10月8日午前に名古屋大学医学部分子病態内科を訪問し、移植血液内科医を交えて養子免疫療法に対する意見交換を行った。

10月8日午後に名古屋第一赤十字病院を訪問し、養子免疫療法に関するミニレクチャー後に病棟を回診し、さらに質疑応答を行った。

10月8日夕刻より名古屋ＢＭＴグループと共催で講演会を行った。（参加者42名）テーマは再発白血病に対する養子免疫療法についてで、受け入れ研究者である赤塚美樹が愛知県がんセンター研究所における現状について、Warren 博士がフレッドハッチンソン癌研究所での7例の症例を中心に討論した。

10月 12 日に岡山大学医学部第二内科を訪問し若手研究者と討論後、講演会を行った。（参加者16名）

10月13日に京都大学医学部血液内科において講演会が開催され、

①再発白血病に対する養子免疫療法

②HLA 不適合ドナーからの移植の際の免疫療法

③マイナー抗原が欠損している場合の養子免疫療法

等についての討議が行われた。（参加者35名）

7. 研究課題の成果

当班のテーマの１つである研究班の目的の一つである「マイナー抗原特異的Ｔ細胞による DLI のための臨床試験体制の確立と実施に関する研究」のために金沢、名古屋、岡山、京都で専門家を集め発表・討議をおこなった。

A. 金沢大学では養子免疫療法実施の際における具体的な問題点について講演・討議が行われた。まずマイナー抗原に特異的な T 細胞を投与前に長期間大量培養する間における機能や特異性の変化の可能性が問題となった。従来腫瘍特異的 T 細胞株を長期培養すると構成成分が変化することがあったが、フレッドハッチンソン癌研究所（以下 FHCRC）の経験によると、T 細胞を単一のクローンとして培養することにより、細胞傷害性試験において特異性や機能が十分保たれていると回答があった。次いで、実際にマイナー抗原に特異的な T 細胞を輸注した患者の末梢血から輸注した T 細胞は検出されたかどうか、またその機能についての議論があった。これに対し T 細胞受容体のクローン特異的部分の PCR 解析により、輸注したと同一の T 細胞が移植片対白血病効果の発現に一致して増加していることが認められたが、細胞数が少量であるため細胞傷害機能の保持の有無については今後の検討課題であることが認識された。最後に養子免疫療法を動物モデルで見るにはどのような動物が良いかが議論となった。FHCRC では NOD-SCID マウスが用いられているが、β2 ミクログロブリンや IL-2 のγ鎖をノックアウトしたマウスの方が良いかに関しては、確かに後者のマウスを用いると白血病細胞の生着率は高くなるが、易感染性が増強

するためマウスの維持が難しいという問題があり、必ずしも後者が優れているとは限らないという結論となった。

B.愛知県がんセンターでは、名古屋市立大学医学部等からの参加者も交え、Warren博士が最近同定した新規のマイナー抗原に関する講演があり、その後質疑応答が行われた。このマイナー抗原は従来のものと違い、タンパク質上の連続したペプチドに由来しないことが極めて新規性に富んでいた。詳細な検討の結果、１つのタンパク質の２箇所のペプチド断端が再結合してマイナー抗原のエピトープペプチドとなることが判明した。この発表に対し、そのメカニズムに関する質問があったが、まだ推論の域を出ないが、ペプチドを産生するプロテアゾームという大きなタンパク質切断酵素の中でタンパク質がループ状になり、その部分が除かれたあと残りが再結合する可能性が示された。ごく最近タンパク質によってはそのようなスプライシングを起こすものがあることが報告されたばかりであり、それが免疫細胞の標的となることが示唆されたことは、今後のエピトープ同定作業に於いて大きな収穫となった。

C-1. 名古屋大学医学部では主に Warren 博士のもとに留学している名古屋大学出身の研究生の研究テーマについて直江知樹教授と会談がもたれ、今後の共同研究についての話し合いが行われた。

C-2. 次いで、名古屋第一赤十字病院において養子免疫療法についてのセミナーが開催された。参加者は血液内科、血液小児科の医師が中心であった。とくに臨床の立場から養子免疫療法開始時には免疫抑制剤を中断する必要の有無について議論となった。FHCRC の経験では、免疫抑制剤を中断することは輸注する T 細胞の体内での生存を高めるために必須であるが、どうしても GVHD が起こることがあり、重症の GVHD が出てしまうと治療はうまくいかないとの経験談が示された。最近増加している骨髄腫に対する養子免疫についての質問が出たが、まだ FHCRC でも経験は無いとの回答で、再発骨髄腫に対しては Thalidomide, Velcade をまず考えるという示唆を受けた。

C-3. ８日夕方より学術講演会を行ったが、主な参加者は名古屋血液骨髄移植グループに属する血液内科・小児科医であった。ここでは受け入れ研究者である赤塚美樹がまず愛知県がんセンター研究所でのマイナー抗原を標的とした養子免疫療法に対する取り組みを講演した。次いで Warren 博士より、FHCRC で７例の患者に対して実際に施行された養子免疫療法に関する詳細な報告があった。全て再発進行白血病症例であったが、４例で良好な反応があり、うち１例が２年以上長期生存していた。ここで示された問題は、大部分の再発が比較的移植後早期に起こり、この時点ではまだ養子免疫に用いるクローン化 T 細胞の準備が間に合わない場合があることであ

った。本研究班の班長であり招へい申請者でもある名古屋第一赤十字病院血液内科部長小寺より、この免疫療法を普及させるためにはどうすればよいかと質問が提示された。これに対し、まず早期再発例が治療対象となるよう、T 細胞クローンの樹立を移植後の患者末梢血から行わず、移植前にドナーから得た T 細胞から直接樹立する試みがなされていることが述べられた。

D. 岡山大学では養子免疫療法の標的となるマイナー抗原の同定法と、その臨床応用について講演会・討議が行われた。特に臨床材料の収集から始まり、細胞傷害性 T 細胞クローンの樹立、特異性の検討、遺伝子の同定に至る綿密な手順の解説は参加者にとって非常に参考となった。

E. 京都大学での研究会では、難治性造血器腫瘍に対するマイナー抗原を標的とした養子免疫療法の実現可能性が示されたことを前提として、今後さらにその臨床現場への普及に向けた課題が議論された。ひとつは、キラーT 細胞の体外増幅過程の迅速化であり、Warren 博士らのプロトコールに参加した症例においても、実際には、T 細胞の準備期間にすでに再発をきたしてしまう場合が相当数認められたことがここでも議論の中心となった。このことはきわめて重要な課題であることが再認識された。また、腫瘍細胞自身が、マイナー抗原を提示する HLA クラスⅠ分子の欠損や発現量抑制などの機序を用いて、キラーT 細胞による細胞傷害を回避する可能性があることから、今後は複数のキラーT 株を準備し、連続的に輸注していくような戦略も検討されるべきであろう、という議論が行われた。

8．外国人研究者のレポートは、別添のとおりである。

Adoptive T Cell Therapy Targeting Minor Histocompatibility Antigens for the Treatment of Leukemia and Renal Cell Carcinoma

Edus H. Warren
Program in Immunology, Fred Hutchinson Cancer Research Center

The graft-versus-host (GVH) and graft-versus-leukemia (GVL) reactions that occur after MHC-identical allogeneic hematopoietic cell transplantation (HCT) are mediated by T lymphocytes derived from the donor. Studies in our laboratory and others have shown that $CD8^+$ cytotoxic T lymphocytes (CTL) specific for minor histocompatibility (H) antigens expressed in recipient hematopoietic cells, including leukemic cells, but not widely expressed in non-hematopoietic tissues, can be isolated from most MHC-identical transplant recipients. A Phase I trial has been initiated at the Fred Hutchinson Cancer Research Center to evaluate the safety and anti-leukemic efficacy of adoptive T cell therapy with $CD8^+$ CTL clones specific for tissue-restricted minor H antigens, in patients with advanced myelodysplastic syndrome and acute leukemia who relapse after HCT from an MHC-identical donor. The CTL clones are generated from transplant recipients prospectively at the time of transplant, and characterized for tissue specificity and class I MHC restriction. To date, 44 infusions of eight different minor H antigen-specific CTL clones have been administered to seven patients. Adoptively transferred CTL have been detected in the blood and bone marrow of recipients up to 21 days after infusion. Graft-versus-tumor (GVT) effects have also been observed after nonmyeloablative MHC-identical HCT for renal cell carcinoma (RCC), and studies to investigate the specificity of effector cells involved in tumor regression are currently in progress.

白血病および腎細胞癌に対するマイナー抗原を標的とした養子免疫療法

フレッドハッチンソン癌研究所　免疫学部門

MHC が一致した同種造血細胞移植（HCT）後に起こる移植片対宿主（GVH）および移植片対白血病(GVL)反応はドナー由来の T 細胞によって引き起こされる。我々や他のグループの研究により、患者の非造血系細胞には広範に発現されていないが、白血病細胞を含む造血系細胞で発現しているマイナー抗原に特異的な CD8 陽性の細胞障害性 T 細胞（CTL）が大部分の移植後患者から樹立できることが示されている。フレッドハッチンソン癌研究所では HCT 後に再発した進行期の骨髄異型性症候群や急性白血病患者において、造血系細胞特異的マイナー抗原に反応する CD8 陽性 CTL を養子移入する治療法の第一相試験が開始されている。CTL クローンは HCT 後に再発に備え前もって樹立され、組織特異性やクラス I MHC 拘束性について検索される。現在までに８種類の異なったマイナー抗原に特異的な CTL クローンが７例の症例に対して合計 44 回投与された。輸注された CTL は末梢血や骨髄中で最長 21 目まで検出された。移植片対腫瘍（GVM）効果もまた骨髄非破壊的 HCT を受けた腎細胞癌の患者で認められており、現在腫瘍の縮小に関わっているエフェクターT 細胞の特異性に関する研究が進行中である。

VI. 公開シンポジウム記録

平成１６年度厚生労働科学研究 ヒトゲノム・再生医療等研究事業
五研究班合同公開シンポジウム

日時：2005年1月29日（土）午後1時30分~午後5時30分
会場：東京慈恵会医科大学 高木2号館南講堂（地下1階）
東京都港区西新橋3-25-8　Tel:03-3433-1111
交通機関：都営三田線 御成門駅より　徒歩3分
日比谷線　神谷町駅より　徒歩7分
ＪＲ　新橋駅 烏森口より 徒歩10分

1:30 **開会の挨拶**　齋藤英彦　主任研究者

厚生労働省挨拶　片岡佳和　厚生労働省健康局疾病対策課臓器移植対策室

発表演題　・五研究班からの発表

Ⅰ.　【座長 高上洋一(主任研究者)】

1. ミニ移植臨床試験の現況
高上洋一　国立がんセンター中央病院薬物療法部

2. 移植後の抗腫瘍免疫回復
平家勇司　国立がんセンター研究所造血幹細胞移植科

Ⅱ.　【座長 齋藤英彦(主任研究者)】

1. 日本さい帯血バンクネットワークを利用した臍帯血移植の臨床成績
加藤俊一　東海大学医学部基盤診療学系再生医療科学

2. 複数臍帯血同時移植の成績
原　宏、甲斐俊朗　兵庫医科大学 内科学/輸血学

Ⅲ.　【座長 小澤敬也(主任研究者)】

1. 骨格筋への脱分化遺伝子導入による新規造血幹細胞ソース開拓
久米晃啓、信吉正治、小澤敬也　自治医科大学分子病態治療研究センター遺伝子治療研究部

Ⅳ.　【座長 小寺良尚(主任研究者)】

1. 強皮症と造血幹細胞移植
坊垣暁之、小池 隆夫　北海道大学大学院医学研究科病態制御学

2. 再生医療・細胞治療実用化のための安全管理体制の確立
清水則夫、森尾友宏、伊藤仁也　東京医科歯科大学 難治疾患研究所ウイルス感染分野／細胞治療センター 先端医療センター再生医療研究部

3. 非血縁者からの移植における組織適合性抗原の意義
森島泰雄　愛知県がんセンター病院血液・細胞療法部

4. 造血幹細胞ドナー(骨髄、末梢血)の安全確保に向けて――日本及び海外の作業状況――
小寺良尚　名古屋第一赤十字病院第四内科、骨髄移植センター

Ⅴ.　【座長 加藤俊一(主任研究者)】

1. 血縁者間骨髄移植ドナーの権利擁護に向けて
加藤俊一　東海大学医学部基盤診療学系再生医療科学

5:25 **閉会の挨拶**　小寺良尚　主任研究者

主催：厚生労働科学研究 ヒトゲノム・再生医療等研究事業
Ⅰ.「骨髄非破壊的前処置療法を用いた同種造血幹細胞移植の確立と普及に関する研究」班
連絡先：国立がんセンター中央病院薬物療法部長室内　《 Tel:03-3248-1510　Fax:03-3248-1510 》
Ⅱ.「臍帯血を用いた造血細胞移植の確立に関する研究」班
連絡先：国立病院機構名古屋医療センター院長室内　《 Tel:052-951-1111　Fax:052-951-0559 》
Ⅲ.「造血系再生医療への応用を目的とした増殖分化制御システムの開発研究」班
連絡先：自治医科大学分子病態治療研究センター遺伝子治療研究部内　《 Tel:0285-58-7402　Fax:0285-44-8675 》
Ⅳ.「骨髄等を利用した効率的な造血幹細胞移植の運用・登録と臨床試験体制の確立に関する研究」班
連絡先：名古屋第一赤十字病院第四内科内　《 Tel:052-481-5111　Fax:052-483-3647 》
Ⅴ.「移植医療におけるドナー及びレシピエントのQOL向上に関する研究」班
連絡先:東海大学医学部基盤診療学系再生医療科学教授室内　《 Tel:0463-93-1121　Fax:0463-91-6235 》

Ⅶ. 研究班会議記録

研 究 班 会 議 記 録

◆ 班 会 議 ◆

・第一回研究班会議

期 日：2004 年 6 月 19 日（土） 午前 10 時～午後 5 時 30 分
会 場：名古屋第一赤十字病院　古川講堂

・第二回研究班会議

期 日：（一日目）2005 年 1 月 28 日（金）午後 1 時～午後 5 時 30 分
（二日目）　29 日（土）午前 9 時～午後 1 時
会 場：東京慈恵会医科大学　高木 2 号館南講堂

◆ 研究打合せ会 ◆

・活性化 CD4－DLI に関する第一回研究打合せ会

期 日：2004 年 7 月 23 日（金）午後 4 時～午後 6 時
会 場：東京医科歯科大学医学部附属病院　会議室

・組織適合性関連第一回研究打合せ会

期 日：2004 年 6 月 19 日（土)午後 12 時 25 分～午後 1 時 25 分
会 場：名古屋第一赤十字病院　第一会議室

・活性化 CD4－DLI に関する第二回研究打合せ会

期 日：2004 年 7 月 23 日（金）午後 4 時 ～ 午後 6 時
会場 ： 東京医科歯科大学医学部付属病院　会議室

・活性化 CD4－DLI に関する第三回研究打合せ会

期 日：2004 年 8 月 6 日（金） 午後 5 時～午後 7 時
会 場：東京医科歯科大学医学部附属病院　第二ゼミナール室

・活性化 CD4－DLI に関する第四回研究打合せ会

期 日：2004 年 8 月 20 日（金） 午後 1 時～午後 3 時
会 場：名古屋第一赤十字病院　第一会議室

・**HLA 2,3** 抗原不一致血縁者間同種造血幹細胞移植に関する研究打合せ会

期 日：2004 年 11 月 26 日（金） 午後 6 時 30 分～午後 8 時
会 場：東京ステーションホテル　「牡丹の間」

・組織適合性関連第二回研究打合せ会

期 日：2005 年 1 月 28 日（金）午前 11 時～12 時
会 場：東京慈恵会医科大学　高木会館Ｆ会議室

Ⅷ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Kanda Y., Izutsu K., Hirai H., Sakamaki H, Iseki T., Kodera Y., Okamoto S., Mitsui H., Iwato K., Hirabayashi N., Furukawa T., Maruta A., kasai M., AtsutaY., Hamajima N., Hiraoka A., Kawa K.	Effect of graft-versus-host disease on the outcome of bone marrow transplantation from an HLA-identical sibling donor using GVHD prophylaxis with cyclosporin A and methotrexate.	Leukemia	18	1013-1019	2004
Nishida T., Hamaguchi M., Hirabayashi M., Haneda M., Terakura S., Atsuta Y., Imagama S., Kanie T., Murata M., Taji H., Suzuki R., Morishita Y., Kodera Y.	Intestinal thrombotic microangiopathy after allogeneic bone marrow transplantation: a clinical imitator of acute enteric graft-versus-host disease.	Bone Marrow Transplantation	33	1143-1150	2004
Ogawa H., Ikegame K., Kawakami M., Takahashi S., Sakamaki H., Karasuno T., Sao H., Kodera Y., Hirabayashi N., Okamoto S., Harada M., Iwato K., Maruta A., Tanimoto M., Kawa K. on behalf of the Japan Society for Hematopoietic Cell Transplantation	Impact of cytogenetics on outcome of stem cell transplantation for acute myeloid leukemia in first remission: a large-scale retrospective analysis of data from the Japan Society for Hematopoietic Cell Transplantation.	International Journal of Hematology	79	495-500	2004
Sakata N, Kawa K, Kato K., Yabe H., Yabe M., Nagasawa M., Mugishima H., Kigawsawa H., Tsuchida M., Akiyama Y., Morishima Y., Kodera Y., Kato S.	Unrelated donor marrow transplantation for congenital immunodeficiency and metabolic disease: an update of the experience of the Japan Marrow Donor Program.	International Journal of Hematology	80	174-182	2004

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Yamamori I., Kanei T., Maeda N., Kodera Y., Matsuyama T. and Hasegawa H.	Appearance of thyroid stimulating and blocking immunoglobulins after bone marrow transplantation: presentation of two contrasting cases.	Endocrine Journal	51 (4)	439-443	2004
Ichinohe T., Uchiyama T., Shimazaki C., Matsuo K., Tamaki S., Hino M., Watanabe A., Hamaguchi M., Adachi S., Gondo H., Uoshima N., Yoshihara T., Hatanaka K., Fujii H., Kawa K., Kawanishi K., Oka K., Kimura H., Itoh M., Inukai T., Maruya E., Saji H., and Kodera Y., for the Japanese Collaborative Study Group for NIMA-Complementary Haploidentical Stem Cell Transplantation.	Feasibility of HLA-haploidentical hematopoietic stem cell transplantation between noninherited maternal antigen (NIMA)-mismatched family members linked with long-term fetomaternal microchimerism.	BLOOD	104 (12)	3821-3828	2004
Torikai H., Akatsuka Y., Miyazaki M., Warren EH Ⅲ, Oba T., Tsujimura K., Motoyoshi K., Morishima Y., Kodera Y., Kuzushima K., and Takahashi T.	A novel HLA-A*3303-restricted minor histocompatibility antigen encoded by an unconventional open reading frame of human TMSB4Y gene[1].	The Journal of Immunology	173 (11)	7046-7054	2004
Kondo E., Akatsuka Y., Nawa A., Kuzushima K., Tsujimura K., Tanimoto M., Kodera Y., Morishima Y., Kuzuya K., and Takahashi T.	Retroviral vector backbone immunogenicity: identification of cytotoxic T-cell epitopes in retroviral vector-packaging sequences.	Gene Therapy	12	252-258	2005
Nakai K., Kanda Y., Fukuhara S., Sakamaki H., Okamoto S., Kodera Y., Tanosaki R., Takahashi S., Matsushima T., Atsuta Y., Hamajima N., Kasai M. and Kato S.	Value of chemotherapy before allogeneic hematopoietic stem cell transplantation from an HLA-identical sibling donor for myelodysplastic syndrome.	Leukemia	19	396-401	2005

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
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Ⅸ. 資 料

Physicians Abstracts

EBMT 2005

Presidential Symposium

66

Severe adverse events of allogeneic peripheral blood stem cell donors –results of nation-wide 2,784 prospectively registered case survey and of its comparison to bone marrow donors in Japan

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In April 2000, we created a system, which was cooperatively steered by The Japan Society of Hematopoietic Cell Transplantation (JSHCT) and G-CSF producing and/or selling companies, to catch up the types and the frequencies of acute and late severe adverse events (SAE) of peripheral blood stem cell (PBSC) donors in Japan. Every PBSC donor was registered to JSHCT and was given unique donor number (UDN) before the PBSC donation. Every harvest center was mandatory required to submit the day 30 report as well as the immediate report of any severe SAE, and also to ask donors Ereceiving annual health check for 5 years. This time, we report the acute SAE observed among 2, 784 consecutive donors in 223 institutes and the late SAE reported by the forth year of post PBSC harvest among 1,746 donors who agreed with this work of the society. As of March 2004, 47 acute SAE out of 2,784 cases (1.7%) were reported. Those were 12 thrombocytopenia, 9 liver damage, 8 febrile episode, 1 interstitial pneumonitis, subarachnoid hemorrhage (SAH), cholecystitis with stone and others. Late SAE were reported at 27 out of 1,746 cases (1.5%). Those were 2 hematological malignancies (acute myelogenous leukemia and myeloproloiferative disorder), 8 other malignancies (4 breast cancer, 1 gastric cancer, uterus cancer, brain tumor, pharygeal cancer), 5 thyroid disorder, 3 myoma uteri, 2 rheumatoid arthritis, 1 brain infarction and SAH and others. To compare these acute and late SAE of PBSC donors to those of bone marrow (BM) donors, the questionnaires shared with The European Group of Blood and Marrow Transplantation were sent to 378 institutes of JSHCT and 203 institutes (53.7%) answered about 10,701 cases and the comparative results were as followings: BM donors: PBSC donors; Death within 30 days = 0:0, Hematological malignancy = 2:2. These results showed that acute SAE, some of which were close to life-threatening, occurred at PBSC donors with certain frequency and that variable late SAE also occured although the frequency of the occurrence of hematological malignancy was not necessarily high at PBSC donors.

ELSEVIER

Seminars in
HEMATOLOGY

Human Leukocyte Antigen Haploidentical Hematopoietic Stem Cell Transplantation: Indications and Tentative Outcomes in Japan

Yoshihisa Kodera,[a] Tetsuya Nishida,[a] Tatsuo Ichinohe,[b] and Hiroo Saji[c]

The stem cell banking system in Japan by the Japan Marrow Donor Program (JMDP) and Japan Cord Blood Bank Network (JCBBN) has provided increased opportunities for patients who might benefit from stem cell transplant from allogeneic sources but who lack human leukocyte antigen (HLA)-matched related donors. Nevertheless, most patients probably do not undergo transplantation because of the absence of suitable stem cell sources. To fulfill this potential need, the outcomes of transplants from HLA-mismatched relatives with or without T-cell depletion were retrospectively analyzed: the rates of engraftment and survival were insufficient in transplants with T-cell depletion, and the actual increase in transplantable donor numbers was small because only a single locus mismatched donor was the realistic choice in those without T-cell depletion. Since prophylaxis with tacrolimus reduced the incidence of grade I–IV acute graft-versus-host disease (GVHD) in HLA class I allele mismatched unrelated donors, we studied transplantation from HLA one haploidentical family donors who showed microchimerism of noninherited maternal antigens, without T-cell depletion but with tacrolimus prophylaxis. The rates of engraftment and survival in this circumstance were similar to those obtained with transplantation from HLA-matched sibling donors.
Semin Hematol 42:112-118

Hematopoietic stem cell transplantation (HSCT) from HLA genotypically identical siblings as well as from HLA allele-matched unrelated volunteer donors has become a standard modality in the treatment of hematologic malignancies and marrow failure syndromes in Japan.[1–8] In addition, the development of a cord blood banking system offers patients who lack suitable donors among relatives or among donors registered in the Japan Marrow Donor Plan (JMDP)[9,10] an opportunity to receive stem cell transplants. Despite stem cell banking systems, about half of patients with hematologic disease are excluded from transplant using alternative stem cell sources, HLA closely matched related, unrelated donors, or cord blood. To meet this demand, certain approaches, such as transplantation from HLA partially- mismatched family members using methotrexate and cyclosporine for graft-versus-host disease (GVHD) prophylaxis[11,12] and $CD34^+$ cell transplant from haploidentical relatives using antithymocyte globulin (ATG) for the prophylaxis of graft failure and GVHD,[13,14] have been investigated. Outcomes were acceptable but hardly optimal.

Tacrolimus appears to reduce the frequency of severe acute GVHD in stem cell transplants from HLA-matched related and unrelated donors,[15] and this drug might improve the outcomes of transplants from other alternative donor sources. Furthermore, van Rood et al[16] and Tamaki et al[17] showed that survival of patients transplanted from mothers was significantly better than from fathers because of feto-maternal tolerance, providing theoretical support for transplantion from HLA haploidentical related donors without T-cell depletion. This review is a report of HLA haploidentical stem cell transplantation from related donors with modern immunosuppressive drugs, as well as of the current status of stem cell transplantation in Japan.

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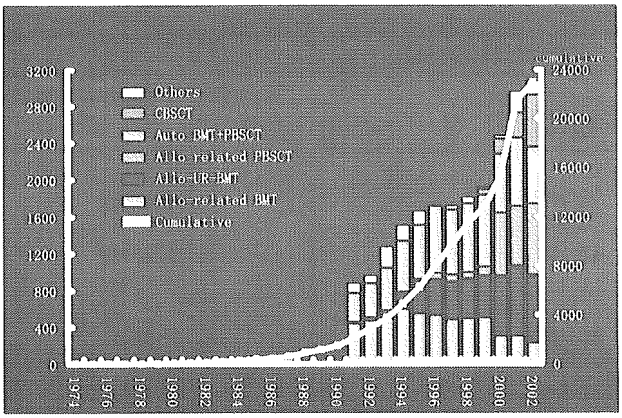

Figure 1 Status of HSCT in Japan. CBSCT, cord blood stem cell transplant; BMT, bone marrow transplant; PBSCT, peripheral blood stem cell transplant; Auto, autologous; Allo, allogeneic; UR-BMT, unrelated donor BMT.

Current Status and Clinical Outcomes of Hematopoietic Stem Cell Transplantation in Japan

Allogeneic Transplant in Japan

Figure 1 shows the annual and cumulative case numbers transplanted from different stem cell sources in Japan; data were obtained by compiling the nationwide case report system of the Japan Society of Hematopoietic Cell Transplantation (JSHCT; capture rate ~70%),[18] the JMDP (capture rate ~100%), the Japan Cord Blood Network (JCBBN) (capture rate near 100%), the nationwide peripheral blood stem cell donor follow-up system conducted by JSHCT (capture rate ~100%), and grant studies supported by the Ministry of Health, Labor and Welfare of Japan. Transplants numbered approximately 3,000 in 2002, and 75% of them were allogeneic, including 8% bone marrow transplants (BMTs) and 25% peripheral blood stem cell transplants (PBSCTs) from related donors, 24% BMTs from unrelated donors, and 19% cord blood stem cell transplants (CBSCTs).

Bone Marrow Transplantation From Unrelated Donors and From Genetically Matched Sibling Donors

Outcomes of transplants from each stem cell source are summarized in Tables 1 through 4. The probability of overall 5-year survival for patients who received bone marrow from HLA-matched siblings, whose data were collected from 331 departments participating in the JSHCT, was separately analyzed for adults (age ≥16) and children (age <16). The probability of 5-year disease-free survival (DFS) of patients who received bone marrow from 6/6 HLA serologically matched unrelated volunteer donors provided an opportunity for bulk analysis. The data were collected from departments registered with the JMDP. As indicated in Tables 1 and 2, the 5-year Kaplan-Meier survival for patients transplanted from unrelated donors was equivalent to that of patients who received marrow grafts from HLA genotypically matched siblings for indications of acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplastic syndrome (MDS). The survival of chronic myelogenous leukemia (CML) patients transplanted in chronic phase from unrelated donors was lower than that for patients transplanted from siblings but was equivalent for patients transplanted at more advanced stages of this disease. For severe aplastic anemia, the survival rate of patients transplanted from unrelated donors was lower than that for HLA genotypically identical siblings, but outcomes were comparable for patients transplanted in relatively early stages of the disease (low-risk BTF group). From these outcomes, we consider BMT from 6/6 HLA serologically matched, unrelated donors as well as from HLA genotypically matched siblings to be the "gold standard" for stem cell transplants.

Table 1 Outcomes of Matched Sibling and Unrelated Donor BMT*

	5-Year Survival		
	Matched Sibling BMT*		Unrelated Donor BMT†
	Adult	Child	
AML			
1 CR	67 (474)	76 (157)	68 (253)
2 CR	59 (171)	69 (27)	52 (190)
> 3 CR	42 (18)	50 (2)	27 (48)
Relapse	20 (224)	49 (48)	10 (278)
ALL			
1 CR	56 (377)	73 (132)	59 (316)
2 CR	26 (84)	39 (27)	46 (210)
> 3 CR	18 (17)	39 (27)	28 (54)
Relapse	16 (154)	13 (95)	15 (227)

Abbreviations: AML, acute myelogenous leukemia; CR, complete remission; ALL, acute lymphocytic leukemia.
*Data from JSHCT, December 2001.
†Data from JMDP, October 2002.

Table 2 Outcomes of Matched Sibling and Unrelated Donor BMT

	5-Year Survival		
	Matched Sibling BMT*		Unrelated Donor BMT†
	Adult	Child	
CML			
1 CP	73 (555)	78 (41)	52 (546)
2 CP	34 (26)	0 (1)	41 (51)
Acc	40 (55)	67 (3)	37 (115)
BC	28 (51)		8 (78)
MDS			
—	65 (167)	81 (39)	57 (75)
—			80 (5)
—	39 (133)	46 (22)	38 (68)
—	35 (167)	51 (35)	31 (93)
—	41 (44)	45 (17)	48 (21)
Aplasia			
Child	93 (19)	90 (89)	Low-risk BTF 73 (97)
Adult	87 (37)	53 (19)	High-risk BTF 56 (125)

Abbreviations: CML, chronic myelocytic leukemia; CP, chronic phase; Acc, accelerated phase; BC, blastic crisis; MDS, myelodysplastic syndrome; BTF, blood transfusion.
*Data from JSHCT, December 2001.
†Data from JMDP, October 2002.

Allogeneic PBSCT Among Siblings for Advanced Leukemia and the Outcome of CBSCT After 2001

Outcomes of PBSCTs from related donors and of CBSCTs were compared to the excellent results obtained with genotypically matched siblings and genotypically compatible matched unrelated donors. PBSCTs from HLA-matched siblings showed 5-year DFS equivalent to that of BMT when transplant was undertaken at an early stage of leukemia (first complete remission of acute leukemia and first chronic phase of CML) and improved survival when transplanted at an advanced stage of the disease (Table 3). Comparing unrelated BMT and CBSCT in patients younger than 15 years, the former is advantageous in terms of 3-year DFS but the difference became smaller for transplants performed after 2001 (Table 4).

Table 3 Outcomes of BMT Versus PBSCT From Matched Siblings

	5-Year DFS (n)	
	BMT	PBSCT
AML		
1 CR	59.8 (192)	61.5 (58)
Other	35.5 (182)*	40.0 (91)*
ALL		
1 CR	56.7 (152)	56.8 (52)
Other	15.2 (88)	26.5 (39)
CML		
1 CP	72.7 (259)	72.7 (61)
Other	36.2 (53)	67.2 (26)

Abbreviation: DFS, disease-free survival.
Data from JSHCT, December 2003.
*Three-year DFS.

Outcomes of Allogeneic Stem Cell Transplantation and Genetic Homogeneity in Japanese

Table 5 summarizes the frequency of grades III–IV acute GVHD after allogeneic BMT from related and unrelated donors according to HLA match status among Japanese patients. The frequency of grades III–IV acute GVHD was 8.1% among 4,701 HLA genotypically identical sibling transplants and 13.1% among 1,371 6/6 HLA serologically matched unrelated donors. These frequencies were almost the same as in our earlier reports[6,19,20] but lower than that of other cohorts.[5,21] Since most of the cases transplanted from 1991 to 1999 received cyclosporine-based GVHD prophylaxis, we consider the low incidence of severe acute GVHD to be a reflection of the genetic homogeneity of Japanese people.

Indications for Allogeneic HSCT

The potential number of new candidates for allogeneic stem cell transplants in Japan was estimated from the current annual number of patients who initiated a donor search with

Table 4 Outcomes of Unrelated Donor BMT Versus Unrelated Donor CBSCT

	3-Year DFS (n)	
	UR-BMT	UR-CBSCT
Transplanted before 2000	50.2 (347)	34.1 (139)
Transplanted after 2001	65.9 (116)	53.5 (96)

NOTE. For patients under age 15, both standard and high risks were included.
Abbreviation: UR, unrelated donor.
Data from *Cord Blood Bank Now*, Vol 19, September 2004.

Table 5 Outcome of Allogeneic CD34+ Cell Transplantation in Japan

Rate of engraftment	82.0%
Rate of acute GVHD (>grade-3)	8.4%
Overall survival	27.4%
Disease-free survival	18.5%
Cause of death	
Infection	38%
Relapse	30%
Other TRM	22%
VOD	5%
GVHD	5%

Abbreviations: TRM, transplant-related mortality; VOD, veno-occlusive disease; GVHD, graft-versus-host disease.
Data from Kato et al.[14]

the JMDP. The number of patients registered with the JMDP was 1,536 in 2000, 1,603 in 2001, and 1,792 in 2002 (when the JCBBN began activity and imatinib was available for CML; therefore, the effects of these factors were excluded). The annual number of unrelated BMTs facilitated through the JMDP was 715 in 2000, 749 in 2001, and 739 in 2002. Thus, some 800 patients remain to be transplanted from donors other than HLA genotypically identical siblings, 6/6 HLA-matched unrelated donors, or HLA closely matched cord blood. In general, the JMDP approves registration only for patients younger than 50 years of age. The number of cases awaiting transplantation would double if transplant eligibility was extended to patients under 65 so that approximately 1,500 new patients would appear each year as candidates for allogeneic transplantation from alternative stem cell sources other than HLA-matched related or unrelated donors, or HLA closely matched cord blood, even if eligibility were restricted to patients younger than 65 with leukemia, myelodysplastic syndrome, and other marrow failure syndromes.

Alternative Stem Cell Sources

Transplants From HLA Single Locus Mismatched Family Members and 6/6 HLA-Matched Unrelated Donors

Following a preliminary survey of the experiences of local transplant teams,[12], Kanda et al recently analyzed 142 patients who underwent allogeneic HSCT from HLA-mismatched family members and compared the outcome to that obtained from 2,805 transplants among HLA-matched siblings and to 1,002 transplants from 6/6 HLA-matched unrelated donors.[22] Among 2,947 adult (age >16) patients who received stem cell transplantation from family donors for the first time between 1991 and 2000 for CML, AML, ALL, and MDS, and for whom serologic HLA datasets for HLA-A, -B, and DR loci were fully available, 112 received the transplants from donors with a single locus mismatch (70 class 1 mismatch; 42 class 2 mismatch; 70 bidirectional mismatch; 15 mismatch at graft-versus-host vector alone; 27 host-versus-graft-vector alone; 89 BMT; 23 PBSCT), and the other 30 patients received the stem cells from donors with more than two mismatched loci. Ninety-one percent of 2,947 patients received cyclosporine-based GVHD prophylaxis. The study included a control group provided by the JMDP of 1,002 patients transplanted from unrelated donors matched for age and background disease. The overall survival of patients who received stem cell transplants from a single locus mismatched family member was equivalent to that of patients who received BMTs from 6/6 HLA-matched unrelated donors for both standard-risk (acute leukemia in first or second complete remission, CML in first or second chronic phase, MDS without leukemic transformation) and high-risk diseases. The outcome following transplantation from a HLA single locus–mismatched family member was comparable to that following standard transplant from 6/6 HLA-matched unrelated donors. However, the availability of donors with a single locus mismatch remains low.

Haploidentical Stem Cell Transplantation of Purified CD34+ Cells

A nationwide survey in Japan of the outcome of haploidentical transplants following negative T-cell depletion using the Isolex CD34+ selection kit,[10] found 135 patients: 110 under age 16 and 25 older than 16. The indications for transplantation were 46 ALL, 32 acute nonlymphocytic leukemia (ANLL), 13 CML, 8 MDS, 6 neuroblastoma, 1 malignant leukemia (ML), 11 severe anaplastic anemia (SAA), 5 Fanconi's anemia, 2 inborn errors of metabolism, 6 severe combined immunodeficiency, 1 Wiskott-Aldrich syndrome, 3 hemophagocytic syndrome, and 1 chronic active Epstein-Barr virus infection. A total of 29 patients with standard-risk and 81 with high-risk malignancies had been grafted with $CD34^+$ cells purified from bone marrow cells (n = 38), granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood (n = 74), or from a mixture of marrow and blood (n = 23) donated from HLA haploidentical family members. The status of HLA disparity between the recipients and the donors was: 0/6–1/6 mismatch: n = 28; 2/6 mismatch: n = 64; and 3/6 mismatch: n = 43. The removal rate of $CD3^+$ cells was log 3.3–3.9, and the concentration of $CD34^+$ cells after purification was 89.6%–93.6%, resulting in the following cell doses: 38 patients received $3.2 \pm 2.3 \times 10^6$/kg $CD34^+$ cells derived from bone marrow, 74 patients received $5.5 \pm 3.6 \times 10^6$/kg $CD34^+$ cells from peripheral blood, and 23 patients received $4.9 \pm 7.1 \times 10^6$/kg $CD34^+$ cells from the mixture of bone marrow and peripheral blood. For prophylaxis of graft rejection and for GVHD, 47 of 135 received ATG and 105 patients received post-transplant GVHD prophylaxis with cyclosporine, tacrolimus, methotrexate, and corticosteroid, either alone or in combination. Nevertheless, in 30 transplants, $CD34^+$ cell selection was the only measure of GVHD prophylaxis. Among these T-cell negatively depleted single-haploidentical transplants from family members, the incidence of grade III–IV acute GVHD was 8.4%, close to that observed for transplants from HLA genotypically matched siblings or from 6/6 HLA-matched unrelated donors without T-cell depletion (Table 6). However,

Table 6 Probability of Acute GVHD According to Donor Type

Donor	Incidence of Grade III–IV Acute GVHD
Syngeneic (n = 85)	2.4%
HLA-matched sibling (n = 4,701)	8.1%
HLA-mismatched sibling (n = 294)	16.7%
HLA-matched unrelated (n = 1,379)	13.1%
HLA-mismatched unrelated (n = 991)	22.8%
HLA-matched relative other than sibling (n = 227)	11.0%
HLA-mismatched related other than sibling (n = 357)	17.9%

Data from JSHCT, December 2001.

the rate of engraftment (82%) was lower than that after transplant from unrelated donors (95%).[6] The observed overall survival of 27.4% and the DFS of 18.5% were insufficient when compared to those obtained from standard transplants (Tables 1 and 2) although most of the patients were transplanted at an advanced stage of disease.

Effect of HLA Allele Mismatches in Unrelated BMT Using Tacrolimus Prophylaxis

A prospective phase II study was performed to investigate the ability of tacrolimus to prevent GVHD after BMT from HLA allele-mismatched unrelated donors. Patients had either leukemia or MDS at various clinical stages, were between the ages of 16 and 50, and had a clinical performance status of 0 to 2. Tacrolimus was administered at a dose of 0.03 mg/kg by 24-hour continuous infusion from day 1 and converted to oral administration with appetite recovery. The dose of tacrolimus was adjusted to keep its blood concentration between 15 and 20 ng/mL. DFS was 70% for standard-risk patients (acute leukemia in first complete remission, CML in first chronic phase, and refractory anemia of MDS; n = 21) and 30% for high-risk patients (n = 31). The frequency of acute GVHD and DFS were compared with age- and disease-matched patients receiving cyclosporine and unrelated transplantation through the JMDP during the same time period (Table 7). The frequency of grades III–IV acute GVHD among patients who were transplanted from single-locus HLA class I allele mismatched donors with tacrolimus was 23.1%, which was much lower than that of the cyclosporine group (58.3%). For patients receiving marrow grafts from single-locus HLA class II allele mismatched donors, the frequency of grades III–IV acute GVHD in the tacrolimus group was 10.5% and that of the cyclosporine group, 16.1%, both of which were lower than that observed for single HLA class I allele mismatch transplants, and the difference between the two groups was small. This can be explained by our previous reports,[23,24] which showed that a single class II allele mismatch did not increase the frequency of severe acute GVHD in Japanese patients receiving cyclosporine-based GVHD prophylaxis. DFS of the patients transplanted from single-locus HLA class I mismatched unrelated donors with tacrolimus was equivalent to that of patients transplanted from single-locus HLA class II mismatched unrelated donors with either cyclosporine or tacrolimus.[25] Our previous reports confirmed that HLA class I allele mismatch but not class II allele mismatch was a significant risk factor for DFS of Japanese patients transplanted from unrelated donors receiving cyclosporine-based GVHD prophylaxis.[23,24] In the present study, tacrolimus improved the outcome of HLA class I mismatch transplants to the level of class II mismatch transplants. Tacrolimus may be more effective for HLA-mismatched transplants such as haploidentical transplants.

Haploidentical Transplantation With Tacrolimus But Without T-Cell Depletion From Related Donors Suspected to be Immunologically Tolerant to Recipients

The presence of microchimerism of noninherited maternal antigens (NIMA) in offspring as well as inherited paternal antigens (IPA) in the mothers of transplant recipients was examined.[26] NIMA or IPA microchimerism exists for more than 50 years in both offspring and mothers, suggesting that the offspring and mother might be immunologically tolerant of each other (Table 7). Preclinical trials of transplants between offspring who shared the paternal but not maternal HLA haplotype with donors who had NIMA microchimerism were performed using tacrolimus-based GVHD prophylaxis.[27] Patients' characteristics are described in Table 8, and the outcomes in Table 9. Engraftment was obtained in all patients. The probabilities of grades III–IV acute GVHD among patients who received stem cells from two- or three-antigen–mismatched donors were 24% and 29%, respectively, which was slightly higher than the overall frequency of grade III–IV acute GVHD in transplants from unrelated do-

Table 7 Probability of Grade III–IV Acute GVHD and DFS After HLA Allele-Mismatched Unrelated Donor BMT: Cyclosporine Versus Tacrolimus

	Probability of Grade III–IV Acute GVHD	3-Year DFS
Class 1 DNA 1 mismatch		
Cyclosporine (n = 12)	58%	25%
Tacrolimus (n = 13)	23%	52%
Class 2 DNA 1 mismatch		
Cyclosporine (n = 31)	16%	55%
Tacrolimus (n = 19)	10%	52%

Data from Nishida et al.[23]

Table 8 Incidence of Long-Term Fetomaternal Chimerism With Reference to Duration

Duration of Chimerism	Maternal Cell Chimerism in Offspring			Offspring Cell Chimerism in Mother		
	No. of Subjects	No. Detected	%	No. of Subjects	No. Detected	%
0–9	39	23	59	42	32	76
10–19	59	46	78	44	36	80
20–29	71	56	79	51	40	78
30–39	39	25	64	17	11	65
40–49	26	13	50	11	8	73
50–59	11	6	55	1	1	100
60–69	1	1	100	0	0	0
Total	246	170	69	166	127	77

NOTE. Represented by offspring's age at blood sampling.
Unpublished data from H. Saji, 2000.

nors (18.4%)[6] but equivalent to the frequency observed for transplants from class I mismatched unrelated donors (HLA -A/B one allele mismatch: 27.8%; HLA-C one allele mismatch: 20.6%).[24] The probability of survival for standard-risk patients was comparable for those with HLA genotypically matched sibling donors and those with HLA allele-matched unrelated donors (Table 10; see Tables 1 and 2).

Conclusion

HLA matching is an essential prerequisite for the success of HSCT from related[12,28] and unrelated[24,29] marrow donors, in part because it predicts GVHD, a significant factor for post-transplant survival. In transplantation of peripheral blood stem cells, GVHD may be more frequent than in marrow transplant[30,31] due to the high dose of T cells in the stem cell preparation. In CBSCT, where requirements of HLA matching are less stringent than for BMT or PBSCT, the outcome is still affected by disparity of HLA.[32,33] Another factor considered to be critical is the timing of transplant in relation to the patient's clinical course and the potential loss of the optimal moment for transplantation due to delays in identifying a suitable donor. Our results demonstrate that when a HLA suitably matched stem cell source cannot be identified among current donor pools, HLA-haploidentical relatives among family members may allow transplantation without delay. Retrospective analysis of transplants from HLA-mismatched siblings among Japanese patients[22] supports the usefulness of such alternative stem cell sources. Our experience shows that transplantation from HLA-haploidentical sibling donors predicted to be immunologically tolerant to the recipients produced survival equivalent to that obtained from transplantation using HLA-matched related or unrelated donors, when tacrolimus was employed for GVHD prophylaxis, without T-cell depletion. Efforts to achieve successful stem cell transplant beyond the conventional HLA barrier must be continued.

Table 9 Characteristics of Recipients

No.	33
Sex (M/F)	14/19
Median age, yr (range)	28 (2–57)
Diagnosis	
AML	10
ALL/LBL	12
CML	7
DLBCL	3
ATL	1
Risk status at HSCT*	
Intermediate	13
High	20

Abbreviations: LBL, lymphoblastic lymphoma; DLBCL, diffuse large B-cell lymphoma; ATL, adult T-cell leukemia.
Data from Ichinohe et al.[26]
*Risk status of hematological malignancies at the time of HSCT was defined as follows: Intermediate, advanced CR/PR/CP, chemosensitive relapse; High, primary refractory disease or chemoresistant relapse.

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Table 10 Probability of Grade III–IV Acute GVHD and DFS After HLA-Haploidentical Related Transplantation

	Probability of	
	Grade III–IV Acute GVHD	DFS
In GVH direction		
HLA 2-antigen mismatch (n = 21)	24%	
HLA 3-antigen mismatch (n = 11)	29%	
Transplanted in remission (n = 13)		62%*
Transplanted in chemorefractory (n = 22)		22%†

Abbreviation: GVH, graft versus host.
Data from Ichinohe et al.[26]
*At 3 years.
†At 2.3 years.