Research Hospital

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Department of Advanced Medical Science was established in September 1997. Our aim is to contribute to the performance and the development of advanced therapeutic approach to the diseases. We have been participating in the potentially important clinical trials and the several projects in line with our principles. 1) First, we participated in the peptide vaccine clinical trial at IMSUT hospital. Our research projects were 2) analysis the role of newly identified non-coding RNA, 3) Analysis of the Gradient Expression of Genes in Human Colonic Mucosa and 4) Gene expression profiles of in acute graft-versus-host disease following cord blood transplantation.

Peptide vaccine clinical trial

1-1) Human leukocyte antigen (HLA)-A*2402/A*0201-restricted vascular endothelial growth factor receptor type 1 (VEGFR1)-specific peptide vaccination for advanced pancreatic cancer (clinical phase I/II trial, ClinicalTrials.gov Identifier NCT 00683358 & NCT00683085)

Nagayama H. et al.

1-2) Human leukocyte antigen (HLA)-A*0201/-A*2402 restricted peptide vaccine therapy in patients with esophageal or gastric cancer (Clinical phase I/IIa trial, ClinicalTrials. gov Identifier NCT00681421 & NCT00681330, NCT00681421 & NCT 00681330)

Ohno H. et al.

Laboratory of Molecular Medicine, Division of Advanced Clinical Proteomics, Human Genome Center, IMSUT (Prof. Nakamura's Laboratory) has identified several new epitope-peptides that were up-regulated in several kinds of cancers. Based on these data, phase I/IIa clinical trial to cancer patients were developed by Nakamura Laboratory. Among these trials, we are engaged in the clinical trials on the patients with either esophageal, gastric or pancreatic cancer from May 2008 at IMSUT hospital.

Outline of the clinical trial

Purpose: Feasibility and efficacy of combined modality intervention using tumor-associated/tumor vessel-specific peptide vaccination with or without chemotherapeutic agents in case of advanced/inoperable or therapy-resistant cancer patients. All peptides are restricted to HLA-A*2402 or HLA-A*0201, both are common HLA alleles among the Japanese human population.

Primary endpoints: Safety of peptide vaccination (phase I) and time to progression of cancer (phase II). As these trial are phase I/IIa study to confirm the feasibility, eligibility criteria is restricted to the end-stage cancer patients with no expected alternative therapy.
Secondary endpoints: Immune response (ELISPOT, Perforin/FoxP3 FACS, in vitro CTL assay etc.) & Tumor regression (basically based on the RECIST criteria).
Design: vaccination of peptides, emulsified with incomplete Freund adjuvant is planned two times weekly for 8 weeks with or without standardized chemotherapy.
Maximum Enrollment: 14. Entry period: one year.

cancer	HLA	peptides	Adjuvant chemotherapy
Esophageal cancer	A2402	URLC10, TTK, KOC1	none
	A0201	URLC10, VEGFR1/R2	none
Gastric cancer	A2402	URLC10, KOC1, VEGFR1/R2	TS-1
	A0201	URLC10, VEGFR1/R2	TS-1
Pancreatic cancer	A2402	VEGFR1	GEM
	A0201	VEGFR1	GEM

2. Analysis of *Dnm3os*, a non-coding RNA for skeletal development

Nakaoka T. et al.

Dnm3os, a gene transcribed into a non-coding RNA (ncRNA), contains three micro RNAs; miR-199a, miR-199a* and miR-214, whose functions remain entirely unknown in mammals. We introduced the lacZ gene into the *Dnm3os* locus to recapitulate its expression pattern and disrupt its function in collaboration with Department of Physiological Chemistry and Metabolism, Division of Biochemistry and Molecular Biology, University of Tokyo. *Dnm3os*$^{+/lacZ}$ heterozygous embryos showed α-galactosidase activity, which reflected the authentic expression pattern of *Dnm3os* RNA. Most of the *Dnm3os*$^{lacZ/lacZ}$ homozygous pups died within one month of birth. After birth, *Dnm3os*$^{lacZ/lacZ}$ mice exhibited several skeletal abnormalities, including craniofacial hypoplasia, defects in dorsal neural arches and spinous processes of the vertebrae, and osteopenia. Importantly, the expression of miR-199 a, miR-199a*, and miR-214 was significantly down-regulated in *Dnm3os*$^{lacZ/lacZ}$ embryos, supporting the assumption that Dnm3os serves as a precursor of these three miRNAs. Now, the project to investigate the molecular mechanisms responsible for several abnormalities in *Dnm3os*$^{lacZ/lacZ}$ mice is under way.

3. Analysis of the Gradient Expression of Genes in Human Colonic Mucosa

Ohno H. et al.

Ulcerative colitis 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. First, we selected the genes whose expression levels were reported to increase toward the distal colon. Next, 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, some genes showed the expression gradient to increase toward the distal colon. We have generated rabbit polyclonal antibodies against the protein encoded by the gene. We are currently examining where the protein localize in the distal colonic mucosa.

4. Gene expression profiles of peripheral blood mononuclear cell subpopulations in acute graft-versus-host disease following cord blood transplantation

Takahashi N.

Compared with allogeneic hematopoietic stem cell transplantation using other sources, cord blood (CB) transplantation (CBT) has clinical advantages in terms of incidence and severity of acute graft-versus-host disease (GVHD) despite using allogeneic stem cells with more human leukocyte antigen mismatches. However, detailed pathophysiology of acute GVHD developed after CBT has not yet been elucidated.

We performed microarray expression profiling of immunoregulatory genes on each of 4 subpopulations ($CD4^+$, $CD8^+$, $CD14^+$, and $CD56^+$) of peripheral blood mononuclear cells (PBMCs), which were taken from 8 patients with hematologic malignancies who suffered from acute GVHD after unrelated CBT. We identified 55 genes, which were differentially expressed during acute GVHD compared to recovery phase. Among them, 22 showed differential expression concurrently in multiple PBMC subpopulations. In particular, 5 genes (TRAIL, IL1RN, IFI27, GZMB, and CCR5) were up-regulated and 3 genes (CLK1, TNFAIP3 and BTG1) were down-regulated in at least 3 out of 4 subpopulations

during acute GVHD. These 8 genes seem to be candidates which may play key roles common to multiple subpopulations in the pathophysiology of acute GVHD. In addition, down-regulation of anti-inflammatory factors, such as TNFAIP3, KLF2, ZFP36, and BTG1, seems to be involved in acceleration of immune response, thus exacerbation of acute GVHD. Further study is required to clarify whether therapeutic augmentation of these factors may ameliorate acute GVHD. Meanwhile, differential expression of several genes, such as CCL5, TNFAIP3, CD161, CD160, and COX2, was assumedly affected by the developmental immaturity of CB-derived cells and this might be somehow involved in the relatively low incidence and low severity of acute GVHD following CBT.

Publications

Takahashi N, Sato N, Takahashi S, Tojo A. Gene-expression profiles of peripheral blood mononuclear cell subpopulations in acute graft-vs-host disease following cord blood transplantation. *Exp. Hematol.* 36: 1760-1770, 2008.

Fujita S, Yamashita N, Ishii Y, Sato Y, Sato K, Eizumi K, Fukaya T, Nozawa R, Takamoto Y, Yamashita N, Taniguchi M, Sato K. Regulatory dendritic cells protect against allergic airway inflammation in a murine asthmatic model. *J Allergy Clin Immunol.* 121: 95-104, 2008.

Watanabe T, Sato T, Amano T, Kawamura Y, Kawamura N, Kawaguchi H, Yamashita N, Kurihara H, Nakaoka T. *Dnm3os*, a Non-coding RNA, is Required for Normal Growth and Skeletal Development in Mice. *Dev. Dyn.* 237: 3738-48, 2008.

Yoshikumi Y, Ohno H, Suzuki J, Isshiki M, Morishita Y, Ohnishi H, Yasuda H, Omata M, Fujita T, Mashima H. Up-regulation of JAM-1 in AR42J cells treated with activin A and betacellulin and the diabetic regenerating islets. *Endocr J.* 55: 757-65, 2008.

Mashima H, Suzuki J, Hirayama T, Yoshikumi Y, Ohno H, Ohnishi H, Yasuda H, Fujita T, Omata M. Involvement of vesicle-associated membrane protein 7 in human gastric epithelial cell vacuolation induced by Helicobacter pylori-produced VacA. *Infect Immun.* 76: 2296-303, 2008.

Sato K, Eizumi K, Fukaya T, Fujita S, Sato Y, Takagi H, Yamamoto M, Yamashita N, Hijikata A, Kitamura H, Ohara O, Yamasaki S, Saito T, and Sato K. Naturally occurring regulatory dendritic cells regulate murine cutaneous chronic graft-versus-host disease. *Blood*, in press, 2009.

Yoshiura K, Nishishita T, Nakaoka T, Yamashita N, and Yamashita N. Inhibition of B16 melanoma growth and metastasis in C57BL mice by vaccination with a syngenic endothelial ceu line. J Exp Cancer Res in press, 2009

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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 200 cases of cord blood transplantation (CBT) for adult patients, which appears a distinguished experience in the world. Recent advance 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 (ABL kinase inhibitor) for CML, rituximab (chimeric anti-CD20 monoclonal antibody) for B cell lymphoma as well as bortezomib (proteasome inhibitor) for multiple myeloma. We extensively apply these molecular targeted therapies for in- and out-patients and are to be involved in clinical trial of newly developed agents including nilotinib (2nd generation ABL inhibitor) and ICL670 (oral iron chelator).

1. Myeloablative cord blood transplantation in adults with AML.

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

We analyzed the disease-specific outcomes of adult acute myelogenous leukemia (AML) patients treated with unrelated cord blood transplantation (CBT) after myeloablative conditioning. Between August 1998 and February 2008, 77 adult patients with AML were treated with unrelated CBT. All patients received 4 fractionated 12 Gy total body irradiation (TBI) and chemotherapy as myeloablative conditioning. The median age was 45 years, the median weight was 55kg, the median number of nucleated cells was 2.44×10^7/kg, and the median number of

CD34-positive cells was 1.00×10^5/kg. All patients received a single and HLA mismatched cord blood unit. The cumulative incidence of neutrophil recovery at day 50 and platelet recovery at day 200 was 94.8% and 91.7%, respectively. A higher CD34-positive cell dose was associated with faster hematopoietic recovery. The cumulative incidence of grade III to IV acute graft-versus-host disease (aGVHD) and extensive-type chronic GVHD (cGVHD) was 25.1% and 28.6%, respectively. With a median follow-up of 78 months, the probability of event-free survival (EFS) at 5 years was 62.8%. The 5-year cumulative incidence of treatment related-mortality (TRM) and relapse was 9.7%, 25.8%, respectively. In multivariate analyses, the risk factor identified for event free survival (EFS) was disease status and cytogenetics. These results suggest that unrelated CBT after myeloablative conditioning could be safely and effectively used for adult patients with AML.

2. Myeloablative cord blood transplantation in adults with ALL.

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

We analyzed the disease-specific outcomes of adult ALL treated with cord blood transplantation (CBT) after myeloablative conditioning. Between October 2000 and November 2007, 27 adult patients with ALL were treated with unrelated CBT. All patients received four fractionated 12 Gy TBI and chemotherapy as myeloablative conditioning. The median age was 36 years, the median weight was 57kg and the median number of nucleated cells was 2.47×10^7/kg. All patients received a single and HLA-mismatched cord blood unit. The cumulative incidence of neutrophil recovery at day 30 and platelet recovery at day 200 was 92.6 and 92.3%, respectively. With a median follow-up of 47 months, the probability of EFS at 5 years was 57.2%. The 5-year cumulative incidence of TRM and relapse was 3.7 and 27.4%, respectively. These results suggest that unrelated CBT after myeloablative conditioning could be safely and effectively used for adult patients with ALL.

3. Myeloablative unrelated cord blood transplantation for acute leukemia patients between 50 and 55 years of age.

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

Increasing recipient age is a well-known risk factor for graft-versus-host disease (GVHD) and treatment-related mortality (TRM) and has a negative impact on allogeneic hematopoietic stem cell transplantation. Since the incidence of severe GVHD after cord blood transplantation (CBT) is lower than that after transplants using bone marrow or mobilized peripheral blood grafts from adult cells, we should expect better outcomes from CBT in older patients. To evaluate the feasibility and efficacy of myeloablative unrelated CBT in patients aged between 50 and 55 years, we performed a retrospective comparison of 100 patients with acute leukemia who received cord blood grafts at our institution. Nineteen older patients (median age, 52; range, 50-55) and 81 younger patients (median, 36; range, 16-49) received a myeloablative conditioning regimen including 12 Gy of total body irradiation and chemotherapy. GVHD prophylaxis included cyclosporine with (n=96) or without (n=4) methotrexate. There were no significant differences in the incidences of grades II to IV acute GVHD, extensive-type chronic GVHD, TRM, and the probability of overall and disease-free survival between these groups. These results suggest that, in patients with acute leukemia, myeloablative CBT might be as safe and effective in patients aged between 50 and 55 years as in younger patients.

4. Second myeloablative stem cell transplantation (SCT) using cord blood for leukemia relapsed after initial allogeneic SCT.

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

There are many reports of second allogeneic stem cell transplantation (allo-SCT) using cord blood (CB) for graft failure after initial allo-SCT. However, the efficacy of second allo-SCT using CB for patients with leukemia relapsed after initial allo-SCT is unknown. We report the results of second allo-SCT using CB in seven adult patients with leukemia relapsed after initial allo-SCT. All patients received a myeloablative conditioning regimen including oral busulfan 16mg/kg, intravenously fludarabine 100mg/m^2 and cyclophosphamide 120mg/kg. All but one patient had myeloid reconstitution and four patients remain alive at between 4 and 40 months after second SCT. We conclude that second myeloablative allo-SCT using CB may be feasible in selected patients with the relatively younger age, less organ damage and longer time interval between first and second allo-SCT.

5. Pneumocystis carinii pneumonia in myeloablative unrelated cord blood transplantation.

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

The incidence of pneumonia caused by Pneumocystis carinii (PCP) (organism now renamed Pneumocystis jiroveci) during the early period after cord blood transplantation (CBT) was studied in 120 adults. Initially 89 patients (74%) received oral administration of 2 single-strength trimethoprim-sulfamethoxazole (TMP-SMZ) tablets twice daily from day -21. In 45 of 89 patients (51%), TMP-SMZ administration for a scheduled duration was completed. In the remaining 44 patients (49%), however, TMP-SMZ administration was discontinued prior to day -3 because of toxicity. Among these patients, 42 subsequently received aerosolized pentamidine (AP) on a median of day -13 (range, -20 to -6). Thirty-one patients (26%) received AP without TMP-SMZ administration on a median of day -14 (range, -21 to -9). None of the 120 patients were diagnosed with PCP within 100 days or 2 years after CBT; however, one patient who received AP before CBT but no prophylaxis after CBT developed cerebral toxoplasmosis on day +91. Pre-transplant prophylaxis against PCP did not significantly affect transplantation-related mortality or disease-free survival at 2 years after CBT. The results suggest that PCP during the early period after CBT can be effectively prevented by any pre-transplant prophylactic method.

6. Early renal injury after myeloablative cord blood transplantation in adults.

Mae H, Ooi J, Takahashi S, Tomonari A, Tsukada N, Tojo A.

We report a retrospective analysis of acute renal failure (ARF) in a group of 54 adult patients with hematological malignancies treated with unrelated cord blood transplantation (CBT) after myeloablative conditioning. All patients received four fractionated 12 Gy total body irradiation and chemotherapy as myeloablative conditioning. ARF was defined as the doubling serum creatinine occurring within the first 100 days after CBT. A statistically significant decrement of renal function from baseline was observed in days between 11 and 20. ARF occurred in 27.8% of patients. Although no difference was seen in maximum cyclosporine trough levels, the maximum of vancomycin (VCM) trough levels were significantly higher in patients with ARF ($p=0.01$). Our result suggests that it is important to monitor VCM dosing more strictly with pharmacokinetic assessment, especially in days 11-20, when the most frequently observed declining renal function.

7. Cardiovascular toxicity of cryopreserved cord blood cell infusion.

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

Although infusion of cryopreserved bone marrow or peripheral blood stem cell is associated with a variety of symptoms, there have been no reports detailing the data of infusion-related toxicities of cryopreserved cord blood (CB) units. We prospectively evaluated the incidence and significance of infusion-related toxicities in 34 adult patients undergoing unrelated CB transplantation. Cryopreserved CB units were thawed and immediately infused, unfiltered, through a central intravenous catheter without further manipulation. Heart rate, blood pressure, oxygen saturation and clinical symptoms were recorded during and after infusion. Twenty-four percent of patients experienced non-cardiovascular toxicities related to infusion. The incidence of systolic and diastolic hypertension and bradycardia was 58, 64 and 32%, respectively. Although three patients (9%) with severe systolic hypertension after the infusion required treatment with antihypertensive agents, no patients experienced life-threatening side effects or needed discontinuation of CB unit infusion. Patient or transplant characteristics had no effect on the hypertension and bradycardia related to the infusion of CB. These data suggest that infusion of cryopreserved CB without further manipulation after thawing is safe and well tolerated. However, cardiovascular toxicities including hypertension and bradycardia were frequently observed.

8. Impact of CMV serostatus on outcome of unrelated cord blood transplantation in adults.

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

Cytomegalovirus (CMV) disease is one of the major infectious complications after allogeneic hematopoietic stem cell transplantation (SCT). Several studies have shown that CMV-seropositive patients have a substantial survival disadvantage after bone marrow transplantation (BMT) or peripheral blood SCT (PBSCT). Between August 1998 and February 2006, 101 adult patients underwent myeloablative cord blood transplantation (CBT) from unrelated donors at our institution. Sixteen and 85 patients were CMV-seronegative and CMV-seropositive,

respectively, prior to CBT. Outcomes of CBT were compared between CMV-seronegative and CMV-seropositive patients. The cumulative incidences of neutrophil engraftment at 60 d after CBT did not differ between CMV-seronegative and CMV-seropositive patients (100% and 94%, P=0.09); however, the cumulative incidence of platelet engraftment at 100 d was higher in CMV-seronegative patients than CMV-seropositive patients (100% vs. 86%, P<0.005). The cumulative incidence of CMV antigenemia at 100 d was lower in CMV-seronegative patients than CMV-seropositive patients (0% vs. 77%, P<0.001); however, the cumulative incidences of CMV disease did not differ between CMV-seronegative and CMV-seropositive patients (0% vs. 1%, P=0.84). The probabilities of disease-free survival at 2 yr also did not differ between CMV-seronegative and CMV-seropositive patients (92% vs. 72%, P=0.16). The outcomes of CBT for CMV-seropositive patients as well as CMV-seronegative patients in our series were favorable. This might be due to effective antiviral therapy for CMV infection. Large-scale studies are needed to determine the impact of recipient CMV serostatus on the outcome of CBT for adults.

9. Factors predisposing to HTLV-1 infection in residents of the greater Tokyo area.

Uchimaru K, Tojo A

Human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent for adult T-cell leukemia. The geographic distribution of HTLV-1 carriers is quite uneven in Japan and the greatest prevalence is in southwestern Japan. Because many people move from endemic areas to the greater Tokyo area, the geographic distribution might have changed. Therefore, we investigated the factors predisposing to HTLV-1 infection, including birthplace, for 88 HTLV-1-infected individuals in greater Tokyo who visited our outpatient clinic. Of these, 39.5% were born in endemic areas, which include Kyushu/Okinawa, south Shikoku, Kii, Tohoku, and Hokkaido, whereas 38.3% were born in greater Tokyo and the proportion is presumed to be increasing. Half of the HTLV-1 infected individuals in greater Tokyo came from endemic areas, whereas around half of the remaining half was presumed to be involved in sexual transmission from a spouse from an endemic area. Overall, they constituted approximately 70% of the HTLV-1 carriers in greater Tokyo. These migration effects may increase the prevalence of HTLV-1 in the greater Tokyo area; nationwide surveillance is warranted.

Publications

Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Kato S, Sato A, Monma F, Kasahara S, Uchimaru K, Iseki T, Tojo A, Asano S. Second myeloablative allogeneic stem cell transplantation (SCT) using cord blood for leukemia relapsed after initial allogeneic SCT. Leuk Res. 2008 Nov 6. [Epub ahead of print]

Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Kato S, Sato A, Monma F, Hongo E, Uchimaru K, Tojo A, Asano S. Donor cell-derived myelodysplastic syndrome after cord blood transplantation. Bone Marrow Transplant. 2008 Nov 3. [Epub ahead of print]

Ooi J, Takahashi S, Tomonari A, Tsukada N, Konuma T, Kato S, Kasahara S, Sato A, Monma F, Nagamura F, Iseki T, Tojo A, Asano S. Unrelated cord blood transplantation after myeloablative conditioning in adults with ALL. Bone Marrow Transplant. 2008 Oct 27. [Epub ahead of print]

Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kato S, Kasahara S, Iseki T, Tojo A, Asano S. No occurrence of Pneumocystis jiroveci (carinii) pneumonia in 120 adults undergoing myeloablative unrelated cord blood transplantation. Transpl Infect Dis. 2008 Jun 18. [Epub ahead of print]

Tamai1 H, Yamaguchi H, Takahashi S, Tojo A, Hamaguchi H, Kobayashi T, Akiyama H, Sakamaki H, Okumura H, Nakao S, Arai A, Miura O, Inokuchi K, Dan K. Treatment of adult acute myeloid leukaemia (AML) with t (6; 11)(q27; q23) by allogeneic haematopoietic stem cell transplantation (allo-HSCT) in first complete remission (CR). Bone Marrow Transplant, 42: 553-4, 2008

Nagamura-Inoue T, Kai S, Azuma H, Takanashi M, Isoyama K, Kato K, Takahashi S, Taniguchi S, Miyamura K, Aoki1 K, Hidaka1 M, Nagamura F, Tojo A, Fang1 XM, Kato S for Japan Cord Blood Bank Network. Unrelated cord blood transplantation in chronic myelogenous leukaemia: Japan Cord Blood Bank Network analysis. Bone Marrow Transplant. 42: 241-51, 2008

Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kato S, Kasahara S, Iseki T, Tojo A, Asano S. Blood eosinophilia after unrelated cord blood transplantation for adults. Bone Marrow Transplant. 42: 63-5, 2008

Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Kato S, Kasahara S, Uchimaru K, Tojo A. Central nervous system leukemia on magnetic resonance imaging. Intern Med. 47: 477, 2008

Watanabe N, Takahashi S, Ishige M, Ishii Y, Ooi J, Tomonari A, Tsukada N, Konuma T, Kato S, Sato A, Tojo A, Nakauchi H. Recipient-derived cells after cord blood transplantation: dynamics elucidated by multicolor FACS, reflecting graft failure and relapse. Biol Blood Marrow Transplant. 14: 693-701, 2008

Mae H, Ooi J, Takahashi S, Tomonari A, Tsukada N, Konuma T, Hongo E, Kato S, Kasahara S, Oiwa-Monna M, Kurokawa Y, Tojo A, Asano S. Early renal injury after myeloablative cord blood transplantation in adults. Leuk Lymphoma. 49: 538-42, 2008

Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Kato S, Kasahara S, Uchimaru K, Iseki T, Tojo A, Asano S. Myeloablative unrelated cord blood transplantation for adult acute myeloid leukemia patients with 11q23 abnormalities. Eur J Haematol. 80: 545-8, 2008

Tamai H, Yamaguchi H, Hamaguchi H, Yagasaki F, Bessho M, Kobayashi T, Akiyama H, Sakamaki H, Takahashi S, Tojo A, Ohmine K, Ozawa K, Okumura H, Nakao S, Arai A, Mitura O, Toyota S, Gomi S, Murai Y, Usui N, Miyazawa K, Ohyashiki K, Takahashi N, Sawada K, Kato A, Oshimi K, Inokuchi K, Dan K. Clinical features of adult acute leukemia with 11q23 abnormalities in Japan: multicenter co-operative study. Int J Hematol. 87: 195-202, 2008

Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Kobayashi T, Sato A, Kato S, Kasahara S, Ebihara Y, Nagamura-Inoue T, Tsuji K, Tojo A, Asano S. Cardiovascular toxicity of cryopreserved cord blood cell infusion. Bone Marrow Transplant. 41: 861-5, 2008

Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kato S, Kasahara S, Iseki T, Yamaguchi T, Tojo A, Asano S. Impact of cytomegalovirus serostatus on outcome of unrelated cord blood transplantation for adults: a single-institute experience in Japan. Eur J Haematol. 80: 251-7, 2008

Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kobayashi T, Takasugi K, Iseki T, Tojo A, Asano S. Preemptive therapy with ganciclovir 5mg/kg once daily for cytomegalovirus infection after unrelated cord blood transplantation. Bone Marrow Transplant. 41: 371-6, 2008

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Founded in 1981, Department of Infectious Diseases and Applied Immunology (DIDAI) started HIV clinic in 1986. In 2008, 59 new patients with HIV infection have visited or been admitted to our hospital and 399 patients in total are currently under our clinical management. The total number of in-patients with HIV-infection during 2008 was 31, and 5-7 beds in our ward have been constantly occupied by patients with not only HIV-infection but also other infectious diseases. 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 and the Department of Infectious Disease Control join the clinic. IMSUT hospital provides the most up-to-date medical treatment to HIV-infected patients in Japan. DIDAI is also a treatment center for international infectious diseases such as malaria and typhoid fever.

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

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

Takeshi, Fujii, Tokiomi Endoh, Tomohiko Koibuchi[1], Toshiyuki Miura[2], Hitomi Nakamura[1], Michiko Koga[1], Takuya Maeda[2], Tadashi Kikuchi, Takashi Odawara[1], and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Department of Infectious Disease Control, International Research Center for Infectious Diseases

59 new patients with HIV-1 infection visited our hospital this year (from January 1 to Decem-

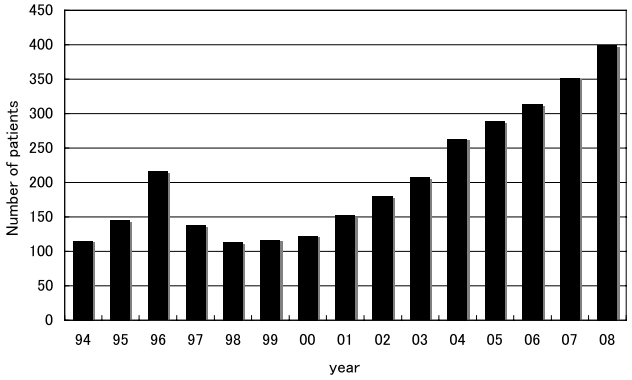

Figure 1. Number of HIV-infected outpatients in IMSUT Hospital

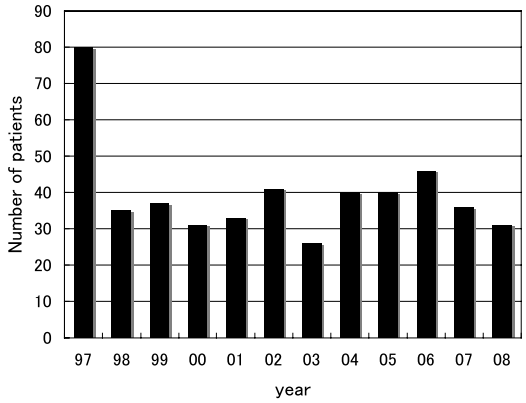

Figure 2. Number of HIV-infected inpatients in IMSUT Hospital

ber 31, 2008), and 399 patients in total are under medical management in our outpatient clinic. The total number of HIV-infected in-patients during 2008 was 31. The number of total patients declined in 1997 because a part of patients as well as medical stuffs moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again after 1998 in accordance with Japanese statistics of HIV-infected patients (Fig. 1). In contrast, the number of admission has decreased since 1997 and stable over the last decade (Fig. 2) because of the introduction of highly active anti-retroviral therapy (HAART) which effectively suppresses the replication of HIV. Anti-retroviral therapy has been introduced to around 280 HIV-infected patients in our hospital, and most of their HIV viral loads have been well controlled. After one year of HAART, the viral loads become less than 400 copies/ml in more than 90% of patients, and their CD4 cell counts increase by approximately 200/μL in average. Consequently, the clinical management of HIV-infected patients changed from how to treat opportunistic infections into how to control patients with HAART.

2. Treatments and Clinical Research of Tropical Diseases

a. Treatment of Tropical Diseases in IMSUT hospital

Takeshi, Fujii, Tokiomi Endoh, Tomohiko Koibuchi[1], Takuya Maeda[2], Toshiyuki Miura[2], Tadashi Kikuchi, Takashi Odawara[1], and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Department of Infectious Disease Control, International Research Center for Infectious Diseases

This year, more than hundred of overseas travelers visited our clinic. The reasons of their visit included prescription of malaria prophylaxis, hepatitis A/B vaccination, other general health consultation, or treatment of tropical diseases such as malaria, amoebic liver abscess, and post-exposure prophylaxis of rabies.

Supported by Department of Pharmacy in our hospital, we are managing the clinical use of orphan drugs for the treatment of tropical diseases in Japan. We take the consultation from the doctors facing tropical diseases all over Japan, and provide them with the appropriate medicines for the treatment of tropical diseases. In 2008, 106 patients were treated with the orphan drugs we provided throughout Japan.

b. Unusual radiological findings of Fasciola hepatica infection with huge cystic and multilocular lesions

Takuya Maeda[2], Takashi Odawara[1], Takeshi, Fujii, Tomohiko Koibuchi[1], and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Department of Infectious Disease Control, International Research Center for Infectious Diseases

We reported a case of hepatic phase Fasciola hepatica infection presenting huge and multilocular lesions. The unique radiological findings mimicked hydatid diseases and also cystic liver neoplasm. Fascioliasis should be included in the differential diagnosis for cystic liver diseases.

3. Development of an early detection method for pathogens causing acute respiratory infection

Takuya Maeda[2], Tadashi Kikuchi, Tokiomi Endoh, Tomohiko Koibuchi[1], Takashi Odawara[1], Aikichi Iwamoto[1], and Takeshi Fujii: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Department of Infectious

Disease Control, International Research Center for Infectious Diseases

To develop a rapid detection method for pathogens causing acute respiratory infection, we have employed Loop-mediated isothermal amplification (LAMP), which is capable of amplifying nucleic acids under isothermal conditions with high specificity and efficiency. Previously we developed appropriate primers for the detection of each pathogen *in vitro.* The target pathogens included RNA viruses causing respiratory infection such as influenzavirus, respiratory syncytial virus (RSV) and human coronavirus. Last year we tested the feasibility of this method for clinical samples, i.e., 236 nasal swab samples (remnants of immunochromatography (IC) test for influenza at clinics), and could detect influenzavirus A or B from 137 samples (58.1%), which was 1.5 times more sensitive than IC detection. These results showed the potential clinical feasibility of genetic diagnostic tools with high specificity and efficiency, but RNA extraction step requires cost and time. This year we tested an RT-LAMP method without RNA extraction and purification (we called this method 'Direct-LAMP') from clinical nasopharyngeal-swab specimens for the detection of influenza virus. Nasopharyngeal swab specimens were collected from 104 Japanese outpatients (age range 0-13 years) presenting influenza or influenza-like illnesses. The samples were tested immediately with commercial IC kits to detect the antigens of influenza A/B virus at clinics. The remaining samples stored at $-4^{\circ}C$ were sent to our laboratory and used for genetic diagnosis. Although the conventional RT-LAMP method demonstrated the highest sensitivity and specificity in comparison with the real-time PCR and IC test, "Direct LAMP" could detect influenza genomes in 51 (94.4%) of 54 positive samples of conventional RT-LAMP. The specificity was also as high as conventional RT-LAMP method. "Direct LAMP" method developed in this study would be useful for clinical diagnosis of respiratory RNA viral diseases, especially in those countries where only minimal laboratory facilities are available and inexpensive techniques are desired.

4. Comprehensive preoperative evaluation of patients with hemophiliac arthropathy

Tomohiko Koibuchi[1], Tokiomi Endoh, Takuya Maeda[2], Takeshi, Fujii, Takashi Odawara[1], and Aikichi Iwamoto[1]: [1] Division of Infectious Diseases, The Advanced Clinical Research Center, [2] Department of Infectious Disease Control, International Research Center for Infectious Diseases

In collaboration with the Department of Joint Surgery we evaluate medical status of patients with hemophiliac arthropathy who are going to be given surgery. The majority of hemophiliac patients who had received factor concentrates in the early 1980s were infected with hepatitis C virus (HCV). A considerable percentage of them were also infected with Human immunodeficiency virus (HIV). Among the hemophiliac patients who had orthopedic surgery in 2008, 85% were HCV-Ab-positive and 30% were HIV-positive. Appropriate preoperative evaluation of liver function and immunological status is essential to reduce the morbidity associated with the surgery and improve the clinical outcomes of these patients.

We have developed a comprehensive preoperative assessment system with a flow chart to evaluate the liver function and immunological status of hemophiliac patients. The flow chart employs several indices for evaluation, such as CD4 cell count, ICG retention test, prothrombin time, fibrosis markers (type IV collagen, hyaluronic acid), the finding of abdominal ultrasound and Child-Pugh score. Enhanced abdominal CT and/or upper gastrointestinal endoscopy are also required when we suspect the existence of hepatocellular carcinoma or esophageal varix. Based on the result of these indices, our multidisciplinary team assesses the risks and benefits of the surgery. The check-up system by the multi-disciplinary team using the flow chart has improved the care of the hemophiliac patients undergoing surgical operations.

Publications

1. Miyazaki E, Kawana-Tachikawa A, Tomizawa M, Nunoya J, Odawara T, Fujii T, Shi Y, Gao FG, Iwamoto A. Highly restricted TCR repertoire in the CD8+ T-cell response against an HIV-1 epitope with a stereotypic amino acid substitution. AIDS. In press.
2. Hou W, Aoki C, Yu L, Wen X, Xue Y, Gao B, Liu W, Gao GF, Iwamoto A, Kitamura Y. A recombinant replication-competent hepatitis C virus expressing Azami-Green, a bright green-emitting fluorescent protein, suitable for visualization of infected cells. Biochem Biophys Res Commun. 377: 7-11, 2008.
3. Zong L, Chen Y, Peng H, Gao F, Iwamoto A,

Gao GF. Rhesus macaque: A tight homodimeric CD8 alphaalpha. Proteins. 75: 241-244, 2008.
4. Nagai H, Iwasaki N, Odawara T, Okada S. Actual status of AIDS-related lymphoma management in Japan. Int J Hematol. 87: 442-443, 2008.
5. Saito T, Nishi M, Lim MI, Wu B, Maeda T, Hashimoto H, Takeuchi T, Roos DS, Asai T. A novel GDP-dependent pyruvate kinase isozyme from *Toxoplasma gondii* localizes to both the apicoplast and the mitochondrion. J Biol Chem. 283: 14041-52, 2008.
6. Maeda T, Yamada H, Akao N, Iga M, Endo T, Koibuchi T, Nakamura T, Odawara T, Iwamoto A, Fujii T. Unusual radiological findings of Fasciola hepatica infection with huge cystic and multilocular lesions. Intern Med. 47: 449-52, 2008.
7. Hosoya N, Miura T, Kawana-Tachikawa A, Koibuchi T, Shioda T, Odawara T, Nakamura T, Kitamura Y, Kano M, Kato A, Hasegawa M, Nagai Y, Iwamoto A. Comparison between Sendai virus and adenovirus vectors to transduce HIV-1 genes into human dendritic cells. J Med Virol. 80: 373-82, 2008.

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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 (HSCT) and regenerative medicine using human embryonic stem cells (hESC), induced pluripotent stem cells (hiPSC) and bome marrow (BM)-derived mesenchymal stem cells (MSC), and analysis of pathogenesis of hematopoietic disorders, especially pediatric myelodysplastic syndrome (MDS).

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

Yasuhiro Ebihara, Kohichiro Tsuji

Although a standard regimen in HSCT 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 HSCT for patients with a vulnerable normal bone marrow reserve. For patients with Fanconi anemia, in particular, we employed a regimen containing fludarabine to reduce the dose of alkylating agents and irradiation to avoid the toxicity, which was otherwise likely to occur in those patients. For patients with NK cell disease, we used a regimen combining alkylating agents (cyclophosphamide and thiotepa) and total body irradiation based on the results that NK leukemic cells strongly expressed multidrug-registant genes. Now we plan to extend our experience in nationwide collaborative studies.

2. Cooperative clinical trial for pediatric myelodysplastic syndrome

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

Pediatric 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.

3. Novel approach to therapy in juvenile myelomonocytic leukemia

Yasuhiro Ebihara, Yoshitoshi Ohtsuka[3], Atsushi Manabe[1], Yuji Zaike[2], Kohichiro Tsuji: [3]Department of Pediatrics, Hyogo College of Medicine

JMML is a clonal myeloproliferative/myelodysplastic disorder of early childhood with poor prognosis. JMML cells are characterized by hypersensitivity to GM-CSF caused by continuously activated GM-CSF receptor-RAS signal transduction pathway through various molecular mechanisms, resulting in spontaneous colony formation *in vitro*. Bisphosphonate zoledronic acid (ZOL), a RAS-blocking compound, suppressed colony formation from 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.

4. Novel method for efficient production of multipotential hematopoetic progenitors from hESC

Feng Ma[5], Yasuhiro Ebihara, Sachiyo Hanada, Yuji Zaike[2], Hiromitsu Nakauchi[5], Kohichiro Tsuji: [5]Division of Stem Cell Therapy, Center for Stem Cells and Regenerative Medicine

ESC are pluripotent cells derived from the inner cell mass of preimplantation embryos. Since ESC have the ability to be maintained in culture indefinitely as undifferentiated cells, yet they are capable of forming more differentiated cell types, hESC recently established are expected as a novel source of human transplantable cells. We then planed to produce HSC for HSCT and functional blood cells for transfusion medicine from human ES cells. This study was started on December 20, 2003 with the permission by the ethical committee of the Japanese Government. On beginning this study, we thought that *in vitro* reconstitution of the circumstance surrounding embryonic hematopoietic cells is important to induce the differentiation of hESC into HSC or functional blood cells. To achieve this, we determined to use stromal cells from murine embryonic hematopoietic tissues to coculture hESC with them, since some mouse-derived stromal cells have been reported to be able to act on human hematopoietic cells.

We then proposed a novel method for the efficient production of hematopoietic progenitors from hESC by co-culture with stromal cells derived from murine FL (mFLSC) at 14 to 15 days post coitus (dpc), in which embryonic hematopoiesis dramatically expands at midgestation. In the co-culture, various hematopoietic progenitors were generated, and this hematopoietic activity was concentrated in cobblestone-like (CS) cells within differentiated hESC colonies. The CS cells expressed CD34 and retained a potential for endothelial cells. They also contained hematopoietic colony-forming cells, especially erythroid and multilineage colony-forming cells at high frequency. The multipotential hematopoietic progenitors abundant among the CS cells produced all types of mature blood cells, including adult type β globin-expressing erythrocytes and tryptase and chymase-double positive mast cells (MC). They showed neither immature properties of ESC nor potentials to differentiate into endoderm and ectoderm at a clonal level. The developed co-culture system of hESC can provide a novel source for hematopoietic and blood cells applicable to cellular therapies and drug screenings.

5. Generation of functional erythrocytes from hESC-derived definitive hematopoiesis

Feng Ma[5], Yasuhiro Ebihara, Sachiyo Hanada, Yuji Zaike[2], Hiromitsu Nakauchi[5], Kohichiro

A critical issue for utilization of hESC in possible clinical use is whether they can derive terminally mature progenies with the normal function. To solve this, we examined hESC-derived erythroid cells in coculture with mFLSC. By the coculture, large quantity of hESC-derived erythroid progenitors allowed us to analyze the development of erythropoiesis at a clone level and to investigate their function as oxygen carrier. The results showed that the globin expression in the erythroid cells in individual clones changed in a time-dependent manner. In particular, embryonic ε globin positive erythrocytes decreased, while adult-type β globin positive cells increased to almost 100% in all single clones we examined, indicating they had already been fated to definitive hematopoiesis. Enucleated erythrocytes also appeared in the clonal erythroid progenies. A comparison analysis showed that hESC-derived erythroid cells took a similar pathway in differentiation to human cord blood $CD34^+$ progenitor-derived erythrocytes when traced by glycophorin A, CD71 and CD81. Furthermore, these hESC-derived erythroid cells could function as oxygen carrier, and had a sufficient glucose-6-phosphate dehydrogenase activity. The present study provided an experimental model to explore early development of human erythropoiesis, hemoglobin switching, erythroid pathogenesis, and to discover drugs for hereditary diseases in erythrocyte development. The quantitative production and their functional maturation indicate that hESC-derived erythrocytes can be a novel potential source for therapeutic transfusion.

6. Differential production of connective tissue-type and mucosal mast cells from hESC for anti-allergy drug screening

Feng Ma[5], Yasuhiro Ebihara, Sachiyo Hanada, Hiromitsu Nakauchi[5], Kohichiro Tsuji

MC function as effector cells in allergy and atopic disease. Therefore, anti-allergy drugs have been established to diminish MC function. However, since the acquisition of an abundance of human MC (hMC) is difficult because of no culture method producing massive hMC, most anti-allergy drugs targeted animal MC. Thus, efficient discovery of effective anti-allergy drugs needs to establish the culture system of massive hMC. Then, hESC are considered as a potential cell source for hMC.

In human, two types of MC have been characterized; connective tissue-type and mucosal MC (CTMC and MMC, respectively). CTMC contain tryptase, chymase, MC carboxypeptidase and cathepsin G in their secretory granules, are predominantly located in normal skin and in intestinal submucosa, and involve in atopic dermatitis. MMC contain tryptase in their secretory granules, but lack the other proteases, are the main type of MC in normal alveolar wall and in small intestinal mucosa, and involve in allergic rhinitis or bronchial asthma. Although MC can be generated from human adult $CD34^+$ hematopoietic progenitor cells *in vitro*, these MC are mainly MMC. So far, there lacks an evidence for the direct derivation of CTMC from adult hematopoietic progenitors.

We achieved successful production of hESC-derived $CD34^+$ hematopoietic progenitors, using co-culture with mFLSC for 1-2 weeks. In suspension culture favoring MC differentiation within 3weeks, hESC-derived progenitors generated mature MC that shared a chymase/tryptase double positive phenotype and strongly expressed c-Kit, similar to human skin derived CTMC. On the other hand, hESC-derived multipotential hematopoietic progenitors obtained in clonal culture developed into MC for a longer time (over 5 weeks) and only expressed tryptase, with no or few chymase, similar to human $CD34^+$ cell-derived MMC. Since the current culture system of hESC can produce differentially a large number of CTMC and MMC, our study may highlight a new understanding for MC development and finally benefit the screening for anto-allergy drugs.

7. Derivation of multipotential hematopoietic progenitors and mature blood cells from human induced pluripotent stem cells

Feng Ma[5], Yasuhiro Ebihara, Sachiyo Hanada, Daisuke Tomizawa, Hiromitsu Nakauchi[5] and Kohichiro Tsuji

The establishments of hESC and hiPSC have constructed a firm base for regenerative medicine. As mentioned above, we developed a culture system for efficient production of hESC-derived multipotential hematopoietic progenitors and functionally mature erythrocytes, thus providing an experimental model to explore early events in human erythropoiesis. We then applied a similar culture system to induce hiPSC to differentiate into hematopoietic progenies.

hiPSC (235G1, 235G4, 201B6 and 201B7, kindly provided from Dr. Yamanaka, Kyoto University) were used in our experiments. To induce differentiation to hematopoietic cells, undifferentiated hiPSC were cocultured with a mouse feeder stromal cell line derived from

AGM region (AGMS-3). On different times, cocultured cells were harvested by 0.05% trypsin/EDTA solution and re-culture in a semisolid culture to produce hematopoietic colonies. In the consequential experiments, erythrocytes developed in colonies were examined with Hb expression, while other myeloid cells, mast cells and magakaryocytes were examined with their maturity.

Although all hiPSC lines were capable of generating hematopoietic cells in clonal culture, 235 G1 revealed most efficiently, which produced approximately a hundred colonies (Mix colonies: 5%) from 30 undifferentiated hiPSC colonies. Nine tenths of clonal erythroid cells expressed definitive β-globin, which is comparable to hESC-derived ones. Furthermore, hiPSC-derived blood cells (granulocytes, mast cells and megakaryocytes) expressed various mature markers, indicating their fully functional maturation.

Thus, we have established a culture system to induce hiPSCs to multipotential hematopoietic progenitors and mature blood cells. Our study may be used as an experimental model and finally help in clinical cures for hereditary blood diseases.

8. hESC-derived MSC capable of efficiently maintaining hESC and hiPSC under animal serum-free conditions

Yasuhiro Ebihara, Feng Ma[5], Sachiyo Hanada, Daisuke Tomizawa, Hiromitsu Nakauchi[5], Haruo Onoda[2], Naoki Oyaizu[2], Kohichiro Tsuji

hESC and hiPSC have the ability to differentiate into all cell types in the body and hold great promise for regenerative medicine. However, the culture maintaining undifferentiated hESC and hiPSC depends on animal feeder cells and/or serum in most cases. Such a culture system is a huge obstacle for clinical applications of these stem cells because of xenogeneic pathogen contamination. To solve this issue, we developed a novel culture method for maintaining undifferentiated hESC and hiPSC using MSC as feeder cells and platlet lysate (PL) instead of animal serum.

When undifferentiated hESC (line H1) cultured on murine embryonic fibroblast (MEF) feeder cells were recultured on gelatin-coated culture dishes with PL-containing media in the absence of MEF feeder cells. Cells were passaged several times with PL containing media, and then stromal cells were induced after 6 to 8 weeks. The stromal cells were spindle-like shaped, revealed a phenotype of CD45-, CD34-, CD14-, CD105+, CD166+, CD31-, and SEA-4-, and had the ability to differentiate into mesenchymal tissues such as bone, cartilage and fat in vitro, indicating these cells were MSC. MEF feeder cells and undifferentiated hESC were undetectable in the hESC-derived MSC by reverse transcription polymerase chain reaction analysis.

In the coculture with the hESC-derived MSC in the presence of PL-containing media, undifferentiated hESC (line H1) were maintained at least for four weeks. The cocultured hESC expressed specific markers for undifferentiated ESC, such as Oct-3/4, Sox-2, and Nanog, and formed teratoma containing ectodermal, endodermal, and mesodermal tissues in the transplantation into non-obese diabetic/severe combined immunodeficient mice. These hESC-derived MSC also have the ability to keep another hESC (line khES-1, kindly provided by Dr. Nakatsuji, Kyoto University) and hiPSC (line 235/G1, kindly provided by Dr. Yamanaka, Kyoto University) undifferentiated state. In addition, khES-1 cells generated MSC with the ability same to H1 cell-derived MSC. Interestingly, hESC or hiPSC isolated by passing through 100 micro-meter filter were capable of forming undifferentiated stem cell colonies in the coculture with hESC-derived MSC.

These results indicate that hESC-derived MSC are able to be substituted for MEF feeder cells in the absence of animal serum but in the presence of PL in the maintenance of hESC and hiPSC, and efficiently support the proliferation of undifferentiated stem cells, even from single hESC or hiPSC. The current culture system can be useful for the clinical application of hESC and hiPSC.

9. Establishment of human BM-derived MSC for the treatment of hemophilic arthropathy

Yasuhiro Ebihara, Hideyuki Takedani[6], Tokiko Nagamura-Inoue[7], Shigeyuki Wakitani[8], Arinobu Tojo[9], Hiromitsu Nakauchi[5], Kohichiro Tsuji: [6]Department of Joint Surgery, Research Hospital, [7]Department of Cell Processing and Transfusion, [8]Department of Orthopaedic Surgery, Osaka City University Graduate School of Medicine, [9]Division of Molecular Therapy, Advanced Clinical Research Center

Hemophilia is the congenital disease with a lack of coagulation factors. One to two thirds of the patients had arthropathy because of recurrent intra-articular bleeding. Most of surgical treatment for the arthropathy, such as synovectomy or total joint arthroplasty, in Japan is performed by Department of Joint Surgery in our hospital. So far, however, the efficacy of the treatment has been insufficient. We then planed

the transplantation of autologous culture-expanded BM-derived MSC into the articular cartilage defect in the hemophilic arthropathy patients. For the project, we are establishing the culture system of MSC from the patient BM using autologous serum.

Publications

Ma F, Ebihara Y, Umeda K, Sakai H, Hanada S, Zhang H, Zaike Y, Tsuchida E, Nakahata T, Nakauchi H, Tsuji K. Generation of functional erythrocytes from human embryonic stem cell-derived definitive hematopoiesis. Proc Natl Acad Sci USA 105: 13087-13092, 2008.

Ma F, Kambe N, Wang D, Shinoda G, Fujino H, Umeda K, Fujisawa A, Ma L, Suemori H, Nakatsuji N, Miyachi Y, Torii R, Tsuji K, Heike T, Nakahata T. Direct development of functionally mature tryptase/chymase double positive tissue-type mast cells from primate ES cells. Stem Cells 26: 705-714, 2008.

Ebihara Y. Identification and clinical application of fibroblasts/myofibroblasts derived from hematopoietic stem cells. Japan J Pediatr Hematol 36: 1-6, 2008.

Mizutani T, Tsuji K, Ebihara Y, Ohba Y, Taniguchi T , and Kenya Honda K. Homeostatic erythropoiesis by the transcription factor IRF2 through attenuation of type I interferon signaling. Exp Hematol 36: 255-264, 2008.

Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Kobayashi T, Sato A, Kato S, Kasahara S, Ebihara Y, Nagamura-Inoue T, Tsuji K, Tojo A, Asano S: Cardiovascular toxicity of cryopreserved cord blood cell infusion. Bone Marrrow Transplant 41: 861-865, 2008.

Tauchi H, Tomizawa D, Eguchi M, Eguchi-Ishimae M, Koh K, Hirayama M, Miyamura N, Kinukawa N, Hayashi Y, Horibe K, Ishii E. Clinical features and outcome of *MLL* gene rearranged acute lymphoblastic leukemia in infants with additional chromosomal abnormalities other than 11q23 translocation. Leuk Res 32: 1523-1529, 2008.

Kulkeaw K, Mizuochi C, Horio Y, Osumi N, Tsuji K, Sugiyama D. Application of whole mouse embryo culture system on stem cell research. Stem Cell Rev & Rep, in press.

Tomizawa D, Koh K, Hirayama M, Miyamura T, Hatanaka M, Saikawa Y, Ishii E. Outcome of recurrent or refractory acute lymphoblastic leukemia in infants with *MLL* gene rearrangements: A report from the Japan Infant Leukemia Study Group. Pediatr Blood Cancer, in press.

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

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

Osamu Hosono, Kei Ohnuma, Noritada Yoshikawa, Hiroshi Kawasaki, Hirotoshi Tanaka, Chikao Morimoto. (Department of Rheumatology and Allergy), Emi kumagai, Akiko Souta-Kuribara, (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-κB activation, followed by upregulation of CD86. In addition, reduced caveolin-1 expression on monocytes inhibits CD 26-mediated CD86 upregulation and abrogates CD26 effect on recall antigen-induced T cell proliferation, and immunohistochemical studies revealed an infiltration of CD26＋ T cells in the sublining region of rheumatoid synovium and high expression of caveolin-1 in the increased vasculature and synoviocytes of the rheumatoid synovium. Taken together, these results strongly suggest that CD26-caveolin-1 interaction plays a role in the upregulation of CD86 on recall antigen-loaded monocytes and subsequent engagement with CD28 on T cells, leading to antigen-specific T cell activation such as the T-cell-mediated antigen-specific response in rheumatoid arthritis (RA).

Currently we are focusing on the translational research of utilization of anti-CD26 mAb as well as recombinant soluble CD26 for treatment of malignant tumors, immune-mediated disorders and immune deficiency diseases. Hopefully we will perform phase I/II clinical trial utilizing humanized CD26 antibody for the treatment of the

above diseases, such as malignant mesothelioma and other CD26 positive malignant tumors soon.

a. Clinical significance of soluble CD26/DPPIV in various disease conditions

(i) Soluble CD26/DPPIV in autoimmune and other immune-mediated disorders

Our previous studies demonstrated that CD 26-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. Possible substrates of CD26/DPPIV include several critical cytokines and chemokines. CD26 could modulate function of several cytokines and chemokines such as RANTES (CCL5), SDF-1 α (CXCL12) and glucagons-like peptide 1 (GLIP-1) through its DPPIV enzyme activity. We have shown that the DPPIV enzyme activity of plasma sCD26 was low in HIV-1-infected individuals, and was inversely correlated with HIV-1 RNA, and that the in vitro addition of recombinant sCD26 could enhance purified protein derivative-induced lymphocyte proliferation. These DPPIV enzyme activity of sCD26 in HIV-1-infected individuals contributes to the immunopathogenesis of HIV infection. Furthermore, we have shown that serum levels of sCD 26 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 examined sCD26 and its specific DPPIV activity in serum of patients with inflammatory bowel diseases (IBD), such as Crohn's disease or ulcerative colitis in collaboration with Gastrointestinal Unit, School of Medicine, Keio University. The DPPIV activity was reduced in patients with IBD and was significantly lower in patients with Crohn's disease compared to with ulcerative colitis ($P < 0.05$). We have also measured sCD26/DPPIV levels in sera and synovial fluid from patients with RA and found significant decrease of serum sCD26 and its specific DPPIV activity. These findings indicate that CD26 may be potentially important for the pathophysiology of IBD and RA. Furthermore, we have investigated autoantibodies against CD26 in serum using ELISA and Western blotting methods. We have not found anti-CD26 autoantibody which could reduce DPPIV activity so far. We plan to examine the effect of TNF-α blocking therapy (infliximab, etanercept, adalimumab) on serum levels of sCD26/DPPIV in patients with RA and its clinical significance.

(ii) Soluble CD26/DPPIV in malignancies associated with asbestos exposure

CD26/DPPIV is able to cleave selected biological factors to alter their functions and regulates topoisomerase II α level in hematologic malignancies, affecting sensitivity to doxorubicin and etoposide. Expressed on various tissues, CD 26 is involved in the development of certain human cancers. We have shown CD26 is highly expressed on the cell surface of malignant mesothelioma and that a newly developed humanized anti-CD26 monoclonal antibody has an inhibitory effect on malignant mesothelioma cells in both in vitro and in vivo experiments.

We examined sCD26 and its specific DPPIV activity in serum of patients with asbestosis in collaboration with Okayama Rosai Hospital. Serum levels of sCD26 and its specific DPPIV activity were significantly increased in patients with pleural plaque compared to healthy individuals ($P < 0.05$). However, serum levels of sCD 26 and its specific DPPIV activity was significantly reduced in patients with both malignant mesothelioma and primary lung cancer associated with asbestos exposure compared to patients with pleural plaque. We also examined sCD26 and its specific DPPIV activity in pleural effusion of patients with mesothelioma, primary lung cancer and tuberculosis. In mesothelioma there seems to be a relationship between pleural CD26/DPPIV and prognosis. We are doing serial studies and measuring sCD26/DPPIV in serum and pleural fluid to confirm their clinical significance.

b. CD26-based molecular target therapy for graft-versus-host disease in hematopoietic stem cell transplantation

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation (alloHSCT). In GVHD, mature donor T cells that accompany the stem cell graft attack recipient tissues, especially the skin, liver, gastrointestinal tract, and lung. Therefore, all patients undergoing alloHSCT receive GVHD prophylaxis to impair T cell function; however, treatment to prevent GVHD can be deleterious since mature donor T cells play a critical role in mediating reconstitution of the adaptive immune system. Recipients of alloHSCT are thus at great risk for infections, particularly when prolonged immunosuppression is required for treatment of GVHD. Although the role of CD26/DPPIV in GVHD needs to be studied in more detail, treatment with a murine antibody against human CD26 was reported to have an effect in patients

with steroid-resistant acute GVHD following alloSCT (Bacigalupo A., et al., Acta Haematol 1985: 73: 185, de Meester, et al., Immunobiology 1993: 188: 145). To examine the efficacy of CD 26-targeting therapy in GVHD more profoundly, we established mouse GVHD model using human peripheral blood lymphocytes (huPBL) (xenograft GVHD mouse model; x-GVHD). After NOD/LtSz-scid or NOD/Cg-Prkdcscidil2 rgtm1Sug/Jic mice were injected with appropriate numbers of huPBL, mice show symptoms of GVHD such as loss of weight, loss of hair, deterioration of activity, and thinning of ear pads. Histopathological examination revealed that CD 3+CD26+ human lymphocytes were infiltrated in the skin, intestinal mucosa, salivary gland, lung and liver of the x-GVHD mice. In this mouse model, humanized anti-CD26 monoclonal antibody (mAb) was injected two weeks later of onset of x-GVHD, and the symptoms of GVHD were improved after ten injections of humanized anti-CD26 mAb. Moreover, x-GVHD was observed to be suppressed when humanized anti-CD26 mAb was prophylactically administered. Taken together, it may be possible that the full therapeutic potential of alloSCT will be realized by approaches that aim to minimize GVHD by targeting CD26-mediated T cell regulation.

II. Therapeutically targeting transcription factors

Hirotoshi Tanaka, Noritada Yoshikawa, Noriaki Shimizu, Chikao Morimoto.

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 (GR) 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 regulations 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 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) Redox Regulation of the GR

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

(ii) Development of Dissociating Ligand for the GR

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. Our recent microarray study demonstrated that GR and MR have differential role in homeostatic regulation in non-classical corticosteroid target tissues including the heart. Notably, collaboration with Professor Miyano's laboratory greatly contributed to development of this program.

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

Expression of HEXIM1 is induced by treatment of vascular smooth muscle cells with a differentiation inducer hexamethylane bisacetamide. It is shown that HEXIM1 binds 7SK snRNA and inhibits P-TEFb-mediated transcriptional elongation process. On the other hand, we have found that HEXIM1 directly associates with the GR in the absence of 7SK and represses GR-mediated transcription. We are currently working on regulation of HEXIM1 expression, physiological role of HEXIM1 in GR action. Indeed, HEXIM1 has differential roles in gene regulation in a context and gene specific fashion. We have recently characterized that HEXIM1 may play an important role in tissue-specific regulation of glucocorticoid-mediated gene expression. Physiological significance of HEXIM1 is being studied using newly generated transgenic mice.

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

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

III. Case Reports

Osamu Hosono, Kei Ohnuma, Noritada Yoshikawa, Tomoki Katayose, Hiroshi Kawasaki, Hirotoshi Tanaka, Chikao Morimoto (Department of Rheumatology and Allergy), Naoki Oyaizu (Department of Laboratory Medicine)

a. An adult case of Henoch-Schönlein purpura complicating common peroneal nerve mononeuropathy.

We present an adult patient with Henoch-Schönlein Purpura who developed mononeuropathy in the common peroneal nerve. Upon admission, the patient had palpable purpura in the arms and legs, polyarthralgia, abdominal pain, and leukocytoclastic vasculitis in the skin biopsy. These symptoms disappeared with 30 mg daily of oral prednisolone. One month later, after induction therapy, fever, livedo reticularis and peripheral mononeuropathy developed with hypocomplementemia and the patient was treated successfully with glucocorticoid pulse therapy.

b. Three cases of MPO-ANCA associated vasculitis: Clinical features and therapeutic options

We report here 3 cases of MPO-ANCA associated vasculitis. [Case 1] A 50-year-old woman with proteinuria and MPO-ANCA for 2 years, developed alveolar hemorrhage. She was improved with PSL 20mg/d, but 1 year later started on hemodialysis. [Case 2] A 64-year-old woman developed myalgia and hematuria with MPO-ANCA in 2006, and then alveolar hemorrhage and renal dysfunction. She was improved by both PSL and cyclophosphamide (CY). [Case 3] A 76-year-old woman had hemoptysis with abnormal CT findings, hematuria and MPO-ANCA, and treated with PSL and IVCY. 7 years ago she was treated with PSL 15mg/d due to SjS and renal impairment. [Discussion] Even if cases of MPO-ANCA associated vasculitis were not diagnosed as MPA, they should be treated early with CY and PSL to prevent renal failure.

IV. Medical Genome Science Program

Satoshi Iwata (Division of Clinical Immunology), Osamu Hosono, Chikao Morimoto.

We have made contribution to Medical Genome Science Program, the Institute of Medical Science, the University of Tokyo. Our mainly involving courses are, "Introduction of Medicine and Medical Ethics" and "Experience and Practice of Medicine", arranged for non-M.D. students of the program. The former is a series of lectures drawing outline of Medicine (past, present & future), and the latter is a weekly practice aiming to make attendants to get experienced in practical Medicine while rounding at the Research Hospital of the Institute of Medical Science. The attendants are supposed to visit a variety of divisions in the hospital, such as Radiology, Laboratory Medicine, Blood Transfusion, Surgical Center, Nursing Quarters, as well as the patient round by Department of Hematology/Oncology, and the joint patient round by Department of Advanced Medical Science/Infectious Disease and Applied Immunology/Rheumatology and Allergy. We especially thank all the people in the Research Hospital of the Institute of Medical Science, who participate in "Experience and Practice of Medicine".

Publications

Nishida, H., Yamada, T., Iwata, S., Dang, NH., Inukai, T., Sugita, K., Ikeda, Y. and Morimoto, C. CD9 correlates with cancer stem cell potentials in human B-acute lymphoblasticleukemia cells. Biochem. Biophys. Res. Commun. In press (2009).

Harima, A., Nakaseko, C., Yokota, A., Kitagawa, M., Morimoto, C., Harigaya, K. and Saito, Y. Fibronectin promotes cell proliferation of human pre-B cell line via its interactions with VLA-4 and VLA-5. Hematology. 13: 236-43, 2008.

Iwata, S., Nori, M., Hashizume, Y., You, K. and Morimoto, C. Effect of H-1-receptor antagonists on proliferative response, cytokine production, and cellular migration of human T cells and macrophages. Clinical and Experimental Allergy Reviews 8: 21-29, 2008.

Ohnuma, K., Hosono, O., Kawasaki, H., Yoshikawa, N., Katayose, T., Oyaizu, N., Tanaka, H. and Morimoto, C. An adult case of Henoch-Schönlein purpura complicating common peroneal nerve mononeuropathy. Mod Rheumatol. 19: 73-9, 2009.

Fujimaki, W., Takahashi, N., Ohnuma, K., Nagatsu, M., Kurosawa, H., Yoshida, S., Dang, NH., Uchiyama, T. and Morimoto, C. Comparative study of regulatory T cell function of human CD25+CD4+ T cells from thymocytes, cord blood, and adult peripheral blood. Clin. Dev. Immunol. 2008: 305859, 2008.

Ohnuma, K., Dang, NH. and Morimoto, C. Revisiting an old acquaintance: CD26 and its molecular mechanisms in T cell function. Trends Immunol. 29: 295-301, 2008.

Yamochi, T., Ohnuma, K., Hosno, O., Tanaka, H., Kanai, Y. and Morimoto, C. SSA/Ro52 autoantigen interacts with Dcp2 to enhance its decapping activity. Biochem. Biophys. Res. Commun. 370: 195-199, 2008.

Yamashita, T., Ohneda, O., Nagano, M., Iemitsu, M., Makino, Y., Tanaka, H., Miyauchi, T., Goto, K., Ohneda, K., Fujii-Kuriyama, Y., Poellinger, L. and Yamamoto, M. Abnormal heart development and lung remodeling in mice lacking the hypoxia-inducible factor-related basic helix-loop-helix PAS protein NEPAS. Mol Cell Biol. 28: 1285-1297, 2008.

Kagawa, T., Watanabe, N., Mochizuki, K., Numari, A., Ikeno, Y., Itoh, J., Tanaka, H., Arias, IM. and Mine, T. Phenotypic differences in PFIC2 and BRIC2 correlate with protein stability of mutant Bsep and impaired taurocholate secretion in MDCK II cells. Am. J Physiol. Gastrointest. Liver Physiol. 294: G58-67, 2008.

Yoshikawa, N., Shimizu, N., Sano, M., Ohnuma, K., Iwata, S., Hosono, O., Fukuda, K., Morimoto, C. and Tanaka, H. Role of the hinge region of glucocorticoid receptor for HEXIM1-mediated transcriptional repression. Biochem. Biophys. Res. Commun. 371: 44-49, 2008.

Shimizu, N., Yoshikawa, N., Wada, T., Handa, H., Sano, M., Fukuda, K., Suematsu, M., Sawai, T., Morimoto, C. and Tanaka, H. Tissue- and context-dependent modulation of hormonal sensitivity of glucocorticoid-responsive genes by hexamethylene bisacetamide-inducible protein 1. Mol Endocrinol. 22: 2609-2623, 2008.

Shibata, S., Nagase, M., Yoshida, S., Kawarazaki, W., Kurihara, H., Tanaka, H., Miyoshi, J., Takai, Y. and Fujita, T. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. Nat Med. 14: 1370-1376, 2008.

Hozawa, S., Nakamura, T., Nakano, M., Adachi, M., Tanaka, H., Takahashi, Y., Tetsuya, M., Miyata, N., Soma, H. and Hibi, T. Induction of matrix metalloproteinase-1 gene transcription by tumour necrosis factor alpha via the p 50/p50 homodimer of nuclear factor-kappa B inactivated human hepatic stellate cells. Liver Int. 28: 1418-1425, 2008.

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Assistant Professor	Naoyuki Takahashi, M.D., D.M.Sc.	助	教	医学博士	高橋直之(併任)

Our department was established to support the translational researches of our hospital. Currently, we are studying a correlation between expression profiles of peripheral blood mononuclear cells in patients with hematologic malignancy and severity of graft-versus-host disease developed after hematopoietic stem cell transplantation. We have been helping prospective studies such as prediction of the sensitivity of Gefenitib to adenocarcinoma of the lung, and that of Imatinib to chronic myeloid leukemia (CML). In addition to the involvement in these projects, we are in charge of outpatient clinic for genetic counseling in Research Hospital, IMSUT.

1. Analysis of gene expression profiles of inflammatory cells in acute graft-versus-host disease following umbilical cord blood transplantation

Naoyuki Takahashi, Noriharu Sato, Satoshi Takahashi[1], and Arinobu Tojo[1]: [1]Department of Hematology-Oncology

Compared with allogeneic hematopoietic stem cell transplantation using other sources, cord blood (CB) transplantation (CBT) has clinical advantages in terms of incidence and severity of acute graft-versus-host disease (GVHD) despite using allogeneic stem cells with more human leukocyte antigen mismatches. However, detailed pathophysiology of acute GVHD developed after CBT has not yet been elucidated.

We performed microarray expression profiling of immunoregulatory genes on each of 4 subpopulations ($CD4^+$, $CD8^+$, $CD14^+$, and $CD56^+$) of peripheral blood mononuclear cells (PBMCs), which were taken from 8 patients with hematologic malignancies who suffered from acute GVHD after unrelated CBT. We identified 55 genes, which were differentially expressed during acute GVHD compared to recovery phase. Among them, 22 showed differential expression concurrently in multiple PBMC subpopulations. In particular, 5 genes (TRAIL, IL1RN, IFI27, GZMB, and CCR5) were up-regulated and 3 genes (CLK1, TNFAIP3 and BTG1) were down-regulated in at least 3 out of 4 subpopulations during acute GVHD. These 8 genes seem to be candidates which may play key roles common to multiple subpopulations in the pathophysiology of acute GVHD. In addition, down-regulation of anti-inflammatory factors, such as TNFAIP3, KLF2, ZFP36, and BTG1, seems to be involved in acceleration of immune response, thus exacerbation of acute GVHD. Further study is required to clarify whether therapeutic augmentation of these factors may ameliorate acute GVHD. Meanwhile, differential expression of several genes, such as CCL5, TNFAIP3, CD161, CD160, and COX2, was assumedly affected by the developmental immaturity of CB-derived cells and this might be somehow involved in the

relatively low incidence and low severity of acute GVHD following CBT.

2. Genetic diagnosis of HNPCC

Yoichi Furukawa, Yusuke Nakamura[1]: [1]Laboratory of Molecular Medicine, Human Genome Center, IMSUT

Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant hereditary disease accompanied by tumors arising mainly in the colon and other associated organs, such as stomach, renal pelvis, and endometrium. The frequency of HNPCC in Caucasian patients with colorectal cancer is estimated between two and five percent. However the frequency in Japanese patients with colorectal cancer remains undetermined. Therefore, Japanese Study Group for Colorectal Cancer started a collaborative project of registration of Japanese HNPCC patients and genetic analysis of mutations in *MSH2*, *MLH1*, and *MSH6*, the responsible genes for HNPCC. All patients with colorectal cancer and those who are diagnosed as HNPCC by Amsterdam's II criteria in the collaborative hospitals have been registered, and the frequency of HNPCC in registered patients with colon cancer has been determined. Collaborating to this project, we have analyzed genetic alteration in a total of 131 patients using PCR-direct sequencing and Multiplex Ligation-dependent Probe Amplification. Among the 131 cases, 69 cases harbored pathogenic mutation in one of the three responsible genes. We have clarified that gastric cancer is frequently observed in HNPCC pedigrees, and that it should be considered as an HNPCC-related tumor in Japanese. In addition, we generated an algorithm to predict patients with a pathogenic mutation. These data will provide valuable information for the understanding of the frequency, penetrance and phenotypes of Japanese HNPCC. The results will be helpful for the identification and diagnosis of Japanese HNPCC patients.

3. Genetic counseling and related activities.

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

In the genetic counseling clinic, we provided genetic counseling for clients who suffered from or had family members of hereditary disease. Genetic diseases and related problems seen at the clinic in 2008 include spinocerebellar ataxia, hereditary breast cancer, and bipolar disease. We also took psychological care of the clients in collaboration with clinical psychologists.

Publications

1. Obama K, Satoh S, Hamamoto R, Sakai Y, Nakamura Y, Furukawa Y. Enhanced expression of RAD51AP1 is involved in the growth of intrahepatic choloangiocarcinoma cells. Clin. Cancer Res 14: 1333-1339, 2008.
2. Silva FP, Hamamoto R, Kunizaki M, Tsuge M, Nakamura Y, Furukawa Y. Enhanced methyltransferase activity of SMYD3 by the cleavage of its N-terminal region in human cancer cells. Oncogene 27, 2686-2692, 2008.
3. Takahashi N, Sato N, Takahashi S, Tojo A. Gene-expression profiles of peripheral blood mononuclear cell subpopulations in acute graft-vs-host disease following cord blood transplantation. Exp. Hematol. 36: 1760-1770, 2008.

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The Department of Radiology works in general diagnostic radiology, neuroradiology, clinical nuclear medicine, radiation therapy, and molecular imaging. For clinical imaging, we have a multi-detector row CT scanner, high-field MRI unit, and gamma camera. We perform all examinations of CT, MRI, angiography, and nuclear medicine, and official reports on all the examinations are made by board-certified radiologists and a nuclear medicine physician. Molecular imaging techniques permit repeated assessment of individual animals to evaluate disease progression, therapeutic effect, and pharmacokinetics. They contribute to preclinical studies and have potentials of application to clinical assessments.

Bioluminescent evaluation of the therapeutic effects of total body irradiation in a murine hematological malignancy model

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

We investigated the utility of in vivo bioluminescence imaging (BLI) in assessing the therapeutic effects of total body irradiation (TBI) in a murine hematological malignancy model. The suspension of Ba/F3 cells transduced with firefly luciferase and p190 BCR-ABL genes was exposed to ionizing radiation, and viable cell numbers and bioluminescent signals were measured serially. Mice intravenously inoculated with the cells underwent TBI at various doses. In vivo BLI was performed repeatedly until spontaneous death, and whole-body bioluminescence signals were determined as an indicator of whole-body tumor burden. In the cell culture study, bioluminescence signals generally reflected viable cell numbers, despite some overestimation immediately after irradiation. Sublethal TBI in mice transiently depressed the increase in whole-body signals and prolonged survival. Spontaneous death occurred at similar signal levels regardless of radiation dose. A significant negative correlation was found between survival and whole-body signal early after TBI. Significant dose dependence was demonstrated for both survival and signal increase early after TBI and was more evident for signal increase. Lethally irradiated mice without bone marrow transplantation died while showing weak signals. In mice receiving lethal TBI and syngeneic bone marrow transplantation, signal reduction and prolongation of survival were prominent, and whole-body signals at death were similar to those in unirradiated or sublethally irradiated mice. In vivo BLI allows longitudinal, quantitative evaluation of the response to TBI in mice of a hematological malignancy model. Antitumor effects can be assessed early and reliably using in vivo BLI.

Comparison of subcutaneous and intraperitoneal injection of D-luciferin for in vivo bioluminescence imaging

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

We compared subcutaneous (SC) injection and intraperitoneal (IP) injection of D-luciferin for in vivo bioluminescence imaging (BLI) to determine the utility of SC injection. Mice bearing SC tumors stably expressing firefly luciferase underwent in vivo BLI using SC and IP injection of D-luciferin. BLI studies were repeated, with an interval of 3 h, using a given injection route to assess repeatability and using different injection routes to assess correlation. In mice bearing both SC and IP tumors, BLI was performed successively using intravenous (IV), SC, and IP injection of D-luciferin. Hematological malignancy model mice underwent BLI using SC and IP injection. In SC tumors, the peak time was slightly shorter and the peak signal was greater using SC injection compared with IP injection. The repeatability of determining peak signals was comparable between the two injection routes, and a good correlation was observed between them. In mice bearing both SC and IP tumors, signals from IP tumors relative to those from SC tumors were much greater using IP injection than using IV or SC injection. In the hematological malignancy model, signals from the spleen relative to those from the bone marrow were greater using IP injection than using SC injection. In addition to rare injection failure, the IP injection of D-luciferin led to the overestimation of signals from IP tissues. For BLI, SC injection was shown to be a convenient alternative to IP injection.

Diet and gastrointestinal signal on T1-weighted magnetic resonance imaging of mice

Shigeru Kiryu, Yusuke Inoue, and Kohki Yoshikawa[2]: [2]Department of Radiotechnical Sciences, Faculty of Radiological Health Sciences, Komazawa University.

Magnetic resonance (MR) imaging provides three-dimensional information about both anatomy and function without radiation exposure, and is accepted as a powerful modality in small animal experiments. In MR imaging of small animals, the gastrointestinal contents may give rise to intense signals on T1-weighted images and it can cause significant problems in MR imaging of the abdomen of a small animal, e.g., by increasing image degradation due to motion artifacts related to peristalsis and respiratory motion. We investigated to determine the optimal dietary preparation to reduce gastrointestinal signals in mice and to evaluate the usefulness of this approach. Images of the mouse trunk were obtained using a T1-weighted, three-dimensional fast low-angle shot sequence under various dietary conditions and were compared with respect to the gastrointestinal signals and image quality. The dietary preparation studied included giving alternative diets for 24 hours, intestinal cleansing, and 6-hour fasting. Mice with and without dietary preparation underwent MR lymphography using gadofluorine 8, and the visualization of abdominal lymph nodes was compared. In the absence of dietary preparation, hyperintense areas were conspicuous in the gastrointestinal system, whereas on the images taken from mice fed potato or sweet potato for 24 hours before imaging, gastrointestinal hyperintensity was less prominent. This preparation also reduced artifactual signals and resulted in higher-quality images of the kidneys. Intestinal cleansing, which consisted of 24-hour fasting and laxative intake, did not reduce the gastrointestinal signals and caused signal changes that were indicative of fatty liver development. Some of the abdominal lymph nodes of the mice that did not receive dietary preparation were visualized on MR lymphography source images but not on maximum intensity projection (MIP) images. In contrast, on the MIP images of mice fed potato, all the lymph nodes delineated on the source images were successfully visualized. In conclusion, feeding mice potato or sweet potato for 24 hours before MR imaging reduces the gastrointestinal signals and image degradation due to artifacts. Appropriate dietary preparations facilitate the display of target structures on MIP images and are expected to enhance the capabilities of small animal MR imaging.

Free-breathing diffusion-weighted imaging for the assessment of inflammatory activity in Crohn's disease

Shigeru Kiryu, Masakazu Takazoe[3], and Rikisaburo Sahara[4]: [3]Inflammatory Bowel Disease Center and [4]Department of Proctology, Social Insurance Central General Hospital.

Crohn's disease is an inflammatory disorder of unknown cause that affects mainly young people and the repeated evaluation to monitor disease activity with minimal burden to the patient is desirable for the assessment of Crohn's disease. Diffusion-weighted MR imaging (DWI) reflects molecular diffusion without the administration of contrast material, and has the capability to detect inflammatory foci. We investigated the application of free-breathing DWI to the assessment of disease activity in Crohn's disease. Thirty-one patients with Crohn's disease

were investigated using free-breathing DWI without special patient preparation or neither IV nor intraluminal contrast agent. The bowel was divided into seven segments, and disease activity was assessed visually on DWI. For quantitative analysis, the apparent diffusion coefficient (ADC) was measured in each segment. The findings of a conventional barium study or surgery were regarded as the gold standard for evaluating the diagnostic ability of DWI to assess disease activity. Upon visual assessment, the sensitivity, specificity, and accuracy for the detection of disease-active segments were 86.0, 81.4, and 82.4%, respectively. In the quantitative assessment, the ADC value in the disease active area was lower than that in disease inactive area in small and large bowels. Free-breathing DWI is useful in the assessment of Crohn's disease. The accuracy of DWI is high in evaluating disease activity, especially in the small bowel, and the ADC may facilitate quantitative analysis of disease activity.

Development of integrated techniques using bioluminescence imaging and magnetic resonance imaging

Yusuke Inoue, Shigeru Kiryu, Yoshitaka Masutani[5], and Arinobu Tojo[1]: [5]Department of Radiology, Graduate School of Medicine, University of Tokyo.

In vivo bioluminescence imaging (BLI) detects luciferase expression in living mice easily and sensitively. However, because of the projectional nature, lack of anatomical information, and low spatial resolution, the localization of bioluminescent sources is often difficult and unreliable. Magnetic resonance imaging (MRI) is another imaging technique applicable to small animal imaging and allows the assessment of detailed morphology. We have demonstrated the feasibility and usefulness of combined BLI and MRI evaluations in evaluating a mouse model of a hematological malignancy. For further sophistication of the multi-modality imaging approach, we are now studying to develop BLI/MRI fusion imaging. MR images suffer from geometric distortion due to gradient nonlinearity and inhomogeneity in static magnetic field. We developed a method to correct such distortion and validated its application to the distortion correction of mouse MR images. Distortion of CCD camera images was also evaluated, and a technique for minimizing the distortion was established. With the aid of point markers that is detectable by fluorescence imaging and MRI, accurate registration was achieved.

Evaluation of disseminated intraperitoneal disease using in vivo bioluminescence imaging

Makoto Watanabe, Yusuke Inoue, and Arinobu Tojo[1]

Quantitative assessment of tumor burden is difficult in animal models of disseminated intraperitoneal disease. In vivo bioluminescence imaging (BLI) allows to assess the tumor burden quantitatively and noninvasively, and has been used for the studies regarding disseminated intraperitoneal disease. We are investigating the techniques for the quantitative evaluation of tumor burden in disseminated intraperitoneal disease. For in vivo BLI, mice commonly receive intraperitoneal injection of D-luciferin, are fixed in the imaging chamber, and are imaged at a fixed time point. We determine the relationship of quantitative indicators with the injection route, mouse posture, and imaging timing. Our aim is to improve the reliability of the experimental results, which should enable to decrease the number of mice to be examined.

Lymph node mapping in the mouse using imaging techniques.

Yusuke Inoue and Shigeru Kiryu

Lymph nodes are important as sites of metastatic involvement, and animal models of lymph node metastasis are being developed. However, lymph nodes of mice are small and difficult to detect. Even normal lymphatic pathway in the mouse has not been well established. We are developing the image-based methods for the assessment of the lymphatic system in living mice and applying them to the investigation of the murine lymphatic system. MRI visualized inflammatory lymph nodes after subcutaneous injection of gadofluorine 8. Fluorescence imaging demonstrated normal lymph nodes after subcutaneous injection of quantum dots. Although fluorescence imaging is generally unsuitable to the delineation of deep structures because of strong scattering and attenuation of light photons, we achieved clear demonstration of deep lymph nodes using a novel, simple technique. Strong signals are originated from gastrointestinal contents in both MRI and fluorescence imaging, and our techniques for reduction in gastrointestinal signals aid the detection of abdominal lymph nodes.

Publications

Inoue, Y., Izawa, K., Kiryu, S., Tojo, A., and Ohtomo, K. Diet and abdominal autofluorescence detected by in vivo fluorescence imaging of living mice. Mol. Imaging. 7: 21-7, 2008.

Inoue, Y., Izawa, K., Kiryu, S., Kobayashi, S., Tojo, A., and Ohtomo, K. Bioluminescent evaluation of the therapeutic effects of total body irradiation in a murine hematological malignancy model. Exp. Hematol. 36: 1634-41, 2008.

Yokoyama, I., Inoue, Y., Kinoshita, T., Itoh, H., Kanno, I., and Iida, H. Heart and brain circulation and CO_2 in healthy men. Acta. Physiol. 193:303-8, 2008.

Kiryu, S., Sundaram, T., Kubo, S., Ohtomo, K., Asakura, T., Gee, JC., Hatabu, H., and Takahashi, M. MRI assessment of lung parenchymal motion in normal mice and transgenic mice with sickle cell disease.J. Magn. Reson. Imaging. 27: 49-56, 2008.

Sakai, M., Aoki, S., Inoue, Y., Ashida, R., Yamada, Y., Kiryu, S., Inano, S., Mori, H., Masutani, Y., Ohtomo, K., and Nakamura, H. Silent white matter lesion in linear scleroderma en coup de sabre. J. Comput. Assist. Tomogr. 32:822-4, 2008.

Inoue, Y., Kiryu, S., Izawa, K., Watanabe, M., Tojo, A., and Ohtomo, K. Comparison of subcutaneous and intraperitoneal injection of D-luciferin for in vivo bioluminescence imaging. Eur. J. Nucl. Med. Mol. Imaging. In press

井上優介，東條有伸．血液学研究における疾患動物モデルの作製と応用：生体イメージング技術を用いた疾患モデルマウスの病態解析．臨床血液　49(7)：437−444，2008.

桐生茂．9 後腹膜・大血管．画像診断臨時増刊号．大友邦，吉岡直己編著．秀潤社　28(4)：206−225，2008.

Research Hospital

Department of Surgery
外科

Associate Professor	Masaru Shinozaki, M.D., D.M.Sc.	准教授	医学博士	篠崎　大
Professor	Hideaki Tahara, M.D., D.M.Sc.	教授	医学博士	田原秀晃
Lecturer	Akihiko Itoh, M.D.	講師		伊藤精彦
Assistant Professor	Giichiro Tsurita, M.D., D.M.Sc.	助教	医学博士	釣田義一郎
Assistant Professor	Akira Kanamoto, M.D., D.M.Sc.	助教	医学博士	金本　彰
Assistant Professor	Keisuke Hata, M.D., D.M.Sc.	助教	医学博士	畑　啓介
Assistant Professor	Masahisa Jinushi, M.D., D.M.Sc.	助教	医学博士	地主将久

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. The principal goal of our department is to provide surgical service of malignancy and inflammatory bowel disease and to develop and conduct clinical trials in the early stages (Phase I and II) at the Research Hospital.

1. Summary of surgical treatment in 2008

Masaru Shinozaki, Akihito Itoh, Giichiro Tsurita, Akira Kanamoto, Kimiyasu Yoneyama, Keisuke Hata

We performed surgical operations on 128 patients. Each procedure was classified and listed in Table 1. Malignancy is the leading indication for operation, followed by benign diseases, such as inflammatory bowel disease (IBD) and hernia. In 2008, three colorectal specialists (M.S., G.T., and K.H.) came to the Department, and we experienced more IBD patients. Two cases with IBD had neoplasm: high grade dysplasia in ulcerative colitis and invasive cancer in Crohn's disease. Laparoscopic surgery for colorectal disease was introduced: ileocecal resection and total proctocolectomy were performed. We updated operative procedures to avoid surgical site infection. Furthermore, routine endoscopic observation after colorectal anastomosis was initiated. Intractable diseases, including malignancy and IBD, are our main target in surgical treatment and we are seeking for less invasive procedures and new strategies for patients' cure/quality of life.

2. Summary of endoscopic examination in 2008

Giichiro Tsurita, Keisuke Hata, Masaru Shinozaki, Akira Kanamoto, Masahisa Jinushi

Under the cooperation with Department of Advanced Medical Science, we performed 485 upper gastrointestinal endoscopies and 340 colonoscopies without major complications. In April, 2008, Dr. Tsurita became the chief of Division of Endoscopy. Since then, the number of endoscopy cases has increased gradually. For the patients' satisfaction, we aggressively perform endoscopic treatment and avoid operation as much as possible.

Table 1. Surgical procedures performed in 2008

Disease	Procedure	
Esophageal cancer	Subtotal esophagectomy	1
Gastric cancer	Total gastrectomy	3
	Distal gastrectomy	4
Colorectal cancer/tumor	Laparoscopic ileocecal resection	1
	Ileoceal resection	2
	Right hemicolectomy	4
	Left hemicolectomy	2
	Anterior resection	4
	Abdomino-perineal resection	2
	Partial resection	3
	Hartmann's operation	1
	Transanal resection	1
Inflammatory bowel disease	Laparoscopic total proctocolectomy	1
	Abdomino-perineal resection	2
	Ileectomy	1
	Total colectomy	1
	Subtotal colectomy	1
	Ileal pouch anal canal anastomosis	1
	Ileostomy closure	1
The other intestinal diseases	Hemorrhoidectomy	3
	Transverse colostomy	2
	Ileectomy	1
	Subtotal colectomy	2
	Partial colectomy+jejunectomy	1
Hepatoma	Partial hepatectomy	2
Metastatic liver tumor	Partial hepatectomy	1
Gallbladder stone	Laparoscopic cholecystectomy	3
	Cholecystectomy	3
Pancreas cancer	Distal pancreatectomy	1
Breast cancer	Mastectomy	1
	Partial mastectomy	7
Breast tumor	Tumor excision	4
	Fistulectomy	2
Hernia		12
Miscellaneous		48
Total		129

3. Clinical trials completed in/before 2008

Akira Kanamoto, Kenji Nakano, Kimiyasu Yoneyama, Akihiko Itoh, Hideaki Tahara

① Phase I clinical trial of tumor specific vaccine using epitope peptides derived from a novel tumor associated antigen RNF43 against advanced colorectal cancer.

② Phase I clinical trial of epitope peptides based vaccine targeting tumor vascular endothelial cells against advanced cancer patients

such as gastric cancer, colorectal cancer, breast cancer and GIST.

③ Phase I clinical trial: Adjuvant use of epitope peptides based vaccine targeting tumor vascular endothelial cells in patients with surgically resected pancreatic cancer.

④ Phase I clinical trial of epitope peptides based vaccines both targeting RNF43 and VEGFR2 in patients with advanced colorectal cancer.

4. Phase I/II colorectal cancer and breast cancer trial

Masaru Shinozaki, Giichiro Tsurita, Kimiyasu Yoneyama, Keisuke Hata

Genome-wide exploration using cDNA Microarray Profiling enabled a new tumor-associated antigen that can induce potent cytotoxic T-cells (CTLs) specific to tumor cells. Among them, we selected RNF43 (ring finger protein 43), TOMM34 (translocase of outer mitochondrial membrane 34), and TTK protein kinase. We also picked up angiogenesis as a critical mechanism of tumor development. VEGFR1 (vascular endothelial growth factor receptor 1) and VEGFR2 were selected as candidates for vaccine therapy. Specific CTLs for each peptide were induced in cancer patients. We set the combination of peptide for colorectal cancer patients and breast cancer patients with HLA-A*0201 and HLA-A*2402.

Three colorectal cancer patients (3 HLA-A*0201 and 1 HLA-A*2402) and four breast cancer patients (3 HLA-A*2402 and 1 HLA-A*0201) were completed without major complications.

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

Akira Kanamoto, Kenji Nakano, Kimiyasu Yoneyama, Akihiko Itoh, Hideaki Tahara

From the results of phase I clinical trial of melanoma vaccine using gp100 derived peptides, phase I/IIa clinical trial of melanoma vaccine using gp100 derived peptides were performed. HLA-A*2402-restricted gp100 derived peptide (gp100-int4: VYFFLPDHL) 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 25 melanoma patients until 2008. So far, the protocols were well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted.

6. 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.

Akira Kanamoto, Kenji Nakano, Kimiyasu Yoneyama, Akihiko Itoh, Hideaki Tahara

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-int4peptide loaded DC vaccination. However, what we found from the results of phase I clinical trial using DC in our institute was 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. We have enrolled 7 melanoma patients until 2008. So far, the protocols were well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted.

7. Clinical trials under development: Development of gene therapy using dendritic cells

Akira Kanamoto, Kimiyasu Yoneyama, Akihiko Itoh, Hideaki Tahara

In close collaboration with Core Facility for Therapeutic Vectors, we are developing clinical application of IL-12 gene transducted dendritic cells for cancer patients.

Publications

Maki T, Carville A, Stillman IE, Sato K, Kodaka T, Minamimura K, Ogawa N, Kanamoto A, Gottschalk R, Monaco AP, Marr-Belvin A, Westmoreland SV, Sehgal P. SV40 infection associated with rituximab treatment after kidney transplantation in nonhuman primates.

Transplantation 85: 893-902, 2008.
Suzuki T, Ogawa S, Tanabe K, Tahara H, Abe R, Kishimoto H. Induction of antitumor immune response by homeostatic proliferation and CD 28 signaling. J Immunol 80: 4596-605, 2008.
Sunami E, Shinozaki M, Sim MS, Nguyen SL, Vu AT, Giuliano AE, Hoon DS. Estrogen receptor and HER2/neu status affect epigenetic differences of tumor-related genes in primary breast tumors. Breast Cancer Res 10: R46, 2008.
Takahashi H, Ishizaki H, Tahara H, Tamaki Y, Yanagi Y. Suppression of choroidal neovascularization by vaccination with epitope peptide derived from human VEGF receptor 2 in an animal model. Invest Ophthalmol Vis Sci 49: 2143-7, 2008.
Saito T, Takayama T, Osaki T, Nagai S, Suzuki T, Sato M, Kuwano H, Tahara H. Combined mobilization and stimulation of tumor-infiltrating dendritic cells and natural killer cells with Flt3 ligand and IL-18 in vivo induces systemic antitumor immunity. Cancer Sci 99: 2028-36, 2008.
Suzuki H, Tsurita G, Ishihara S, Akahane M, Kitayama J, Nagawa H. Resovist-Enhanced MRI for Preoperative Assessment of Colorectal Hepatic Metastases. Case Rep Gastroenterol 2: 509-16, 2008.

Research Hospital

Department of Joint Surgery
関節外科

Lecturer Hideyuki Takedani, M.D. D.M.Sc.

講 師 医学博士 竹 谷 英 之

Department of Joint Surgery was established in 2006. Our mission is evaluation and treatment of hemophilic arthropathy. In Japan, some hospitals are able to control bleeding for hemophilia using concentrates, however there are no hospitals focus on surgical treatments except us. Many hemophilia patients come to our department from all over Japan. We evaluate their joint function and condition roentgenographically and physiotherapically and explain joint status and indication of surgical treatments. After that, we make surgical plans for some of them and performed surgical treatments mainly joint arthroplasty and arthroscopic synovectomy to improve their quality of life.

Surgical treatment for haemophilia

Hideyuki Takedani

In 2008, there are 20 surgical treatments for hemophilia (15 for hemophilia A, five for hemophilia B) included three inhibitor cases (one hemophilia A and two hemophilia B). We performed 15 joint arthroplasties (eight primary total knee arthroplasties, one revision total knee arthroplasties, seven total hip arthroplasties, one bipolar hip arthroplasties and one total elbow arthroplasties) and three arthroscopic synovectomies and two others.

Publications

1）松下正，天野景裕，瀧正志，岡敏明，酒井道夫，白幡聡，高田昇，高松純樹，竹谷英之，花房秀次，日笠聡，福武勝幸，藤井輝久，田中一郎，三間屋純一，吉岡章，嶋緑倫，インヒビターのない血友病患者の急性出血，処置・手術における凝固因子補充療法のガイドライン，日本血栓止血学会誌，510–519，2008

2）田中一郎，天野景裕，瀧正志，岡敏明，酒井道夫，白幡聡，高田昇，高松純樹，竹谷英之，花房秀次，日笠聡，福武勝幸，藤井輝久，松下正，三間屋純一，吉岡章，嶋緑倫，インヒビター保有先天性血友病患者に対する止血治療ガイドライン，日本血栓止血学会誌，520–539，2008

Research Hospital

Surgical Center
手術部

Associate Professor	Mieko Chinzei, M.D., M.D.Sc.	准教授	医学博士	鎮西 美栄子
Assistant Professor	Sachiko Imamura, M.D.	助 教	医学博士	今村 佐知子
Clinical Engineer	Osamu Ichimura		臨床工学技士	市村 理

Our clinical practice and clinical 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, and (4) assessment of the impact of anesthesia and surgery on autonomic nervous activity. We have published several works on these subjects.

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 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 useful to detect cerebral ischemia during pediatric and adult cardiac surgery sepecially 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/analgsics. 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 mechanism underlying the pain. We also reviewed usefulness of epiduloscopy in pain management in patients with chronic intractable low back pain.

4. Assessment of the impact of anesthesia and surgery on autonomic nervous activity.

It is generally accepted that the parameters

derived from power spectral analysis (PSA) of heart rate variability (HRV) can provide a non invasive measure of autonomic nervous activity. We published several works on assessment of the impact of anesthetics on autonomic nervous activity during perioperative period using real time monitor for PSA of HRV.

Publications

1) Oshima, N,.Chinzei, M. Application of heart rate variability analysis for evaluation of the effects of psychiatric occupationa therapy. J Jpn Soc Balneol Climatol Phys Med, 71 (2), 97-100, 2008,

2) 長友香苗，富岡俊也，金信秀，小川真，鎮西美栄子，山田芳嗣．トリーチャー・コリンズ症候群とピエール・ロバン症候群における気管挿管難易度の加齢による推移について．麻酔58巻；165–169，2009

Research Hospital

Department of Clinical Trial Safety Management
附属病院・医療安全管理部

Associate Professor Fumitaka Nagamura, M.D., D.M.Sc.
Project Assistant Professor Seiichiro Kobayashi, M.D., D.M.Sc.

准教授 医学博士 長 村 文 孝
特任助教(兼) 医学博士 小 林 誠一郎

Division of Clinical Trial Safety Management (DCTSM) owes two major missions. One is the risk management of the Research Hospital (RH) and the other is the support for the conduct of clinical trials, especially for Translational Research (TR). Our roles on TR varies from the assistance for planning study design and writing protocol to the data confirmation by Case Report Form which is managed by Translational Research Coordinator (TRC) and the quality assurance of TRs by monitoring/audit. To protect the right of participants into TR and to conduct TR scientifically and ethically appropriately, we have organized TRC, which consists nurse, pharmacist, clinical laboratory technologist, dietitian, and clinical psychotherapist.

1. Risk management of Research Hospital

Fumitaka Nagamura, Seiichiro Kobayashi, Hatsue Narita

We engage in the risk management of RH. We have promoted the report system on medical incidents and accidents, and quick corresponding scheme such as "Medical Accident-Response Meeting" and "Council of Risk Management in the RH". We take place at least two seminars for staffs of RH on medical safety every year. Almost all of the workers of RH participated into these seminars. We have revised manuals on the risk management and Standard Operating Procedures (SOP) on operations of RH every year. As the result of these actions, no severe medical accident has been observed in 2008.

2. Assistance and oversight of Clinical trials/ TRs

Kazufumi Matsumoto, Kumiko Sumino, Noriko Fujiwara, Ikue Nakajima, Minako Kohno, Makiko Tajima, Fumitaka Nagamura

The assistance by TRC is indispensable for the conduct of clinical trials, especially for TR. In 2008, we participated in 31 protocols, and thirteen of them were newly started in this period. Details of protocols are summarized in Table 1. Table 2 shows the number of patients enrolled into clinical trials at RH in 2008. In addition to these patients, around two hundred patients visited RH for the purpose of the screening. Furthermore, around 500 patients called to ask on participation into clinical trials. TRCs managed the visit for screening and telephone consultation.

3. The development of the scholastic program for the graduate students of nurses in the area of Translational Research.

Kazufumi Matsumoto, Fumitaka Nagamura.

High-educated nurses are required to perform TRs in terms of the protection of study patients and the conducts of scientifically appropriate

Table 1.

Number of protocol	Started in 2008	Continuation before 2008	Total
TR	12	11	23
Clinical trials from pharmaceutical companies	0	4	4
Multi-center studies	1	3	4
	13	18	31

Table 2.

Number of patients	Enrolled in 2008	Continuation before 2008	Total
TR	52	7	59
Clinical trials from pharmaceutical companies	4	2	6
Multi-center studies	3	6	9
	59	15	74

studies. We developed the scholastic program for the graduate students of nurses in the area of TR. We planed and implemented the two-weeks program aimed to acquire the expertise of research nurse for the graduate students of nurses. It consists of lectures on the feature points of TR (e.g. ethical considerations of TRs, and the role of research nurse), role-plays of Institutional Review Board and obtaining Informed Consent, case conference, and the experience of the actual operations. We evaluated the reports and questionnaires from students to explore the degree of their understandings and satisfactions for this program. These reports and questionnaires were analyzed in accordance with the qualitative method.Six students participated in the program this year, and we received the reports and questionnaires. Students could understand the role of research nurse including the supports of investigators, cares and the protection of participants, the coordination between participants and medical staffs, and the necessary ability to conduct appropriately. They were satisfied with the content and the quality of lectures and role-plays, however, the experiences of the actual operations did not meet their demands due to the less acquisition of the practical expertise. Generally, our program meets the demands of students, however, the improvement of the content on the experience of the actual operations is the next issue.

4. Preliminary research for patients participating in translational research on the influence of conflict of interest factors on their decision-making.

Momoyo Ohki[1], Fumitaka Nagamura. [1]Department of Psychology, Faculty of Human Science, Bunkyo University.

We analyzed results in the preliminary research stage for participating in Translational Research (TR) on the influence of Conflict of Interest (COI) factors on their decision-making. Analysis revealed the following tendencies: (1) patients participating in TR paid more heed to intrinsic COIs, which are ubiquitous in research and include an investigator's perceived need to engage in and publish research to achieve career advancement, to receive accolades from peers and professional societies, and to be competitive for grand funding, rather than financial COIs; (2) Information on COI has little effect on decision-making as part of TR participation; (3) Desired methods of specifically describing COIs differ among participants; (4) Opinions on the relationship to the company also differed among participants; (5) efforts of the Institute of Medical Science, the University of Tokyo, along with the activities of Translational Research Coordinators, in dealing with COI problems, have been recognized to an extent from the perspective of compliance with ethics.

Publications

Nagamura-Inoue T, Kai S, Azuma H, Takanashi M, Isoyama K, Kato K, Takahashi S, Taniguchi S, Miyamura K, Aoki K, Hidaka M, Nagamura F, Tojo A, Fang X, Kato S; Japan Cord Blood Bank Network. Unrelated cord blood transplantation in CML: Japan Cord Blood Bank Network analysis. Bone Marrow Transplant. 42: 241-51, 2008

Ooi J, Takahashi S, Tomonari A, Tsukada N, Konuma T, Kato S, Kasahara S, Sato A, Monma F, Nagamura F, Iseki T, Tojo A, Asano S. Unrelated cord blood transplantation after myeloablative conditioning in adults with ALL. Bone Marrow Transplant. (in press)

Ooi J, Takahashi S, Tomonari A, Tsukada N, Konuma T, Kato S, Kasahara S, Sato A, Monma F, Nagamura F, Iseki T, Tojo A, Asano S. Unrelated cord blood transplantation after myeloablative conditioning in adults with acute myelogenous leukemia. Biol Blood Marrow Transplant. 14: 1341-7, 2008.

Futami M, Hatano T, Soda Y, Kobayashi S, Miyagishi M, Tojo A. RNAi-mediated silencing of p190Bcr-Abl inactivates Stat5 and cooperates with imatinib mesylate and 17-allylamino-17-demetoxygeldanamycin in selective killing of p190Bcr-Abl-expressing leukemia cells. Leukemia. 22: 1131-8, 2008.

Inoue Y, Izawa K, Kiryu S, Kobayashi S, Tojo A, Ohtomo K. Bioluminescent evaluation of the therapeutic effects of total body irradiation in a murine hematological malignancy model. Exp Hematol. 36: 1634-41, 2008.

大木桃代，長村文孝．探索型臨床研究において利益相反問題が参加者の意思決定と人権に及ぼす影響の予備的検討　人間科学研究　30：115-120，2008．

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 established in 1990, in order to manage the transfusion medicine and the cell processing for hematopoietic stem cell transplantation. We are transferring the processing facility of Tokyo Cord Blood Bank (Tokyo CBB) for clinical use to Yotsugi Facility and instead we have newly established the research cord blood stem cell bank corporate with Tokyo CBB.
Also we have been engaged to study for the development of various cell therapies together with other departments, as follows.

1. Expansion and regulatory T cells for immunosuppressive therapy and immunological analysis of the patients with hematological disease:

Tokiko Nagamura-Inoue, Kazuo Ogami, Kazuaki Yokoyama[1], Seiko Izawa, Seiichiro Kobayashi and Arinobu Tojo: [1]Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo

Regulatory T cells harbored the immunosuppressive effects and were related to the onset of graft-versus-host disease (GVHD), rejection of organ transplantation and autoimmune disease. We developed the system of *ex vivo* expansion of CD25+FOXP3+regulatory T cells from the small amount of peripheral blood, to apply the cell therapy for severe GVHD, autoimmune diseases (Collaboration with Division of Molecular Therapy). In addition, we have been monitoring the immunological change of the patients with hematological disease, especially CML.

2. Research Cord Blood Stem Cell Bank:

T. Nagamura-Inoue, I. Ishige, Miki Yuzawa, K. Ogami and A. Tojo

"Research Cord Blood Stem Cell Bank" (former named 'Research Stem Cell Resource Bank') was established with the support of MEXT (Ministry of Education, Culture, Sports, Science and Technology) for the achievement of the development of the medicine including Regenerative Medicine and drug discovery in Japan since 2004. The research banks perform cord blood processing for non-conforming units for clinical use incorporated public clinical cord blood bank and cryopreserve and provide to domestic researchers for research use via RIKEN Bioresource Center. Visit our home page http://scb.ims.u-tokyo.ac.jp/

3. Exploring mesenchymal stem cells derived from Umbilical Cord:

Ikuo Ishige[1], Tokiko Nagamura-Inoue, Arinobu Tojo: [1]Department of Stem cell processing, Lab. of Stem cell Therapy, The Institute of Medical Science, The University of Tokyo

In addition to contribute the research use of cord blood banking as the regenerative leading project, we have been explored the new source, mesenchymal stem cells derived from umbilical cord (Warton Jelly) (Collaboration with Division

of Molecular Therapy and Department of Stem cell processing).

4. Room for Clinical Cellular Technology (RCCT):

Tokiko Nagamura-Inoue, Kazuo Ogami, Masako Hirai, Arinobu Tojo and RCCT Execution Committee

To promote the cell therapy related to translational research, RCCT has been established in 1997. Until now, the following projects were implemented; 1) Cord blood cell processing for banking (for Tokyo Cord Blood Bank and Research cord blood stem cell bank), 2) Dendritic cell therapy, 3) Regenerative therapy of alveolar bone derived from bone marrow mesenchymal cells, 4) Gene therapy for renal cancer.

Publications

Ikeda K, Nagamura-Inoue T, Kai S, Fujii Y, Ozaki S, Sagawa K, Takamatsu J, Takahashi K, Ohto H. Questionnaire surveys on cell processing in Japan by the Japanese Society of Transfusion Medicine and Cell Therapy/Japanese Association of Medical Technologists (2007) and by Working Group for Adverse Events of the Transfusion Conference of the University Hospitals (2006)., Jap. J. Transfusion and cell Therapy, in press

Atsuta Y, Suzuki R, Nagamura-Inoue T, Taniguchi S, Takahashi S, Kai S, Sakamaki H, Kouzai Y, Kasai M, Fukuda T, Azuma H, Takanashi M, Okamoto S, Tsuchida M, Kawa K, Morishima Y, Kodera Y, and Kato S, for the Japan Marrow Donor Program and the Japan Cord Blood Bank Network; Disease-specific analyses of unrelated cord blood transplant compared with unrelated bone marrow transplant in adult patients with acute leukemia. Blood, 113, 1631-8. 2009.

Yoshimi A, Kojima S, Taniguchi S, Hara J, Matsui T, Takahashi Y, Azuma H, Kato K, Nagamura-Inoue T, Kai S, Kato S; Japan Cord Blood Bank Network., Unrelated cord blood transplantation for severe aplastic anemia. Biol Blood Marrow Transplant. 9, 1057-63. 2008

Nagamura-Inoue T, Kai S, Azuma H, Takanashi M, Isoyama K, Kato K, Takahashi S, Taniguchi S, Miyamura K, Aoki K, Hidaka M, Nagamura F, Tojo A, Fang X, Kato S; Japan Cord Blood Bank Network. Unrelated cord blood transplantation in CML: Japan Cord Blood Bank Network analysis. Bone Marrow Transplant. 42, 241-51, 2008

Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Kobayashi T, Sato A, Kato S, Kasahara S, Ebihara Y, Nagamura-Inoue T, Tsuji K, Tojo A, Asano S. Cardiovascular toxicity of cryopreserved cord blood cell infusion. Bone Marrow Transplant. 41, 861-5. 2008

Research Hospital

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

Professor	Hideaki Tahara, M.D., D.M.Sc.		教授(室長)	医学博士	田 原 秀 晃(併)
Guest Researcher	Takafumi Nakamura, Ph.D.		客員研究員	生命科学博士	中 村 貴 史
Project Assistant Professor	Hisako Katano, D.D.S., Ph.D.		特任助教	歯学博士	片 野 尚 子

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 or cells under current Good Manufacturing Practice (cGMP) conditions defined by FDA of USA. The CFTV is the first facility established in Japanese academia to produce genetic or cellular vectors of clinical grade. Using this facility, clinical lots of the adenoviral vector and herpes vector are scheduled to be produced in CFTV in 2008-2009.

1. Preparation of Standard Operating Procedures (SOPs)

The cGMP compliance is maintained using written SOPs prepared by ourselves. The SOPs codify all aspects of laboratory activities including facility design and operations of the personnel. The SOPs enables the staff not only to produce the reagents with high quality in the stable manners but also to help identify areas for improvement.

2. Adoption of ISO

In order to continuously improve our activities, quality management system of the CFTV has been assessed and found to be in accordance with the requirements of the quality standards detailed ISO9001:2000; in the scope of development and manufacture of cell and gene therapy products.

3. Validation of CFTV

The CFTV consists of 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 generating dendritic cells for immunotherapy and gene therapy. There are two self-contained vector production suites in the Vector Unit and two self-contained tissue culture suites in the Cell Unit. These suites are kept Class 10,000. There are many features incorporated into the design of this CFTV to minimize the risk of cross-contamination between products; i. e., unidirectional traffic flow, individual airlocks to each production suite, single-pass HEPA filtered supply air, 100 percent exhaust from the biological safety cabinets through dedicate ducts, among others. Periodical validation has been performed on the facility and the equipments in CFTV to ensure cGMP compliance.

4. Projects in CFTV

Four projects are now in progress in the CFTV.

I. Cancer gene therapy using dendritic cells transfected with IL-12 genes

Collaboration with Division of Bioengineering of Advanced Clinical Research Center (ACRC)

and Department of Surgery of the research hospital.

・Preparation of the Clinical Lot

We have been preparing the replication-defective recombinant adenoviral vector encoding human interleukin-12, which is an immunestimulatory cytokine. The backbone of this vector 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. The IL-12 genes are driven by a CA promoter (CMV-IE enhancer with the chicken β-actin promoter). The master virus seed stock (MVSS) and purified final material have been prepared following the optimization of purified method for the production of high-titer vector. The purified material is now in the process of quality examination for use of early phase trials.

II. Vaccine therapy with peptide-loaded dendritic cells for advanced melanoma

Collaboration with Division of Bioengineering of ACRC and Department of Surgery of the research hospital.

・Preparation of Peptide-Loaded Dendritic Cells (DCs)

We have been supporting phase I clinical trials against melanoma. Based on the results of the basic research performed in Division of Bioengineering, the SOPs of the DC preparation have been written and used. The cellular reagents have been successfully prepared in the Cell Unit and offered for clinical trials without serious problems.

III. Oncolytic viral therapy using genetically engineered herpes simplex viruses for malignant brain tumors.

Department of Neurosurgery, Graduate School of Medicine, The University of Tokyo,

・Manufacture of the viral vector

In collaboration, we have been preparing oncolytic herpes simplex virus. This preparation will be used for phase I clinical trial for brain cancer patients. We have supported the establishment of the master and working cell banks of Vero cells to produce genetically engineered herpes simplex viruses. The cGMP compliant MVSS, which contains a replication-competent herpes simplex virus type 1 vector defective for the α47 gene, was successfully produced. The purified final products have been successfully prepared and are now in the process of regulatory approvals for use of early phase trials.

IV. Development of robotized cell culture system

In collaboration with Dr. Wakitani of Osaka City University and Kawasaki Heavy Industries, Inc., we are developing robotized cell culture system which could be applied to a variety of procedures including virus production as a funded project by NEDO.

5. Financial Support

This CFTV has been supported in large by 21 st Century COE Program (P.I. Dr. Yusuke Nakamura) from Japan Society for the Promotion of Science (2003-2007). From 2007, it has been also supported by Coordination, Support and Training Program for Translational Research from Ministry of Education, Culture, Sports, Science and Technology (2007-2011).

Publications

Hummel, HD., Kuntz, G., Russell, SJ., Nakamura, T., Greiner, A., Einsele, H. and Topp, MS. Genetically engineered attenuated measles virus specifically infects and kills primary multiple myeloma cells. J. Gen. Virol. 90: 693-701, 2009.

Jing, Y., Tong, C., Zhang, J., Nakamura, T., Iankov, I., Russell, SJ. and Merchan, JR. Tumor and vascular targeting of a novel oncolytic virus retargeted against the urokinase receptor. Cancer Res. 69: 1459-1468, 2009.

Pham, L., Nakamura, T., Gabriela Rosales, A., Carlson, SK., Bailey, KR., Peng, KW. and Russell, SJ. Concordant activity of transgene expression cassettes inserted into E1, E3 and E4 cloning sites in the adenovirus genome. J. Gene Med. 11: 197-206, 2009.

Takeda, M., Ohno, S., Tahara, M., Takeuchi, H., Shirogane, Y., Ohmura, H., Nakamura, T. and Yanagi, Y. Measles viruses possessing the polymerase protein genes of the Edmonston vaccine strain exhibit attenuated gene expression and growth in cultured cells and SLAM-knockin mice. J. Virol. 82: 11979-11984, 2008.

Inoue, H., Iga, M,., Xin, M., Asahi, S., Nakamura, T., Kurita, R., Nakayama, M., Nakazaki, Y., Takayama, K., Nakanishi, Y. and Tani, K. TARC and RANTES enhance antitumor immunity induced by the GM-CSF-transduced tumor vaccine in a mouse tumor model. Can-

cer immunology immunotherapy. 57: 1399-1411, 2008.
Nakamura, T. and Russell, SJ. Chapter 18 of engineering oncolytic measles viruses for targeted cancer therapy. molecular targeting in oncology. Edited by Kaufman, HL., Wadler, S. and Antman, K. (Humana Press). pp 431-445, 2008.

Research Hospital

Department of Laboratory Medicine
附属病院　検査部

Associate Professor Naoki Oyaizu, M.D., Ph.D.
Assistant Professor Naoyuki Isoo, M.D., Ph.D.

准教授，部長　医学博士　小柳津　直　樹
助　教　医学博士　磯　尾　直　之

The Department of Laboratory Medicine consists of eight divisions-clinical physiology, hematology, biochemistry, serology, bacteriology, molecular diagnosis and pathology, and a division of flow cytometrical analysis which has been added recently. This Department engages in the laboratory analysis and gives diagnosis of clinical materials in the hospital. While facilitating the ongoing translational research (TR) projects in the research hospital, the Department also functions as an integrated diagnosis & monitoring laboratory that evaluates the safety and effectiveness of experimental therapeutic approaches. These functions are now offcially operating under the name of "laboratory of TR vererification".

Overview

Our basic research strategies include the following approaches: characterizing molecular mechanisms underlying the pathology, developing a novel method to measure the disease-defining mechanism in the clinical materials and evaluating the effectiveness of molecular-targeted therapies thereby contributing to the translational research conducted in the institute. Integrating molecular-/biochemical-based laboratory assays on the solid background of pathological examinations enables us to evaluate the effectiveness of experimental clinical trials and leads to correct experimental therapies that further promote translational research. Our department also functions as an integrated diagnosis & safety-monitoring laboratory as well as the division of quality control by examining/evaluating the safety of investigational new drugs under GMP conditions.

1. Evaluation of "proof of concept" of the TR clinical trials and validate safety of the biomedical reagents under GMP standard

Evaluation of "proof of concept" of the TR clinical trial is one of our important missions which are described as follows. In addition to this, the missions include validate the safety of the biomedical reagents such as vector constructs are cellular therapeutic materials which will be used for patients as gene therapy and cell therapy, respectively. These are critical to conduct TR clinical trials in a safe manner. We thus established a new division, which specifically aimed to fulfill this purpose under the quality of GMP regulation. We are now ready to validate the safety by conducting aseptic test, endotoxin-free test and micoplasma-free test under strictly regulated GMP facility.

2. Pathological evaluation of cancer immunotherapy

We have initiated the analysis of surgical specimen obtained from the patients under cancer immuno-therapy conducted in the research hospital. By applying sophisticated immunohistochemical techniques, we now are intensively analyzing materials from cases including GM-CSF-based gene therapy for renal cell carcinoma

and dendritic cell-based or peptide-pulsed anti-melanoma immuno-therapy. One of our goals is to evaluate the effectiveness of the therapies and to elucidate the mechanisms of anti-tumor immune response elicited by the therapy *in situ*.

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

We found the presence of characteristic pathological findings in bone marrow specimen from some patients with MDS-RA, aplastic anemia, or pure red cell aplasia, which implicates 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, the Department of Laboratory Medicine will elucidate molecular mechanisms based on the pathological consideration to establish new disease entities and develop new therapeutic interventions.

4. Analysis of the chimeric gene expression of hematological disorder

We have initiated the analysis of bcr-abl gene expression in specimen from patients with CML and Ph1+ve ALL by real-time PCR and nested RT-PCR techniques. In addition, we sequenced the amplified products to provide information for the molecular resistance to STI571 treatment. We are expanding the target molecules to non-hematological disorder, which includes c-kit, PDGF-R genes that is associated with gastro-intestinal stromal cell tumor (GIST).

5. Developing quick & inclusive diagnosis system for infectious disease

Since the introduction of new therapeutic maneuver, host-pathogen interactions have drastically altered drawing attention. This has resulted in altered recognition and molecular interaction of infected cells with immune cells, leading to atypical pathological as well as clinical manifestations. While distinguishing infectious disease and immunological disorder calls for urgent attention, it may be difficult to achieve these tasks in some cases due to modified manifestations. To avoid such cases, it is imperative to establish a comprehensive diagnosis system of infectious disease to the earliest possible opportunity.

6. Immunopathogical analysis of hematopoietic cell transplantation

The number of allogeneic hematopoietic stem cell transplantation (HSCT), mainly cord blood transplantation, has been performed for the treatment of hematological malignancies. Graft-versus-host disease (GVHD), a life-threatening complication, occurs as a complication of allogeneic HSCT. Our prime goal is to develop a new way to detect GVHD and make an accurate evaluation of GVHD at our laboratory.

Publications

1. Ohnuma K, Hosono O, Kawasaki H, Yoshikawa N, Katayose T, Oyaizu N, Tanaka H, Morimoto C. An adult case of Henoch-Schönlein purpura complicating common peroneal nerve mononeuropathy. Mod Rheumatol. 2009; 19: 73-9.
2. Togo M, Hashimoto Y, Iso-O N, Kurano M, Hara M, Kadowaki T, Koike K, Tsukamoto K. Identification of a novel mutation for phytosterolemia. Genetic analyses of 2 cases. Clin Chim Acta. 2009 401 (1-2): 165-9.