[1]: [1]Department of Rheumatology and Allergy, and Division of Clinical Immunology

Our laboratory has been showing that β1 integrins and their associating molecules, play crucial roles in the activation of lymphocytes. One of the cruicial elements of β1 Integrin-mediated lymphocyte activation is the tyrosine phosphorylation of Cas-L by Src-family kinase and FAK. The phosphorylated Cas-L ultimately enhances a panel of transcriptional activity through MAP kinase pathway. The ligation of

chemokine receptors by respective ligands also activates MAP kinase pathway via G-protein coupled Rac/Ras/Rho, resulting in the upregulation of cell migration, motility, and proliferation. We have already shown that overexpression of Cas-L induced the enhancement of T lymphocyte migration in vivo and in vitro. We are investigating the functional linkage of Cas-L and signaling pathway of chemokine receptors. The manipulation and utilization of Cas-L function might contribute to the elucidation of not only lymphocyte activation but also the development of inflammation.

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Department of Advanced Medical Science was established in September 1997. We are investigating, (1) Analysis of the mechanisms of cardiac outflow tract development, (2) Analysis of the role of newly identified gene, Hag2, for organ development, (3) Analysis of the expression gradient of genes in human colonic mucosa, (4) Adenovirus-mediated gene transfer and lipoprotein-mediated protein delivery of plasma PAF-AH in rat model of glomerulosclerosis, and (5) Identification of a tumor-associated antigen targeted by the dendritic cell therapy. We are planning and progressing several projects described below to develop new therapies for several diseases, including cancer and ischemic disorders.

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 death 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. One is the subtractive hybridization using a portion of E9.5 mouse embryo, which comprises primary and secondary heart field. The other is candidate gene approach using several markers which represent the primary (Mlc2a, Mlc2v and etc.) and secondary (isl-1, FGF8 and etc.) heart field and the neural crest cells (Tfap2a, and etc.).

Analysis of the role of newly identified gene, Hag2 (Hdf affected gene 2), for organ development

Watanabe T. et al.

To understand the process of organ develop-

ment is of importance with respect to the possibility of medical approach for congenital diseases. Recently, using subtractive hybridization method, our colleague, Nakaoka et al., identified a novel gene, Hag2, which was downregulated at some stage of fetal development in the thoracic region of heart defect mouse, which is embryonic lethal due to the cardiac anomaly. 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 noncoding RNA. Hag2 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 connective tissues around 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 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 the expression pattern of the protein in rat, mouse, and human tissue and also trying to elucidate the role of this protein.

Adenovirus-mediated gene transfer and lipoprotein-mediated protein delivery of plasma PAF-AH in rat model of glomerulosclerosis

Iso-O N. et al.

High density lipoprotein (HDL) is well known for its antiatherogenic effect through reverse cholesterol transport. We are elucidating another function of HDL as a transporter of antioxidative enzymes, such as PAF-acetylhydrolase (PAF-AH), paraoxisonase-1, etc. The *in vivo* study using adenovirus vector coding cDNA of human PAF-AH showed that liver-directed gene transfer of PAF-AH in rodents resulted in the deposition of PAF-AH protein in mesangial cells of the glomeruli and smooth muscle cells of the arteries, although mRNA of PAF-AH was not detected in these organs. On the other hand, the plasma PAF-AH was detected almost exclusively on HDL. Injection of purified HDL abundant in PAF-AH to rodents also resulted in the deposition of PAF-AH protein in mesangial cells and smooth muscle cells, which suggests that HDL is an active transporter of PAF-AH. Overexpression of PAF-AH in the model animals for spontaneous glomerulosclerosis led to the significant reduction of oxidative stress and urine protein excretion. These data show lipoprotein-mediated glomeruli-targeted protein delivery promises to be a novel therapeutic approach to glomerulosclerosis.

Recently, it was reported that βTC3 cells, deriving from human pancreatic β cells, express the scavenger receptor classB type1 (SR-B1), which mediates cholesterol uptake from HDL particles (Roehrich et al: JBC, 2003). This encouraged us to study how to deliver PAF-AH to β cells *in vivo*, which may be a therapeutic treatment for β cell dysfunction in diabetic patients by reducing oxidative stress in β cells.

Identification of tumor-associated antigens in melanoma patients treated by dendritic cell therapy

Yoshiura K. et al.

We previously conducted dendritic cell therapy on 10 malignant melanoma patients and shrinkage or disappearance of metastatic tumors with massive necrosis occurred in two patients. We explored serum antibodies reacting with autologous tumor lysate by Western blots, and found a 29-kDa protein against which antibody was elicited by dendritic cell therapy in the patients who had good clinical outcome. Matrix-assisted laser desorption ionization-time of flight /mass spectrometry analysis of the protein isolated by two-dimensional electrophoresis combined with Western blots revealed that the 29-kDa protein was carbonic anhydrase II (CA-II). Immunohistochemistry of the tumors and normal tissues showed that CA-II was expressed in the tumor vessel but not in normal vessel endothelium. CA-II expression in tumor endothelium was observed as well in other cancers including esophageal, renal, and lung cancers. In an in vitro angiogenesis model, CA-II expression of normal human vein endothelial cells was significantly up-regulated when cells were cultured in the acidic and hypoxic conditions indicative of a tumor environment. These findings suggest that CA-II is a tumor vessel endothelium-associated antigen in melanoma and other cancers, and elicitation of serum anti-CA-II antibody by dendritic cell therapy may be associated with good clinical outcome including tumor reduction.

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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 2005

Hideaki Tahara, Akihiko Itoh, Takuya Takayama, Takuya Tsunoda, Hiroyuki Mushiake, Koji Yoshida, Hajime Ishikawa, Shigenori Nagai, Eri Ichikawa.

Surgical operations have been performed in 186 cases under general anesthesia and spinal or epidural and/or local anesthesia. As shown in Table 1, major operations were performed in 118 patients with malignant diseases and with benign diseases. We especially endeavor in the treatment of the far advanced malignant tumors. In this period, the resection of the peritoneal seeding and splenic metastasis of GIST under the chemotherapy with STI571, esophagectomy after chemo-radio therapy, removing the peritoneal seedings of the colo-rectal cancer combined chemotherapy and right lovectomy against huge hepato cellular carcinoma (the diameter of the tumor was over 18 cm) were performed. In addition, minimum invasive surgery and operations without complications were tried to performed. As a result of our efforts, the average hospitalization days of surgical inpatient 2005 was 16.6 days.

Table 1. 118 major operations performed in 2005

Esophagus*	4	*: 3 cancer and 1 carcinosarcoma
Stomach**	2	**: 1 GIST
Colo-rectum***	9	***: 1 metastatic gastric cancer
Liver	4	
Biliary Tract****	29	****: 24 laparoscopic cholecystectomy
Pancreas	1	
Spleen*****	1	*****: Metastatic GIST
Breast	16	
Thyroid	6	
Miscellaneous	55	
Total	118	

Procedures other than surgical operations performed in 2005 were as follows: gastroduodenal endoscopy (353 cases), and colorectal endoscopy

(207 cases).

2. Phase I clinical trial of melanoma vaccine using gp100 derived peptides restricted to HLA-A*0201

Takuya Tsunoda, Marimo Sato, Takuya Takayama, Akihiko Itoh, Hiroyuki Mushiake, Kohji Yoshida, Eri Ichikawa, Shigenori Nagai, Hideaki Tahara

Phase I clinical trial has been performed to evaluate safety, immunological response and clinical response against advanced malignant melanoma patients. Epitope peptides derived from gp100, a melanoma associated antigen, are used for the cancer vaccine to treat the patients with advanced malignant melanoma. Patients with HLA-A*0201 were treated with a gp100 derived peptide (ITDQVPFSV) and another peptide with a mutation (IMDQVPFSV). All of the peptides were used with incomplete Freund's adjuvant (IFA) in order to augment for anti-tumor immunity. So far, five patients with stage IV melanoma have been immunized with a vaccine consisting of HLA-A*0201-restricted epitope peptide derived from gp100 melanoma differential antigen emulsified with IFA. No adverse effects without grade I toxicity were observed in these patients. Immunological monitoring was performed to determine IFN-g production using PBMC stimulated with the vaccinated peptides. Immunological response and clinical response have not been obtained so far. We continue to perform the immunological response.

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

Takuya Tsunoda, Marimo Sato, Takuya Takayama, Akihiko Itoh, Hiroyuki Mushiake, 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.

4. Phase I/IIa clinical trial of epitope peptides based vaccine against gastrointestinal malignancy targeting HER2/neu and MAGE3

Takuya Tsunoda, Marimo Sato, Takuya Takayama, Akihiko Itoh, Hiroyuki Mushiake, Kohji Yoshida, Eri Ichikawa, Shigenori Nagai, Hideaki Tahara

Epitope peptides derived from MAGE3 and HER2/neu are used for the cancer vaccine to treat the patients with advanced gastrointestinal malignancy. Patients with HLA-A*0201 were treated with MAGE3 and HER2/neu derived peptide (FLWGPRALV, KIFGSLAFL). Patients with HLA-A*2402, were treated with MAGE3 and HER2/neu derived peptide (IMPKAGLLI,

RWGLLLALL). All of the peptides were used with IFA and IL-2 in order to augment for anti-tumor immunity. To analyze the immune response of the vaccinated patients, HLA-Tetramer was prepared and used for staining of the peripheral blood lymphocytes taken from the patients enrolled in this protocol. 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 1 esophageal cancer patient for MAGE3-HLA-A*2402 peptide until now. So far, the protocols were well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted.

5. 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 Tsunoda, Takuya Takayama, Marimo Sato, Akihiko Itoh, Hiroyuki Mushiake, 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.

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

Takuya Takayama, Takuya Tsunoda, Marimo Sato, Akihiko Itoh, Hiroyuki Mushiake, 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.

7. 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, Akihiko Itoh, Hiroyuki Mushiake, 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 (gp100-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.

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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.

Propeller diffusion tensor MRI: Feasibility study of cervical spondylotic myelopathy in an early clinical stage

Toshiyuki Okubo

To implement propeller diffusion tensor MR imaging (PRDTI) on a 1.5 Tesla MR imager, and investigate the findings in the spinal cord of patients with cervical spondylotic myelopathy in an early clinical stage. Patients with clinical symptoms of cervical myelopathy underwent PRDTI. The signal-to-noise ratio (SNR) in the spinal cord and cerebrospinal fluid (CSF) was evaluated. The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were measured. We classified the ROIs into two groups: 1) unaffected (no clinical symptoms and no abnormality on conventional images) and 2) affected (some clinical symptoms but no abnormal signal on conventional images). The ADC value in group 2 increased and the FA in group 2 decreased on average, compared to those in group 1. PRDT images may be a sensitive method for elucidating the structural characteristics of spinal cord pathology in vivo. However, clinical correlation and a long-term follow-up study will be needed.

In vitro validation of bioluminescent monitoring of disease progression and therapeutic response in leukemia model animals

Yusuke Inoue, Arinobu Tojo[1] and Rieko Sekine[1]: [1]Division of Molecular Therapy, Advanced Clinical Research Center

The application of in vivo bioluminescence imaging (BLI) to noninvasive, quantitative monitoring of tumor models relies on the positive correlation between the intensity of bioluminescence and tumour burden. We conducted cell culture studies to investigate the relationship between bioluminescent signal intensity and viable cell numbers in murine leukemia model cells. IL-3-dependent murine pro-B cell line Ba/F3 was transduced with firefly luciferase to generate cells expressing luciferase stably under the control of a retroviral long terminal repeat. The luciferase-expressing cells were transduced with p190 BCR-ABL to give factor-independent proliferation. The cells were cultured under various conditions, and bioluminescent signal intensity was compared with viable cell numbers and the

cell cycle stage. The Ba/F3 cells showed autonomous growth as well as stable luciferase expression following transduction with both luciferase and p190 BCR-ABL, and in vivo BLI permitted external detection of these cells implanted into mice. The bioluminescence intensities tended to reflect cell proliferation and responses to imatinib in cell culture studies. However, the luminescence per viable cell was influenced by the IL-3 concentration in factor-dependent cells and by the stage of proliferation and imatinib concentration in factor-independent cells, thereby impairing the proportionality between viable cell number and bioluminescent signal intensity. Luminescence per cell tended to vary in association with the fraction of proliferating cells. Although in vivo BLI would allow noninvasive monitoring of leukemia model animals, environmental factors and therapeutic interventions may cause some discrepancies between tumor burden and bioluminescence intensity.

Monitoring of disease progression by in vivo bioluminescence imaging and magnetic resonance imaging in an animal model of leukemia

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

Imaging technologies are increasingly used for animal experiments of tumor models. We examined the feasibility and reliability of a multimodality approach using in vivo bioluminescence imaging (BLI) and magnetic resonance imaging (MRI) for the monitoring of disease progression in an animal model of leukemia. Murine pro-B cell line Ba/F3 was transduced with firefly luciferase and p190 BCR-ABL, and mice were inoculated with the cells intravenously. Imaging studies, including in vivo BLI and MRI of living mice and ex vivo BLI of excised organs, were performed one, two, three, and four weeks after inoculation. Disease progression in a given mouse was observed longitudinally by in vivo BLI and MRI. The in vivo BLI demonstrated extensive light emission throughout the body, and the whole-body signal on in vivo BLI increased with time after inoculation. The ex vivo BLI showed predominant light emission in the liver, spleen, and bone marrow, and the signal for each organ correlated with the whole-body signal. MRI enabled accurate volume measurement of the liver and spleen, visualized hepatic nodules, and aided in localizing sources of light emission on in vivo BLI. The volumes of the liver and spleen measured by MRI correlated with the signals of the respective organs measured by ex vivo BLI. Longitudinal imaging studies allowed the assessment of disease progression for each mouse. BLI and MRI allow repetitive, whole-body, quantitative evaluation of extensive disease induced by the intravenous inoculation of leukemia model cells.

Imaging of living mice using a 1-T compact magnetic resonance (MR) imaging System

Yusuke Inoue, Yukihiro Nomura, Kohki Yoshikawa[2], and Tomoyuki Haishi[3]: [3]Department of Radiotechnical Sciences, Faculty of Radiological Health Sciences, Komazawa University, [4]MRTechnology Inc

To determine the feasibility of imaging living mice with a 1-T compact MR imaging system and to investigate appropriate imaging techniques for use in routine animal experiments. An MR imaging system, consisting of a 1-T permanent magnet and compact console, was used. 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 0.26×0.26×0.52 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.

Gadobenate dimeglumine as a contrast agent for magnetic resonance (MR) imaging of the mouse liver

Yusuke Inoue and Kohki Yoshikawa[2]

The small size of the mouse liver disturbs the acquisition of high-quality MR images during a reasonable scan time. In this study, we examined the characteristics of gadobenate dimeglumine (Gd-BOPTA) as a contrast agent for mouse liver imaging. MR images were acquired with a T1-weighted 3D FLASH sequence under isoflurane anesthesia on a 1-T compact MR imaging system. First, the time course of contrast effect was assessed by sequential imaging. After anesthesia induction, Gd-BOPTA was injected intravenously or subcutaneously, followed by repeti-

tive imaging up to 60 min. Next, the contrast effect was assessed at a single time point after subcutaneous injection into awake mice. Demarcation of the liver was evaluated at different doses of Gd-BOPTA. On sequential imaging after intravenous injection, the contrast effect increased for initial 15 min and remained almost constant up to 60 min. The subcutaneous injection induced delayed but significant contrast effect, and maximal contrast enhancement, comparable to that after intravenous injection, was obtained at 60 min. When Gd-BOPTA was injected subcutaneously into awake, the liver signal peaked at 30 min, followed by rapid reduction. The subcutaneous Gd-BOPTA injection increased liver signal in a dose-dependent manner and tended to improve the visualization of the liver border. A dose of 0.1 mmol/kg was required to identify the entire liver border easily and confidently in all mice. The injection of Gd-BOPTA causes significant contrast effect for the mouse liver. Subcutaneous injection can be substituted for intravenous injection although the waiting time is longer for subcutaneous injection. Washout from the liver is more rapid in awake mice than in mice under isoflurane anesthesia. The injection of 0.1 mmol/kg of Gd-BOPTA improves the visualization of the liver border and would aid the volumetry of the liver.

Diffusion tensor tractography of the corticospinal tracts in patients with arteriovenous malformations

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Diffusion tensor imaging (DTI) is a unique and relatively new technique to evaluate the integrity of the white matter. Diffusion tensor tractography (DTT) can visualize the white matter pathways according to diffusion tensor anisotropy, derived from DTI. This imaging technique has become progressively common in the past few years. Arteriovenous malformations (AVMs) are one of the major causes of hemorrhagic stroke in young population. Since rupture of AVM often causes miserable impairment, surgical, radiosurgical or neurointerventional treatment is usually required. Specific information about the coritcospinal tracts (CSTs) provided from DTI may be essential since knowing the precise location of the CSTs would enable safer treatment. Furthermore, in vivo evaluation of AVM and white matter enabled by DTI may contribute to the understanding embryological aspects of AVM. We performed MRI including diffusion tensor imaging in 20 patients who had their AVM near the CST were included in our study. Eleven patients had history of rupture of AVM. Eight patients had hemiparesis at the time of MRI. We used a free software (dTV/VOLUME-ONE) to visualize the CST based on DTT (DTT-CST). We evaluated the spatial relationships of the DTT-CST to the AVMs qualitatively and examined the correlation between the spatial relationships and clinical symptom. The study was designed to test the hypthesis that the DTT can predict patients clinical symptom in patients with AVMs.

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Research Hospital

Department of Laboratory Medicine
検査部

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

助教授 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 research 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-tumor 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 the 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 Ph[1] ALL by real-time PCR and nested RT-PCR techniques. In addition, we sequence the amplified products to provide information for the molecular resistance to STI571 treatment. We are now expanding target molecules to non-hematological disorder, which includes c-kit, PDGF-R genes that is associated with gastro-intestinal stromal cell tumor (GIST).

4. Developing quick & inclusive diagnosis system for infectious disease

Since the introduction of new therapeutic maneuver, host-pathogen interactions altered drastically and came into aspects, which resulted in altered recognition and molecular interaction of infected cells with immune cells. These alternations 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 for infectious diseases.

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 and finally started FCM analysis this year. Currently, we are conducting lymphocyte-subset analysis on a routine basis. We are planning to expand FCM analysis for hematological disorders in the near future.

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Research Hospital

Department of Applied Genomics
ゲノム診療部

Associate Professor Noriharu Sato, M.D., Ph.D.
Research Associate Naoyuki Takahashi, M.D., Ph.D.

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 in April, 2001 to support the translational researches of our hospital. We are studying whether expression profiles of CD4＋, CD8＋, CD14＋ and CD56＋ cells, respectively, in cord-blood transplanted patients could be useful to explain 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＋, CD14＋, 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 and Noriharu Sato, Reiko Sada[1], Momoyo Ohki[2], Kohichiro Tsuji[3], Koichiro Yuji[4], Yuhko Ogami[5], Masae Ono[6], Shiro Ikegawa[7], Toshihiro Tanaka[7], Mayumi Tamari[7], Tsuyoshi Sakamoto[8], Yoichi Furukawa[9] and Yusuke Nakamura[9]: [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]Laboratory of Molecular Medicine

At the 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 spinocerebellar ataxia, consanguineous marriage, familial adenomatous polyposis, von Hippel-Lindau syndrome, a client of suspected balanced chromosomal translocation and so on.

As an initial step to perform individualized medicine, Human Genome Center has started microarray analysis 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.

Research Hospital

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

Professor	Aikichi Iwamoto, M.D., D.M.Sc.		教授(併) 医学博士	岩 本 愛 吉
Lecturer	Fumitaka Nagamura, M.D., D.M.Sc.		特任講師 医学博士	長 村 文 孝
Clinical Associate	Seiichiro Kobayashi, M.D., D.M.Sc.		特任助手 医学博士	小 林 誠一郎

Division of Clinical Trial Safety Management (DCTSM) was established in 2001. The missions of DCTSM are divided into two areas. One is the risk management of research hospital, and the other is the support/monitoring of clinical studies. The staffs of DCTSM (doctors and nurses) are doing their work in collaboration with Translational Research Coordinators (TRC), which are organized by co-medical staffs, including 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, Mitsui Kobayashi

Prevention and management of medical accidents are the critical problem at Research Hospital. The demand to avoid and to reduce the accidents has been increasing year by year. One aim of the Research Hospital is to promote the translational researches, and reliance of the Hospital 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. When the urgent action is required, the meeting (Iryoujiko Kinkyu Taisaku Kaigi) is immediately held to discuss the tentative actions, to protect the involved patient and to promote the clinical circumstances. This meeting was held for 6 times in this year. Medical accidents and the responses of DCTM are reported in the Council of Risk Management in the Research Hospital, which is held monthly.

Educational seminars on risk management are required by the law 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. Although medical accidents were reported, no serious events with prolonged/irriversible influences were seen this year, and no suit had seen.

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 2005 to December 2005, we received seven entirely protocols and numerous

questions within the research hospital. All the protocols were either Phase I or Phase I/IIa studies. Pre-review of these protocols 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 clinical studies. Final decision should be made at the Institutional Review Boards. Furthermore, we performed these activities for other institutes. We received requirements from four 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. Thirty-seven 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.

Trend of Drug Approval on Hematological Malignancies in the U.S. and Japan

Fumitaka Nagamura

The trends of drug approval on hematological malignancies in the U.S. and in Japan were analyzed. The purpose of this study is to clarify the problems on the drug evaluation and approval methods for fastening approval periods and evaluating efficacies/safeties more precisely, especially in cases of entirely new concepts of drugs by the comparison of two countries. Forty-six drugs were approved in the U.S., and 43 were in Japan for hematological malignancies. Twenty-seven drugs were approved in both countries. Twenty-two of 27 drugs were approved earlier in the US, and the dates of approval were earlier in the U.S. (median: 46.0 Mo, mean: 54.7 Mo). These differences have not been shortened when compared in every 10-year period. In the U.S., eight drugs were approved as "Accelerated Approval". Seven of eight "accelerated approval" drugs were approved only in the U.S. However, only one drug approved as "accelerated approval" could have shown its clinical benefit in the designated clinical trial. The ratio of non-U.S. studies was considerably low in hematological malignancies (7.0%) when compared with all oncologic drugs (23.7%). Five drugs approved only in Japan were approved in the US for diseases other than hematological malignancies, while no drug was approved in the reverse case. Accelerated approval is considered to be useful for fastening the period until approval, although the problem, that "surrogate markers" could predict the "survival and/or QOL benefit", has not been clarified, yet. The outstanding result that most of pivotal studies were "not non-U.S." study may be caused by the superiority of drug development, especially in new era of drugs for hematological malignancies and the ability to conduct the appropriate clinical trials in the U.S., or approval based on "accelerated approval". On the contrary, the expansion of the indication would be the problem in the U.S.

Activities of Translational Research Coorinator (TRC) and clinical Research Coorinator (CRC)

Hajime Kotaki, Fumitaka Nagamura, Momoyo Ohki, Miho Tabata, Kumiko Sumino.

The activities of research coordinators are important to conduct clinical studies smoothly and to manage the relationship with participants. In TR, sufficient concerns on the rights and the understandings of participants themselves should be paid compared with other clinical researches. TRCs have been organized to solve these problems, and they consist of nurse, pharmacist, psychologist, dietician and clinical laboratory technologist. DCTSM collaborates with chief of TRC, Director of Pharmacy, on the activities of TRC.

Exclusive CRCs belong DCTSM and take part in clinical trials from pharmaceutical companies and medical doctor-initiative studies to maintain GCP requirements.

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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) (3). Our on-going researches are (1) Analysis of immune reconstitution post-stem cell transplantation and cancer patients, (2) Expansion of T, NK and NKT cells for GVL/T effect and expansion of regulatory T cells for prevention of GVHD after HSCT.

(1) Analysis of immune reconstitution post stem cell transplantation and cancer patients

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Immune reconstitution following unrelated cord blood transplantation (UCBT) in adult patients is of great concern because of immaturity of cord blood immunological cells. We analyzed the twenty-six adult patients (15 to 58 year-old) with hematological malignancies, who underwent UCBT and sustained engraftment were enrolled in this study. Infused number of immunological cells in thawed CB units including T cells (CD3+), B cells (CD19+), NK cells (CD3-CD56+), monocyte (CD14+) and also CD34+ cells was analyzed using bead-contained TRUCOUNT tube (BD, CA). Dead cells after thawing were excluded by gating out with 7AAD dye. Immune reconstitution was analyzed every 30 days by 120 days after CBT. Four-color FACS Caliber and TRUCOUNT tube were utilized to calculate the absolute number of immune cells concentration in blood after UCBT. We put strict volume of 50ml fresh unmanipulated blood in each TRUCOUNT tube. RESULTS: Thawed-transplanted NC $2.3\pm\times10^7$/kg, CD34 was $0.72\pm0.3\times10^5$/kg (4.1×10^6 total), T cells; $3.1\pm1.6\times10^6$/kg with CD4/8 ratio of 3.2 ± 2.0, B cells; $1.2\pm0.5\times10^6$/kg, NK cells; $1.0\pm0.5\times10^6$/kg and monocyte; $1.6\pm0.6\times10^6$/kg. There were no correlations between infused CD34+ cells number and T, B, NK and monocyte numbers. Monocyte increased in blood rapidly after CBT at 30 days, then, declined to the normal value. NK cells was recovered in the early after CBT and then did not so change in number from 30 to 120 days after CBT, while T cells increased time dependent manner, and B cells appeared late but influenced by acute GVHD grade. Within 120 days after CBT, T cells showed also CD4+dominant in most cases with relatively high CD25+CD4+ regulatory T (rT) cells compared to normal control. The patients with grade II to IV aGVHD showed significantly higher number of rT cells on 30 days ($P<0.05$) compared to those with

grade 0-I aGVHD. On day 30, the number of rT cells showed 7.7±5.9/ l in grade 0-I aGVHD and 19.4±13.3/ml in grade II-IV. The patients with grade II to IV aGVHD showed significant delayed recovery of B cells on 90 days after CBT compared to those with 0-I aGVHD ($P < 0.001$). Conclusively aGVHD in adult patients may influence on the number of regulatory T cells in the early period after UCBT and delayed recovery of B cells. This technique is also useful for the monitoring of immune cells after immune therapy after HCST.

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

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Cord blood transplantation (CBT) is rapidly increasing in number. However, graft failure, viral infections such as CMV and the relapse after CBT remains 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 CD25+CD4+T (rT) expansion using the patient peripheral blood after CBT.

(3) 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 project 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) accompanied with clinical clean room and P3 facilities 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.

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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.

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Research Hospital

Core Facility for Therapeutic Vectors
治療ベクター開発室

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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.

2. Production of Master Virus Seed stock (MVSS)

We have prepared the cGMP compliant MVSS which contained 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.

3. 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.

4. 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.

5. Acceptance of projects

(1) Takuya Takayama, Hideaki Tahara
Cancer gene therapy using IL-12-transduced dendritic cells.
(2) Marimo Sato, Takuya Takayama, Hideaki Tahara
Vaccine therapy with peptide-loaded dendritic cells for advanced melanoma
(3) Tomoki Todo, Yasushi Ino
Oncolytic virus therapy using genetically engineered herpes simplex viruses for malignant brain tumors.

6. Support

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