-DQB1*0601 -DPB1*0901)は 108 人、homozygous HP-P2 (HLA-A*3303 -Cw*1403 -B*4403 -DRB1*1302 -DQB1*0604 -DPB1*0401) は 24 人、homozygous HP-P3 (HLA -A*2402 –Cw*0702 –B*0702 –DRB1*0101 –DQB1*0501 –DPB1*0402)は 15 人認められた。

- SNPs 解析が可能であった homozygous HP-P1 の 72 人中の 65 人及び、homozygous HP-P3 の 8 人中 4 人は HLA-A から HLA-DPB2 の 3.2Mb の範囲で 99%以上が一致した連続性の homozygous SNP allele を認めた。Homozygous HP-P2 の 10 人全てで解析した少なくとも 4.9Mb の範囲で 99%以上が一致した連続性の homozygous SNP allele を認めた。これらのデータより、SNPs の consensus sequence を決定することができた。
- Common HLA HP と同じ HLA allele を持つ個人 (heterozygous HP)の検討では、HP-P1 の 1053 人中 1000 人 (95.0%)、HP-P2 では 387 人中 380 人 (98.2%)、HP-P3 では 437 人中 381 人 (87.2%)が HLA-A から HLA-DPB2 の 3.2Mb の範囲で consensus sequence を認めた。さらに、HP-P2 では 80%で 4.9Mb の範囲にわたって consensus sequence を認めた。
- これらの結果より HLA アリルの combination で common HLA-HP を同定した場合、HLA allele 以外の領域も含めて一致している可能性が高いと考えられた。

(2) HLA haplotype と急性 GVHD の発症の解析

- 特定の HP が GVHD 発症頻度に及ぼす影響を解析するため、各々の HP を持つ群と持たない群に分けて、grade2-4 の急性 GVHD 発症リスクを検討した。HLA 型が全て一致したドナーから移植を受けた 712 例のうち、HP-P1 は 331 例に認め、P1(-) 群と比較して差はなかった (HR=1.06, p=0.665)。HP-P2 (n=111)は P2(-)と比較して優位に発症リスクが低かった (HR=0.63, p=0.032)。一方、HP-P3 (n=104) は P3 (-)と比較して発症リスクが高い傾向が認められた (HR=1.38, p=0.07)。
- HP-P1(+)の患者を多数認めたため、これらの患者においてもう一方の HP の影響を検討した。Homozygous HP-P1 (n=36)の急性 GVHD 発症頻度は 16.2%、HP-P1/P2 (n=25) では 12.0%であり、HP-P1/P3 (n=19, 49.9%)や HP-P1/other (n=251, 34.2%)と比べて有意に低かった (p=0.0052)。多変量解析でも同様に、homozygous HP-P1 と比較して HP-P1/P2 (HR=0.71, p=0.64)は発症リスクに差は認めなかったが、P1/P3 (HR=3.35, p=0,024)及び P1/other (HR=2.49, p=0.036)は有意に発症リスクが高かった。

【まとめ】

(1) 大規模な JMDP の HLA データ及び SNPs データを用いることにより、日本人の common HLA haplotype が高度に保存されていることが示された。
(2) HP-P2 では GVHD 発症のリスクを減少し、一方 HP-P3 では増加する傾向が認められたことより、HLA ハプロタイプ自身すなわち genetic background の違いが GVHD 発症と関連すると考えられた。
(3) 特定の HLA ハプロタイプが GVHD のリスクを減少あるいは増加させるメカニズムについて今後検討していく必要がある。

全ゲノム関連解析による GVHD 関連遺伝子座の探索

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腫瘍細胞に対するアロ免疫反応は、造血幹細胞移植の主要な治療効果を担うメカニズムであるが、同様の反応は患者のレシピエントの健常組織に対しても惹起され、GVHD として知られる重篤な合併症を引き起こす。GVHD の誘導にはドナーとレシピエントの遺伝学的背景の相違が本質的であるが、HLA 適合移植の場合、このアロ免疫反応は、ドナーT 細胞が HLA 分子を介してレシピエントの細胞上に提示される不適合抗原(マイナー組織適合性抗原，mHag)を認識することにより誘導され、ドナーないしレシピエントの遺伝的背景や前処置・GVHD 予防などの環境因子により修飾される。本研究では、日本骨髄バンクを通じて行われた非血縁骨髄移植のうち、HLA A，B，C，DR，DQ 座が DNA レベルで肝前適合し、かつ GVHD 予防としてメトトレキセートおよびシクロスポリンないしタクロリムスが用いられた 1598 移植のドナーおよびレシピエントの DNA 試料について、Affymetrix GeneChip を用いて 50 万 locus の SNP タイピングを行い、全ゲノム関連解析により、GVHD の発症に関わるアロ抗原その他の遺伝的多型の探索を行った。HLA DP 座については 1033 移植(63%)で GVHD 方向の不適合を認めており、654 例(41.7%)に II 度以上の、また、254 例(14.9%)に III 度以上の急性 GVHD の発症が認められた。

直接タイピングを行った 50 万 SNP に加え、HapMap PhaseII データに基づいて未観測の SNP を推定したのち、95%以上の call rate を有し、Hardy-Weinberg 平衡を満たす SNP のうち、5%以上のアレル頻度を有する SNP、1,276,699 SNP について、GVHD との関連を各 SNP 座について LogRank 統計量を算出することにより、検定した。極端な多重解析が不可避なため、random permutation (N=1000)を行うことにより、経験的な genome-wide p=0.05 を与える統計量を算出して閾値を決定し、有意な SNP を抽出した。また、関連解析は、ドナーおよびレシピエントの SNP に加え、両者の SNP で定義される遺伝型不適合についても検討を行った。

遺伝型不適合の解析では、全ゲノムで唯一の有意なピークとして DPB1 遺伝子座

に一致する rs6937034 が同定され($P=1.81 \times 10^{-9}$)、DPB1 不適合と急性 GVHD の既知の関連(HR=1.91, $P=2,88 \times 10^{-13}$)を捕らえることができたことから、本方法論の有効性が確認された。そこで、マイナー組織適合性抗原の HLA 拘束を考慮して腫瘍な HLA サブタイプについて同様の関連解析を行ったところ、A*2402/B*5201/C*1201/DRB1*1501/DQB1*0601 アレルに拘束される rs17473423(12 番染色体)の不適合と III 度以上の GVHD の関連($P=3.99 \times 10^{-13}$)、および A*3303/B*4403/C*1403 アレルに拘束される rs9657655(9 番染色体)の不適合と III 度以上の GVHD の関連($P=8.56 \times 10^{-10}$)を含む 6 遺伝子座と急性 GVHD との関連が抽出された。同様に慢性 GVHD および再発のリスクと関連する遺伝子座も同定された。これらの SNP については、独立な症例セットを用いた検証研究が必要であるが、今回の結果は、全ゲノム関連解析により、造血幹細胞移植の成績に影響する遺伝的多型を同定できる可能性を示唆するものと考えられる。

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「組織適合性に基づく非血縁者間造血幹細胞移植の成績向上に関する研究」班（H20-免疫-一般-014）
平成21年1月17日

「HLA-Cw不適合非血縁者間骨髄移植を受けた患者より分離したCTLクローンの解析」

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HLA-A, B, DRB1遺伝子型適合ドナーからの非血縁間骨髄移植におけるHLA-Cw不適合は、重症急性GVHDの発症危険度を上昇させる。急性GVHDは、主としてドナーTリンパ球によって誘導されるが、しかしHLA-Cw抗原は一般に細胞表面上の発現レベルが低く、従ってその抗原性は低いと考えられている。事実、HLA-Cw不適合移植を受けかつ実際に急性GVHDを発症した患者体内において、不適合Cwに対するTリンパ球免疫応答が誘導されているかどうかについて詳細な解析はまだなされていない。最近、日本骨髄バンクを介した非血縁者間骨髄移植を対象とした統計学的解析により、特定のHLA不適合の組み合わせが重症急性GVHDの発症危険率と相関すること、さらに特定の部位のアミノ酸相違が重症急性GVHDの発症危険率と相関することが報告された（Kawase et al. Blood 2007）。具体的には、HLA-Aの9番、116番のアミノ酸相違、およびHLA-Cwの9番、77番、80番、90番、116番、156番のアミノ酸相違が有意な部位として抽出されており、特にHLA-Cwから多くのアミノ酸部位が抽出されたことは興味深い。

今回我々は、上記報告で急性GVHDの発症危険度が高いと分類されたHLA-Cw不適合（患者Cw*0303、ドナーCw*0801）の非血縁者間骨髄移植を受け、実際にgrade IIの急性GVHDを発症した白血病患者の末梢血中T細胞のin vitro解析を行い、この不適合Cw分子に対するT細胞応答の存在を確認し得たので報告する。

Non-HLA genetic associations with GVHD in Japanese HSCT recipients: High density screening of the immunogenome with microsatellite markers)

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Background

The genetics of outcomes of haematopoietic stem cell transplantation (HSCT) extend far beyond HLA matching or mismatching. Studies in various settings and from various populations have implicated more than 150 genetic polymorphisms in non-HLA genes with outcomes after HSCT, namely graft-versus-host disease (GVHD). GVHD is the strongest determinator of morbidity and mortality in survivors of HSCT, hence identification of genetic variation that pose a risk, or protect against GVHD would potentially be useful to stratify recipient GVHD risk at a pre-transplant stage. Previous studies investigating non-HLA polymorphisms in HSCT outcomes often have methodological limitations: focus on a narrow set of candidate markers, highly heterogenous populations (genetically and clinically), limited statistical power due to small sample size, and lack of independent confirmation. As a result data are lacking consistency between populations and settings. Therefore, more systematic explorations using statistically more robust methodologies are required to evaluate to a full extend the significance of non-HLA polymorphisms for HSCT outcomes.

Aims and Objectives

This study investigates the effect of non-HLA genetic polymorphisms on GVHD in a Japanese HSCT population by systematically scanning the immunogenome. We are applying the methodology of a multi-step genomic screening using high-density microsatellite markers in pooled DNA. Thie methodology has been derived from case-control whole genome association (WGA) studies, and is employed here for the first time in a transplantation setting.

Methods

Study population: We aim to include approximately 1100 HSCT recipient and donor pairs into this study. Systematic statistical exploration of a larger dataset was used to identify a cohort in which variation from known clinical and genetic risk factors was minimised. Inclusion criteria are a diagnosis of ALL or ANLL, age between 4-40 years, myeloablative conditioning, and a CSA/MTX or Tacrolimus/MTX-based GVHD prophylaxis.

Genes and markers: An extensive literature search identified a set of n=2956 genes of key immunoregulatory function and relevance in an HSCT context. N=4108 microsatellite markers were selected to cover these genetic regions in high density.

Study design and procedures: This is a case-control study with a nested cohort study; modifying a design previously used in WGA studies with microsatellites. DNA is pooled by degree of GVHD (Grade 0-I v Grade II-IV) using a highly accurate pooling method. Three independent screening steps are applied to verify the results, using n=460 (pooled DNA 1st screening), n=300 (pooled DNA 2nd screening) and a further n=300 donor-recipient pairs (individual genotyping). The first screening step tests the entire marker set, involving the largest cohort for maximised power and sensitivity. Only markers positively associated in 1st screening are transferred to subsequent screening steps, applying rigorous statistical tests, thus minimising association by chance and multiple testing error. The remaining positive markers after 3rd screening each indicate a candidate gene region of approximately 100kB in

size, which will be investigated further by SNP typing on the combined cohort of all three screening steps.

Data analysis: Allele frequency number in pools is derived from the peak height of the flourescent signal from the genotyping process. After each screening step, data are analysed in four directions: Comparing recipients with grade 0-I and recipients with grade II-IV GVHD ('intrinsic recipient risk for severe GVHD'), donors accordingly ('intrinsic donor risk to induce recipient GVHD'), donors grade 0-I with recipients grade 0-I GVHD, and donors grade II-IV with recipients grade II-IV GVHD. The latter two analyses are combined to identify specific markers that indicate protection (grade 0-I GVHD analysis) or risk (grade 2-4 GVHD) by allele frequency mismatch. While with the first screening step only simple statistical methods (Hardy-Weinberg, Chi square, Fishers exact test for 2x2, 2xm) are applied to achieve high sensitivity, more stringent tests are applied in subsequent screening steps (including Bonferroni correction for multiple testing).

Results

The 1st screening step has been completed, yielding preliminary results which are consistent with both a set of SNP markers used for internal validation, as well as with findings from previous studies on genetic polymorphisms and gene expression in the context of GVHD.

免疫ｱﾚﾙｷﾞｰ疾患等予防・治療研究事業「組織適合性に基づく非血縁者間造血幹細胞移植の成績向上に関する研究」班（H20-免疫-一般-014）平成20年度第２回班会議

「NK細胞受容体およびサイトカイン遺伝子多型と非血縁者間骨髄移植成績」

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１、KIR遺伝子型、HLAリガンド型適合性

昨年度までに非血縁者間骨髄移植症例においてHLA-C抗原のKIRリガンド型（C1,C2）GVH方向不適合の場合に急性GVHDの重症化および全生存率の低下が見られること[1]、その効果はATG投与およびドナーKIR2DS2遺伝子の有無に影響されること[2]を報告してきた。今年度からはHLA-A,B抗原上に存在するKIRリガンド(Bw4I80)と認識受容体KIR3DL1/S1遺伝子の組み合わせについての解析を開始している。今回はKIRリガンドGVH方向不適合症例のドナーについてKIR3DL1/S1アリルタイピングを実施し移植成績との関連を解析した。

２、LILR遺伝子型多型

マウスMHCクラスI抗原認識受容体PIR-Bが骨髄移植急性GVHD重症化と関連することを東北大学の中村らが報告している[3]。ヒトの相同分子と考えられるLILRはNK,DC,顆粒球細胞などで発現する活性化型ならびに抑制型の11種類からなるHLAクラスＩ抗原認識ペア型受容体ファミリーである。このうち機能的にPIR-Bと類似するLILRB2に注目し、遺伝子多型解析系を構築し健常者を解析し細胞表面高発現性と関連するSNPを検出した[4]。このSNPについてHLA6座アリル一致非血縁者間骨髄移植症例ペアを判定し移植成績との関連を統計解析した。

３、サイトカイン/サイトカイン受容体遺伝子多型

昨年度までに非血縁者間骨髄移植において、患者の抑制性のサイトカインであるIL-10遺伝子のプロモーター領域3箇所のSNPハプロタイプが急性重症GVHD発症と関連することを報告した。血縁者間HLA一致骨髄移植においてはIL-10型に加えてIL-10受容体遺伝子多型も成績に影響することが報告されており（Linら[5]、佐治ら[6]）、今年度はHLA6座アリル一致非血縁者間骨髄移植症例ペアのIL-10受容体SNP(IL-10R2, rs2834167)を判定し移植成績との関連について統計解析を行った。

４、HLAタイピング法の構築と検証

JMDPではドナー登録時におけるHLA-C座検査導入が検討されており、高精度DNAタイピング法によるHLA-C座の検査法について比較検討した。健常者503人を対象として最も高精度とされているSBT法と現在HLA-A,-B,-DR査に用いられている蛍光ビーズ法とによる大規模頻度調査を実施した

5、検体保存事業協力

後方視野的研究のためにJMDPが収集、保存する患者ドナー検体の血液から抽出されたDNAを用いてHLA-AからDPの6座を蛍光ビーズ法でアリルタイピングしており、本年は2006年度に保存された1300検体について行った。また今後の解析のための試料確保を目的に、これまでに保存されていたDNAの全ゲノム増幅（WGA）系の構築と検証作業および解析希望検体セットをロボットシステムで作成、配布する系の構築作業（東海大学担当）にも協力した。

文献

1. Morishima Y et al. 2007. Effects of HLA Allele and Killer Immunoglobulin-Like Receptor Ligand Matching on Clinical Outcome in Leukemia Patients Undergoing Transplantation With T-cell-Replete Marrow From an Unrelated Donor. *Biol Blood Marrow Transplant* 13: 315-28
2. Yabe T et al. 2008. Donor killer immunoglobulin-like receptor (KIR) genotype-patient cognate KIR ligand combination and antithymocyte globulin preadministration are critical factors in outcome of HLA-C-KIR ligand-mismatched T cell-replete unrelated bone marrow transplantation. *Biol Blood Marrow Transplant* 14: 75-87
3. Nakamura A et al. 2004. Exacerbated graft-versus-host disease in Pirb-/- mice. *Nat Immunol* 5: 623-9
4. Hirayasu K et al. 2008. Evidence for natural selection on leukocyte immunoglobulin-like receptors for HLA class I in Northeast Asians. *Am J Hum Genet* 82: 1075-83
5. Lin MT et al. 2005. Genetic variation in the IL-10 pathway modulates severity of acute graft-versus-host disease following hematopoietic cell transplantation: synergism between IL-10 genotype of patient and IL-10 receptor beta genotype of donor. *Blood* 106: 3995-4001
6. 佐治ら. IL-10/IL-10 Receptor β多型性と急性GVHD. 平成17年度厚生労働研究班報告

小腸特異的ケモカイン受容体遺伝子 *CCR9* の一塩基多型は皮膚急性 GVHD 発症と相関する

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【緒言】CCR9 はリンパ球が小腸特異的に homing する際に発現する受容体である。我々はこの遺伝子 *CCR9* の nonsynonymus な一塩基多型の 1 つである 926A/G に着目し、同種移植においてドナー*CCR9* の遺伝子型が臓器特異的な急性 GVHD 発症および移植予後に及ぼす影響を解析した。【対象と方法】1987 年から 2006 年に連続的に施行された HLA 完全一致同胞間同種移植のうち CsA+MTX を GVHD 予防法とする非 T 細胞除去移植を対象とした。ドナーの *CCR9* 926A/G 遺伝子型は PCR-RFLP 法を用いて決定した。臓器別急性 GVHD 発症率、II 度以上の急性 GVHD 発症率、生存率、再発率、非再発死亡率に影響する因子を Cox 比例ハザードモデルを用いて解析した。共変数は *CCR9* 遺伝子型、年齢、RIST、TBI、疾患リスク、移植前寛解状態、移植片種（骨髄/末梢血幹細胞）とした。【結果】症例数は 167 例。年齢中央値 38 歳（15-62 歳）、疾患は悪性疾患 148 例、良性疾患 19 例であった。遺伝子型は AA 型 94%、AG 型 6%であった。急性 GVHD は I 度 22%,II 度 12%,III 度以上 6.6%で認めた。AG 型は stage2 以上の皮膚急性 GVHD 発症率と有意な相関を示したが(hazard ratio 4.6; 95%CI, 1.7-12)、腸・肝臓の急性 GVHD 発症率、II 度以上の急性 GVHD 発症率、生存率、再発率、非再発死亡率とは有意な相関が見られなかった。急性 GVHD を発症した 69 例に限って検討しても AG 型は stage2 以上の皮膚急性 GVHD 発症率のみと有意な相関を示した(hazard ratio 3.3; 95%CI, 1.2-8.7)。遺伝子型によるリンパ球の機能的相違を明らかにするため、レトロウイルスベクターを用いて Jurkat 細胞株にそれぞれの遺伝子型の cDNA を導入した。走化性試験を施行したところ,926G 導入細胞は 926A 導入細胞と比べて走化性が高いことが示された。【考察】*CCR9* の遺伝子型が腸ではなく皮膚の急性 GVHD 発症と相関したことは興味深い。最近の報告によれば、ある臓器に GVHD を起こすリンパ球が生じる場所は特定ではなく、様々な臓器の二次リンパ組織でありうるとされる。腸管のパイエル板は抗原提示の場として重要であり、*CCR9*-926G の遺伝子型によってドナーリンパ球のパイエル板への homing 能が増すことで腸以外の臓器の急性 GVHD 発症率に差が生じた可能性がある。

HLA 一致同胞間移植における予後因子、IL-6、MBL-2 の SNPs 多型性

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HLA 以外の予後因子を探るため、マイナー組織適合性抗原適合性や、接着分子の SNPs 多型性、各種サイトカインの SNPs 多型性、自然免疫関連物質の SNPs 多型性などと、HLA 一致同胞間移植の予後との関連を検索している。今回は炎症性サイトカインである IL-6 と、自然免疫関連物質である MBL-2（mannose binding lectin 2）の SNPs を検討した。

方法と材料

1、IL-6 の SNPs（-572(G/C),-174(G/C)）は、自から開発した Luminex 法（未発表）でタイプした。IL-6 のプロモーター領域には -597 にも SNPs(G/A)があるが、-174 の SNPs と強い連鎖不平衡があり、-174 のタイピングから推定した。

2、MBL-2のSNPs（-619,-290,-66, cdn52, cdn54, cdn57）を自ら開発したLuminex法（Human Immunology 69 (2008) 877-884)によりタイプし、表2により、High producer, Intermediate producer, Low producer, Deficiency に分類した。

3、HLA 一致同胞間移植ペア 91 例の DNA を被検体とした。a-GVHD と一年以内の生存により群わけをして解析した。感染についてはデータに不備があり解析できなかった。

結果 1、表 1、IL-6 SNPs の頻度

Position	Nucleic acid	Japanese N=109	Caucasian N=45	Position	Nucleic acid	Japanese N=109	Caucasian N=45
-174 or -597	G/G	1.00	0.40	-572	G/G	0.05	0.95
	G/C	0	0.42		G/C	0.45	0.05
	C/C	0	0.18		C/C	0.49	0
	G	1.00	0.61		G	0.28	0.98
	C	0	0.39		C	0.72	0.02

日本人には-174 の多型性はないが、-572 に多様性がある。GGG ハプロタイプは日本人は 28%、白人は 60%に検出される。（GGG ハプロタイプは IL-6 low producer とされる）

結果 2、表 2、MBL-2 SNPs ハプロタイプとその機能

Function	Promoter			Exon 1			Frequency			
	-619	-290	-66	52	54	57	Japan. n=57		Cauc. n=49	
High producer	C	G	T	C	G	G	0.079	0.483	0.163	0.449
	G	G	C	C	G	G	0.403		0.286	
Intermed	C	G	C	C	G	G	0.132	0.132	0	0
Low pro.	C	C	C	C	G	G	0.105	0.105	0.265	0.265
Deficiency	C	G	C	C	A	G	0.263	0.263	0.133	0.255
	C	G	T	C	G	A	0		0.020	

	G	G	C	T	G	G	0		0.102	

MBL-2 のプロモーターの 3 箇所、Exon 1 の 3 箇所の SNPs のハプロタイプは 7 種あり、High-, Intermediate-, Low- producer および、Deficiency に分類できる。High producer（45~48%）と Deficiency（26%）の頻度は人種で大差はない。

結果 3、表 3、GVHD と Donor の IL-6 SNPs (2×2 表)

	GGG(+)	GGG(-)	Total
≧II GVHD	5	11	16
< II GVHD	32	43	75
Total	37	54	91

ハプロタイプ GGG をもつとき、GVHD の relative risk （Odds）=0.6

ハプロタイプ GGG negative、 GVHD の relative risk （Odds）=1.63 P> 0.05

結論：GGG ハプロタイプを持つ Donor は GVHD を起こしにくい傾向があるが、有意さは認められない。

結果 4、表 4、MBL-2 High producer と Deficiency が移植後生存に与える影響

Haplotype	1 year Alive	Death within 1 year
Deficiency/ Deficiency	3/ 67 4%	3/ 24 12%
High pro./ High pro.	24/ 67 36%	4/ 24 17%
other	40/ 67 60%	17/ 24 70%

Deficiency allele homozygote の Recipient は 1 年以内の死亡率が高く、High-producer allele homozygote は生存率が高い傾向がある。P<0.05 （Yates 補正後は有意差なし）

考察とまとめ

1、炎症性サイトカイン IL-6 のプロモーター領域 SNPs は人種により多型性が異なる。日本人は白人に多様性のある「-174 (および-597)」については多型性がなく、むしろ、白人に多様性の低い「-572」に多様性が高い。

2、IL-6 のプロモーター領域の SNPs haplotype 「GGG」は、IL-6 Low producer とされ、腎移植において、低い拒絶率が報告されている。日本人集団の造血幹細胞移植においては、GGG haplotype が GVHD 抑制的（Odds=0.6）に働く可能性が示唆された。

3、自然免疫の最前線にあるとされる MBL-2 は、ウイルス、細菌、真菌などの感染防御に primary な役割を果たしている。MBL-2 は 6 個の SNPs haplotype により、High producer と Deficiency に分類できることがわかっている。Deficiency allele の頻度は無視できない高さ（26%）にあり、予後因子になり得る。

4、感染と MBL-2 SNPs の関連は、臨床データの不備で解析できなかった。移植後 1 年の生存率で見るとき、Deficiency allele homozygote は低く、High-producer allele homozygote は高い傾向にあった（P=0.05）。

移植免疫反応と遺伝子多型の解析

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目的と方法

がん監視機構や自己免疫疾患の疾患感受性・感染免疫・同種移植への影響が示唆され、TaqMan PCR 法で解析可能な免疫関連遺伝子多型を解析し、同種移植後転帰との関連を後方視的に解析した。

対象

HLA-A/B/C/DRB1 一致非血縁者間骨髄破壊的前処置骨髄移植を受けた前移植歴の無い血液がん患者とそのドナー(145 ペア)。

結果

1. NKG2D 遺伝子多型：HNK1 ハプロタイプ陽性(高 NK 活性) vs. 陰性(低 NK 活性)

再発低リスク群では、HNK1 陽性ドナーを有する患者の生存率(OS)・移植関連死亡(TRM)は有意に優れていた(図 1・2)。これは多変量解析でも確認された(表 1)。一方、再発高リスク群では、このような影響はみられなかった。

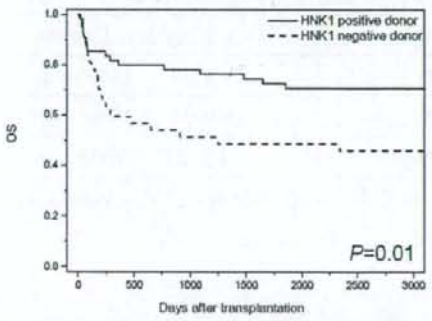

図1. ドナーNKG2D多型とOS (低リスク群)

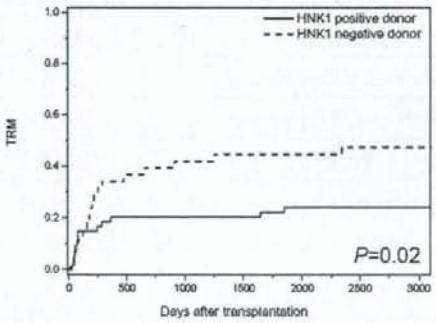

図2. ドナーNKG2D多型とTRM (低リスク群)

表 1. NKG2D 遺伝子多型と移植後転帰　多変量解析

		ドナーHNK1 陽性	患者 HNK1 陽性
		Adjusted hazard ratio (95% confidence interval), *P*	
Low risk disease	OS	**0.44 (0.21-0.92), 0.03**	1.12 (0.49-2.56), 0.78
	TRM	**0.39 (0.17-0.89), 0.03**	1.07 (0.43-2.62), 0.89
	Relapse	0.69 (0.15-3.09), 0.62	1.25 (0.24-6.42), 0.79
	II-IV aGVHD	0.95 (0.39-2.33), 0.91	1.33 (0.56-3.20), 0.52
	cGVHD	0.89 (0.34-2.29), 0.81	0.94 (0.40-2.23), 0.89
High risk disease	OS	0.88 (0.27-2.85), 0.83	0.99 (0.40-2.48), 0.99
	TRM	0.66 (0.14-3.11), 0.60	0.70 (0.18-2.81), 0.62
	Relapse	1.89 (0.16-22.95), 0.62	2.76 (0.52-14.52), 0.23
	II-IV aGVHD	3.02 (0.70-12.99), 0.14	**0.22 (0.07-0.72), 0.01**
	cGVHD	0.58 (0.11-3.03), 0.52	0.72 (0.21-2.46), 0.61

2. FCGR3A 遺伝子多型：158V ハプロタイプ陽性(高 ADCC) vs. (低 ADCC)

骨髄系腫瘍の場合、158V 陽性患者の TRM は有意に低く(*P*=0.02)、OS も良好であった(図3)。リンパ系腫瘍では逆に、158V 陽性患者の OS は有意に不良であった(図 4)。これらは多変量解析でも確認された(表 2)。ドナーの FCGR3A 多型は、腫瘍の種類にかかわらず、移植後転帰に影響しなかった。

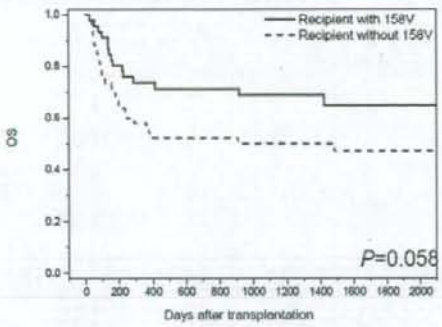

図3. 患者FCGR3A多型とOS (骨髄系腫瘍)

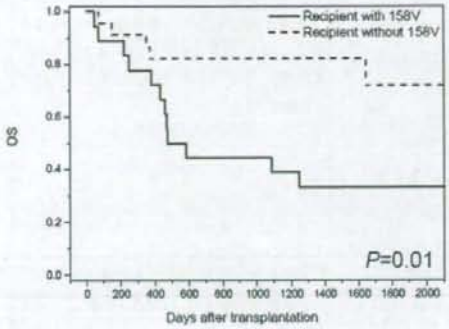

図4. 患者FCGR3A多型とOS (リンパ系腫瘍)

表 2. FCGR3A 遺伝子多型と移植後転帰　多変量解析

		ドナー158V 陽性	患者 158V 陽性
		Adjusted hazard ratio (95% confidence interval), *P*	
Myeloid malignancy	OS	1.29 (0.70-2.38), 0.42	0.49 (0.26-0.93), 0.03
	TRM	1.73 (0.84-3.54), 0.14	0.30 (0.14-0.67), 0.003
	Relapse	0.82 (0.27-2.55), 0.74	0.67 (0.22-2.02), 0.47
	II-IV aGVHD	1.24 (0.63-2.46), 0.53	0.55 (0.27-1.10), 0.09
	cGVHD	0.55 (0.25-1.21), 0.14	0.45 (0.20-0.99), 0.049
Lymphoid malignancy	OS	1.17 (0.40-3.42), 0.78	3.93 (1.28-12.08), 0.02
	TRM	1.01 (0.29-3.48), 0.99	2.72 (0.80-9.30), 0.11
	Relapse	1.52 (0.39-5.89), 0.54	1.65 (0.43-6.39), 0.47
	II-IV aGVHD	0.80 (0.18-3.51), 0.77	1.83 (0.50-6.73), 0.36
	cGVHD	0.82 (0.30-2.27), 0.71	1.71 (0.64-4.59), 0.29

別紙4

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kawase T, Matsuo K, Kashiwase K, Inoko H, Saji H, Ogawa S, Kato S, Sasazuki T, Kodera Y, Morishima Y;	HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism.	Blood.	113(12)	2851-8.	2009
Atsuta Y, Morishima Y, Kato S. et al.	Disease-specific analyses of unrelated cord blood transplant compared with unrelated bone marrow transplant in adult patients with acute leukemia.	Blood	113	1631-38	2009
Kawase T, Nannya Y, Torikai H, Onizuka M, Morishima S, Morishima Y, Ogawa S, Akatsuka Y. et al.	Identification of human minor histocompatibility antigens based on genetic association with highly parallel genotyping of pooled DNA.	Blood	111(6)	3286-94.	2008
Yabe T, Matsuo K, Hirayasu K, Kashiwase K, Inoko H, Saji H, Ogawa S, Juji T, Sasazuki T, Morishima Y et al.	Donor killer immunoglobulin-like receptor (KIR) genotype-patient cognate KIR ligand combination and antithymocyte globulin preadministration are critical factors in outcome of HLA-C-KIR ligand-mismatched T cell-replete unrelated bone marrow transplantation.	Biol Blood Marrow Transplant.	14(1)	75-87	2008

HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism

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The finding that the risk of relapse in hematologic malignancy decreases after allogeneic hematopoietic stem cell transplantation (HSCT) has lead to the concept of a graft-versus-leukemia (GVL) effect. However, this beneficial effect is considered to be frequently offset by graft-versus-host disease (GVHD). Thus, improving HSCT outcomes by separating GVL from GVHD is a key clinical issue. This cohort study registered 4643 patients with hematologic malignancies who received transplants from unrelated donors. Six major human leukocyte antigen (HLA) loci were retrospectively genotyped. We identified 4 HLA-Cw and 6 HLA-DPB1 mismatch combinations responsible for a decreased risk of relapse; of these, 8 of 10 combinations were different from those responsible for severe acute GVHD, including all 6 of the HLA-DPB1 combinations. Pairs with these combinations of HLA-DPB1 were associated with a significantly better overall survival than were completely matched pairs. Moreover, several amino acid substitutions on specific positions responsible for a decreased risk of relapse were identified in HLA-Cw, but not in HLA-DPB1. These findings might be crucial to elucidating the mechanism of the decreased risk of relapse on the basis of HLA molecule. Donor selection made in consideration of these results might allow the separation of GVL from acute GVHD, especially in HLA-DPB1 mismatch combinations. (Blood. 2009;113:2851-2858)

Introduction

The use of allogeneic hematopoietic stem cell transplantation (HSCT), an established treatment for hematologic malignancies, is associated with several immunologic events with contrary effects in the recipient. In graft-versus-host disease (GVHD), for example, graft immune cells attack host organs, whereas in the graft-versus-leukemia (GVL) effect, they eradicate residual leukemia cells.[1-3] GVL is likely to function not only in hematologic malignancies but also in solid tumors, particularly breast cancer and renal cell carcinoma,[4-6] in which it is referred to as the graft-versus-tumor (GVT) effect. Because both GVL and GVHD are caused by either or both major and minor histocompatibility antigen mismatches between donor and recipient, the beneficial effect of allogeneic HSCT due to GVL is thought to be frequently offset by GVHD. Thus, improving HSCT outcome by separating GVL from GVHD is a key clinical issue. Importantly, however, while most such efforts have been in the area of minor histocompatibility antigen,[7] few researchers have approached this problem in terms of the major histocompatibility antigen.

We recently identified 16 human leukocyte antigen (HLA) mismatch combinations associated with a high risk of severe acute GVHD. Results showed that the overall number of these high-risk mismatches was strongly associated with the occurrence of severe acute GVHD and poor overall survival (OS).[8] We speculated that the intensity of GVL and acute GVHD in any particular mismatch might not necessarily be parallel, and that among HLA mismatch combinations not inducing severe acute GVHD, those that induce strong GVL might occur. In other words, the hypotheses of this study were that particular mismatch combinations allow the separation of GVL from acute GVHD and that specific amino acid substitutions in HLA molecules contribute to this mechanism.

As part of efforts to improve donor selection and allogeneic HSCT outcomes, we identified HLA mismatch combinations that resulted in a decreased risk of relapse in all 6 major HLA loci and compared them with mismatch combinations carrying a high risk of severe acute GVHD. Further, we investigated specific amino acid substitution positions in the HLA molecule responsible for a decreased risk of relapse.

Methods

Patients

This study was conducted using clinical data that were collected prospectively at transplant centers participating in the Japan Marrow Donor Program. Patients who received a first transplant of T cell–replete marrow for a hematologic malignancy from a serologically HLA-A, -B, and -DR antigen-matched unrelated donor between January 1993 and December 2005 through the Japan Marrow Donor Program (n = 4643) were registered. Eligible diagnoses included acute lymphoblastic leukemia (ALL); acute myeloid leukemia (AML), which included only de novo AML;

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Table 1. Patient characteristics

		A locus		B locus		C locus		DRB1 locus		DQB1 locus		DPB1 locus	
	Total	Match	Mismatch	Match	Mismatch	Match	Mismatch	Match	Mismatch	Match	Mismatch	Match	Mismatch
	4643	4018	625	4351	292	3308	1335	3718	925	3597	1046	1584	3059
Median age, y	31.5	31.8	29.6	31.7	28.3	31.8	30.9	31.7	30.9	31.7	30.8	31.8	31.4
Sex, donor/patient													
Male/male	1904	1673	231	1769	135	1387	517	1551	353	1492	412	678	1226
Male/female	923	789	134	874	49	650	273	734	189	704	219	299	624
Female/male	894	747	147	843	51	634	260	693	201	672	222	268	626
Female/female	922	809	113	865	57	637	285	740	182	729	193	339	583
Disease													
ALL	1464	1267	197	1372	92	1051	413	161	303	1132	332	452	1012
AML	1571	1360	211	1478	93	1114	457	1255	316	1224	347	574	997
CML	979	827	152	905	74	682	297	779	200	746	233	343	636
ML	564	507	57	536	28	43	146	468	96	49	118	192	372
MM	65	57	8	60	5	418	22	55	10	446	16	23	42
Risk of leukemia relapse*													
Standard risk	1684	1485	199	1588	96	1184	500	1375	309	1322	362	572	1112
High risk	1909	1607	302	1772	137	1365	544	1485	424	1451	458	642	1267
Disease other than leukemia	1050	926	124	991	59	759	291	858	192	824	226	370	680
GVHD prophylaxis													
Cyclosporine-based	2503	2159	344	2346	157	1802	701	2107	396	2030	473	881	1622
Tacrolimus-based	2140	1859	281	2005	135	1506	634	1611	529	1567	573	703	1437
ATG													
ATG	152	112	40	135	17	102	50	110	42	118	34	51	101
Non-ATG	4491	3906	585	4216	275	3206	1285	3608	883	3479	1012	1533	2958
Preconditioning													
TBI regimen	3687	3175	512	3445	242	2623	1064	2933	754	2834	853	1242	2445
Non-TBI regimen	956	843	113	906	50	685	271	785	171	763	193	342	614

ATG indicates antithymocyte globulin; and TBI, total body irradiation.

*Standard risk for leukemia relapse was defined as the status of the first complete remission of AML and ALL and the first chronic phase of CML at transplant, while high risk was defined as a more advanced status than standard risk in AML, ALL, and CML. Disease other than leukemia was defined as other than ALL, AML, and CML.

chronic myeloid leukemia (CML); malignant lymphoma (ML); and multiple myeloma (MM).

Patient characteristics are shown in Table 1. A final clinical survey of the patients was completed by December 2006. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and approval for the study was obtained from the Institutional Review Board of Aichi Cancer Center and the Japan Marrow Donor Program.

HLA typing of patients and donors

Alleles at the HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci were identified by previously described methods in all 4643 pairs at the Japanese Red Cross Tokyo Metropolitan Blood Center.[8,9]

Matching of HLA allele between patient and donor

HLA allele mismatch among the donor-recipient pair was scored when the recipient's alleles were not shared by the donor (graft-versus-host vector) for all analyses.

Definition of relapse

Relapse was defined as the recurrence of malignancy as detected by the parameter by which the malignancy was first detected, namely marrow morphology; flow cytometry; cytogenetic studies, including fluorescence in situ hybridization; electrophoresis; immunofixation assays; polymerase chain reaction-based assays for disease markers; or imaging results. The day of relapse was defined as the day on which the respective clinical, hematologic, cytogenetic, or molecular relapse was recognized.

Definition of amino acid substitution

Amino acid sequences of HLA-Cw and -DPB1 molecules were obtained from the IMGT/HLA sequence database.[10] For example, Tyr99C-Phe99C indicated an amino acid substitution at position 99 in the HLA-C molecule in which the donor had tyrosine and the patient had phenylalanine. Substituted amino acids in HLA-Cw and -DPB1 are summarized in Tables S1 and S2 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Statistical analysis

OS rate was assessed using the Kaplan-Meier product limit method. To eliminate the effect of competing risk, the cumulative incidence of relapse was assessed using a previously described method.[11,12] The competing event for relapse was defined as death without relapse. Impact by the factor of interest was assessed using the log rank test. The impact of HLA allele mismatch combinations and the position and type of amino acid substitution (for example, alanine, arginine, and asparagine) in HLA molecules were evaluated using multivariable Cox regression analysis[13] for OS and the occurrence of acute GVHD, while the risk of relapse was evaluated using the multivariable proportional hazard modeling of subdistribution functions in competing risks.[14]

HLA mismatch combinations were evaluated for each locus separately. When the locus of interest was evaluated, we allowed the other loci to be mismatched, with the status of such mismatches adjusted for in the same way as other confounders. The HLA match and HLA one-allele mismatched in every locus were analyzed. For example, the A*0206-A*0201 mismatch combination meant that the donor had HLA-A*0206, the recipient had HLA-A*0201, while another HLA-A allele of the donor and recipient was identical. This mismatch was compared with the HLA-A allele match. Mismatch combinations that had 9 or fewer pairs were combined together as "other mismatch." The model was constructed with mismatch combinations, mismatch status in other loci (match, 1 allele mismatched, and 2 alleles mismatched, as an ordinal variable), and potential confounders. Confounders considered were sex (donor-recipient pair), patient age (linear), donor age (linear), transplant year, type of disease, risk of leukemia relapse (standard, high, and diseases other than leukemia), GVHD prophylaxis (cyclosporine [CSP] vs tacrolimus [FK]), ATG (vs no ATG), and

preconditioning (TBI vs non-TBI). These confounders were used in all analyses to maintain the comparability of results.

The impact of position and type of amino acid substitutions in HLA molecules was evaluated in pairs with oneallele mismatched in HLA-Cw and -DPB1 separately. The amino acid positions we analyzed were all positions at which an amino acid was substituted in the respective locus. We analyzed the impact of each amino acid substitution on each position separately. Multivariable models were constructed to include the position and type of amino acid substitution, mismatch status in other loci (match, 1 allele mismatched, and 2 alleles mismatched as an ordinal variable) and the confounders described above. A P value less than .05 was considered statistically significant. All statistical tests were 2-sided. All analyses were performed using STATA version 10.0 (StataCorp, College Station, TX) and R version 2.5.1 (The R Foundation for Statistical Computing, www.r-project.org).

Validation of statistical analysis

Statistical analyses were validated using the bootstrap resampling method.[15] Briefly, we estimated the measure of association with resampled data drawn repeatedly from the original data. Although approximately 100 to 200 bootstrapped samples are generally sufficient,[16] we used 1 000 bootstrap samples for all analysis validations. Further, we judged the results of analysis as statistically significant only when the results of both base analysis and analysis validation using bootstrap resampling were significant; cases in which the result of base analysis was significant but that of analysis validation using bootstrap resampling was not are indicated by an asterisk next to the P value of the base analysis.

Results

Impact of HLA allele mismatches in locus level on relapse

The number of mismatched alleles of HLA-Cw (1 allele mismatched: hazard ratio [HR], 0.68; 95% confidence interval [CI], 0.58-0.80; 2 alleles mismatched: HR, 0.43;95% CI, 0.24-0.75) and HLA-DPB1 (1 allele mismatched: HR, 0.80; 95% CI, 0.70-0.92; 2 alleles mismatched: HR, 0.62; 95% CI, 0.51-0.75) was strongly associated with a decreased risk of relapse. In contrast, no associations were seen for HLA-A (1 allele mismatched: HR, 1.00; 95% CI: 0.82-1.22; 2 alleles mismatched: HR, 0.79; 95% CI, 0.28-2.28), HLA-B (1 allele mismatched: HR, 1.06; 95% CI, 0.79-1.41; 2 alleles mismatched: not applicable), HLA-DRB1 (1 allele mismatched: HR, 0.93; 95% CI, 0.74-1.18; 2 alleles mismatched: HR, 1.18, 95% CI: 0.53-2.63) or HLA-DQB1 (1 allele mismatched: HR, 1.12; 95% CI, 0.90-1.40; 2 alleles mismatched: HR, 0.73; 95% CI, 0.35-1.52; Figure 1; Table 2).

Impact of HLA mismatch combinations on relapse

Four mismatch combinations in HLA-Cw and 6 in HLA-DPB1 were significantly associated with a decreased risk of relapse (Tables 3 and S3). In contrast, mismatch combinations in HLA-A, -B, -DRB1, and -DQB1 were not significantly associated with differences in risk of relapse (data not shown). The 10 HLA mismatch combinations associated with lower risks of relapse were Cw*0102-Cw*1402 (HR not estimated due to no event), Cw*0801-Cw*0102 (HR not estimated), Cw*1402-Cw*0304 (HR not estimated), Cw*1502-Cw*1402 (HR, 0.28; 95% CI, 0.09-0.88), DPB1*0402-DPB1*0201 (HR, 0.32, 95% CI, 0.12-0.87), DPB1*0501-DPB1*0201 (HR, 0.67; 95% CI:0.50-0.91), DPB1*0501-DPB1*0401 (HR, 0.36; 95% CI, 0.13-0.98), DPB1*0501-DPB1*0402 (HR, 0.55; 95% CI, 0.33-0.93), DPB1*0901-DPB1*0201 (HR, 0.37; 95% CI, 0.14-0.96), and DPB1*1301-DPB1*0201 (HR not estimated; Tables 3 and S3). All 10 HLA mismatch combinations were also significant on validation analysis using the bootstrap resampling method. We speculated that these mismatch combinations would mainly decrease the risk of relapse due to GVL, so we tentatively call them GVL mismatch combinations.

Evaluation of clinical importance of GVL mismatch combinations

We evaluated the clinical importance of GVL mismatch combinations in HLA-Cw and -DPB1. All analyses in this section were conducted in matched pairs other than the evaluated locus. In HLA-C mismatch, the small number of patients with GVL mismatch combinations (n = 13) in matched pairs at the allele level for HLA-A, -B, -DRB1, -DQB1, and -DPB1 prevented comprehensive analysis. We evaluated the GVL mismatch combinations of HLA-DPB1 in matched pairs for HLA-A, -B, -Cw, -DRB1, and -DQB1. Pairs with HLA-DPB1 mismatch were divided into 2 groups, those with a GVL mismatch combination and those with mismatch combinations other than GVL mismatch combinations. These were then compared with 12/12 matched pairs for association with severe acute GVHD, relapse, and OS (Table 4). The curve of the cumulative incidence of OS is shown in Figure 2. Multivariable analysis revealed that although OS was similar between the 12/12 matched pairs and the pairs with mismatch combinations other than GVL mismatch combinations, it was significantly improved in pairs with a GVL mismatch combination (Table 4). In terms of mortality due to relapse according to HLA-DPB1 matching status and whether the mismatch combinations were GVL mismatch combinations, the HLA-DPB1 matched group, HLA-DPB1 1 allele mismatched group, and GVL mismatch combination group showed an expected decreased mortality due to relapse (20.0%, 15.3%, and 10.5%, respectively). Further, mortality due to relapse in the GVL mismatch combination group was significantly lower than that in the HLA-DPB1 1 allele mismatched group (P = .049). We conducted the same analyses with stratification by leukemia type (ALL, AML, or CML) and found that the myeloid malignancies (AML and CML) had the same tendency (Table 4). In particular, in CML, GVL mismatch combinations in HLA-DPB1 were associated with a significantly reduced risk of relapse (HR, 0.14; 95% CI, 0.03-0.55) and significantly improved OS relapse (HR, 0.50; 95% CI, 0.25-0.98).

Impact of position and type of amino acid substitutions of HLA molecules on relapse

We surveyed all substituted positions in HLA-Cw and -DPB1 and found 159 specific amino acid substitutions at 55 positions in HLA-Cw and 55 specific amino acid substitutions at 19 positions in HLA-DPB1 (Tables S1,S2). Analysis revealed 3 specific amino acid substitutions responsible for a decreased risk of relapse in HLA-C, namely Ser9C-Tyr9C (HR, 0.53; 95% CI, 0.30-0.92), Phe99C-Tyr99C (HR, 0.52, 95% CI, 0.30-0.91), and Arg156C-Leu156C (HR, 0.59; 95% CI, 0.37-0.92). In contrast, no decrease in the risk of relapse was seen for substitutions in HLA-DPB1 (Table 5). However, Tyr9C-Ser9C and Tyr99C-Phe99C were strongly linked (see "Discussion"). These specific amino acid substitutions were all significant on validation analysis using the bootstrap resampling method.

Discussion

Improving outcomes in allogeneic HSCT for hematologic malignancies by separating GVL from GVHD is considered a key clinical

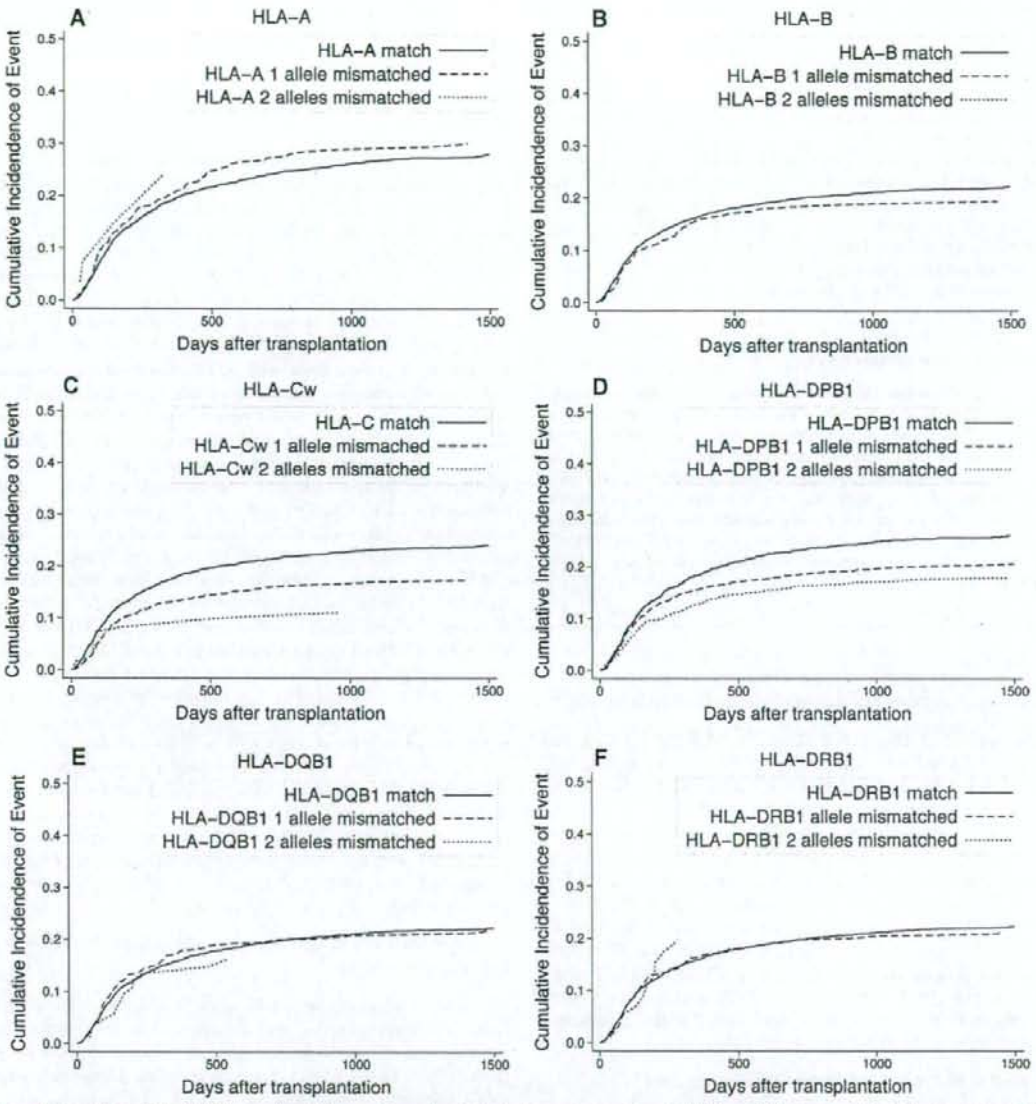

Figure 1. Impact of individual HLA locus mismatches on relapse. Cumulative incidence of relapse for each HLA locus. [—] indicates matched pairs in each locus; [---], 1-allele mismatched pairs in each locus; and [...], 2-allele mismatched pairs in each locus.

challenge. Here, our analysis demonstrated that several donor-recipient HLA mismatch combinations and specific amino acid substitutions in HLA molecules were associated with a decreased risk of relapse, and, in some cases, no significant increase in the risk of severe acute GVHD. These findings suggest that GVL might be separated from severe acute GVHD by selection of suitable HLA mismatch combinations.

We recently reported 16 significant high-risk HLA allele mismatch combinations for severe acute GVHD in 6 HLA loci, a number of which were highly associated with the occurrence of severe acute GVHD and worse OS.[8] Of note, a group of pairs with mismatches other than severe acute GVHD high-risk mismatches showed an incidence of severe acute GVHD and OS rates almost equal to those of 12/12 matched pairs. In the present study, we elucidated a total of 10 mismatch combinations that were significantly associated with a decreased risk of relapse, which we termed GVL mismatch combinations. Of course, it is possible that some mismatch combinations not classified as GVL mismatch combinations might actually induce strong GVL. Misclassification might have occurred as a result of insufficient statistical power due to the relatively small number of patients in the subcategories. Among these mismatch combinations, 2 of 4 in HLA-Cw were identical to the severe acute GVHD high-risk combinations; a third had a marginal effect on the occurrence of severe acute GVHD, while the

Table 2. Impact of HLA mismatches in allele level on relapse

	All diseases		
	n	HR (95% CI)	P
HLA-A matched	4018	1.00 (ref)	
HLA-A 1 allele mismatched	597	1.00 (0.82-1.22)	.99
HLA-A 2 alleles mismatched	26	0.79 (0.28-2.28)	.67
HLA-B matched	4351	1.00 (ref)	
HLA-B 1 allele mismatched	288	1.06 (0.79-1.41)	.7
HLA-B 2 alleles mismatched*	4	ND	ND
HLA-C matched	3308	1.00 (ref)	
HLA-C 1 allele mismatched	1212	0.68 (0.58-0.80)	<.001
HLA-C 2 alleles mismatched	123	0.43 (0.24-0.75)	.003
HLA-DRB1 matched	3718	1.00 (ref)	
HLA-DRB1 1 allele mismatched	866	0.93 (0.74-1.18)	.56
HLA-DRB1 2 alleles mismatched	59	1.18 (0.53-2.63)	.68
HLA-DQB1 matched	3597	1.00 (ref)	
HLA-DQB1 1 allele mismatched	958	1.12 (0.90-1.40)	.30
HLA-DQB1 2 alleles mismatched	88	0.73 (0.35-1.52)	.40
HLA-DPB1 matched	1584	1.00 (ref)	
HLA-DPB1 1 allele mismatched	2190	0.80 (0.70-0.92)	.002
HLA-DPB1 2 alleles mismatched	869	0.62 (0.51-0.75)	<.001

Each group was compared with the matched group in each locus after adjusting for other matching status of HLA, sex (donor-recipient pairs), patient age (linear), donor age (linear), type of disease, risk of leukemia relapse (standard, high, and diseases other than leukemia), GVHD prophylaxis (CSP vs FK), ATG vs no ATG), and preconditioning (TBI vs non-TBI).

ref indicates reference; and ND, not determined.

*Comprehensive analysis could not be performed due to the small number of cases.

fourth combination was different from acute GVHD high-risk mismatch combinations. In contrast, all 6 mismatch combinations in HLA-DPB1 were different from acute GVHD high-risk mismatch combinations (Table 3). As expected, HLA-A, -B, -Cw, -DRB1, and -DQB1 matched pairs with GVL mismatch combinations of HLA-DPB1 were associated with significantly better OS than 12/12 matched pairs (Table 4; Figure 2), indicating that the beneficial antitumor effect of GVL mismatch combinations in HLA-DPB1 would not be offset by the effect of severe acute GVHD. We speculate that conformational changes of HLA molecules in each mismatch combination control the intensity of the acute GVHD and GVL effect, as described later in "Discussion" and in our previous report[8]; namely, conformational changes of HLA molecules in GVL mismatch combinations in HLA-DPB1 induce strong GVL with mild or no acute GVHD. These findings suggest that HLA mismatch selection according to these results might improve HSCT outcomes over those obtained with a complete match. The same tendency was seen for AML and CML, whereas the effect of GVL mismatch combination in the HLA-DPB1 allele in ALL patients would be weaker than in the other leukemia types (Table 4). Comprehensive analyses for ML and MM could not be done because of the small number in each group. Thus, the effects of GVL mismatch combination vary according to disease type and may also change according to other factors, including particular cytogenetic abnormalities.

Recent research has shown that HLA-Cw and -DPB1 mismatch at the allele level is strongly associated with a decreased risk of relapse.[17,18] These findings were confirmed in the present large cohort. In addition, the present study also clarified that the mismatching of 2 alleles in either the HLA-Cw or -DPB1 locus had a stronger association with decreased risk than respective mismatching of one allele. Moreover, no association whatsoever was seen for

Table 3. GVL mismatch combinations

Mismatch combination, donor-recipient	n	HR (95% CI)	P
Cw*0102-Cw*1402*†	13	ND	ND
Cw*0801-Cw*0102*†	10	ND	ND
Cw*1402-Cw*0304†	20	ND	ND
Cw*1502-Cw*1402	43	0.28 (0.09-0.88)	.030
DPB1*0402-DPB1*0201*	54	0.32 (0.12-0.87)	.026
DPB1*0501-DPB1*0201*	301	0.67 (0.50-0.91)	.009
DPB1*0501-DPB1*0401*	48	0.36 (0.13-0.98)	.046
DPB1*0501-DPB1*0402*	112	0.55 (0.33-0.93)	.026
DPB1*0901-DPB1*0201*	43	0.37 (0.14-0.96)	.042
DPB1*1301-DPB1*0201*†	20	ND	ND

As an example of the mismatch combination analysis, the Cw*0102-Cw*1402 mismatch combination meant that the donor has HLA-Cw*0102, the recipient has HLA-Cw*1402 and another HLA-Cw allele of each donor and recipient was identical. Each mismatch pair in HLA-Cw was compared with the HLA-Cw allele match, and each mismatch pair in HLA-DPB1 was compared with the HLA-DPB1 allele match. All indicated results were concurrently significant in both the base analysis and validation analysis using bootstrap resampling.

ND indicates not determined.

*Mismatch combinations that were not significantly associated with a higher occurrence of severe acute GVHD in our previous study.[8] However, the Cw*0102-Cw*1402 mismatch combination has a marginal effect on the occurrence of severe acute GVHD; that is, Cw*0102-Cw*1402 was significantly associated with a higher occurrence of severe acute GVHD in base analysis, but not in validation analysis.

†HR was not estimated due to the lack of an event in this group.

Table 4. Clinical importance of GVL mismatch combinations in HLA-DPB1 mismatch

		Acute GVHD		Relapse		OS*	
All diseases	n	HR (95% CI)		HR (95% CI)	P	HR (95% CI)	P
HLA-DPB1 matched	864	1.00 (ref)		1.00 (ref)		1.00 (ref)	
HLA-DPB1 1 allele mismatched	808	1.34 (1.03-1.74)	.028	0.83 (0.68-1.01)	0068	0.96 (0.83-1.12)	.62
GVL mismatch combination	258	1.18 (0.81-1.73)	.375	0.47 (0.33-0.67)	<.001	0.75 (0.59-0.94)	.012
ALL							
HLA-DPB1 matched	250	1.00 (ref)		1.00 (ref)		1.00 (ref)	
HLA-DPB1 1 allele mismatched	263	1.56 (0.96-2.54)	.067	0.85 (0.6-1.19)	.33	1.10 (0.85-1.43)	.48
GVL mismatch combination	80	1.27 (0.63-2.57)	.5	0.75 (0.45-1.26)	.28	0.95 (0.65-1.39)	.8
AML							
HLA-DPB1 matched	308	1.00 (ref)		1.00 (ref)		1.00 (ref)	
HLA-DPB1 1 allele mismatched	264	1.47 (0.9-2.39)	.13	0.83 (0.61-1.14)	.26	0.95 (0.74-1.23)	.72
GVL mismatch combination	89	1.25 (0.62-2.5)	.54	0.44 (0.24-0.78)	.006	0.71 (0.48-1.06)	.1
CML							
HLA-DPB1 matched	176	1.00 (ref)		1.00 (ref)		1.00 (ref)	
HLA-DPB1 1 allele mismatched	162	1.25 (0.74-2.14)	.41	0.69 (0.40-1.20)	.19	0.93 (0.65-1.33)	.69
GVL mismatch combination	54	1.13 (0.51-2.47)	.66	0.14 (0.03-0.55)	.005	0.50 (0.25-0.98)	.041

Each group was compared with the HLA-DPB1 matched group. Confounders considered were sex (donor-recipient pairs), patient age (linear), donor age (linear), type of disease, risk of leukemia relapse (standard, high, and diseases other than leukemia), GVHD prophylaxis (CSP vs FK), ATG vs no ATG), and preconditioning (TBI vs non-TBI). ref indicates reference.

*The HR indicates the likelihood that OS will be shorter (if HR > 1) or longer (HR < 1) than when the HLA type matches (ie, the Ref condition).

HLA-A, -B, -DRB1, or -DQB1 (Figure 1; Table 2). Furthermore, all 10 GVL mismatch combinations were elucidated from mismatch combinations of HLA-Cw and HLA-DPB1 (Tables 3 and S3), although we also analyzed HLA-A, -B, -DRB1, and -DQB1. These findings indicate that GVL after allogeneic HSCT is mainly induced by HLA-Cw and -DPB1, not HLA-A, -B, -DRB1 or -DQB1, although the role of each HLA locus might vary with the type of disease.[18] There are 3 possible explanations for this. First, the relative expression of HLA-Cw and -DPB1 on malignant cells may be higher than that on normal hematopoietic cells; second, HLA-Cw and -DPB1 may be preferentially expressed on malignant stem cells; and third, surface expression of a few key molecules—such as major histocompatibility complex (MHC), adhesion, and costimulatory molecules—on malignant cells may determine the effect of each HLA locus on GVL.[19-21] In other words, some molecules might stimulate GVL of HLA-Cw or -DPB1, and other molecules might block GVL of other than HLA-Cw and -DPB1. Further investigation of this question is warranted.

In this study, 3 specific amino acid substitutions responsible for GVL at positions 9, 99, and 156 were identified in HLA-Cw, of which only 2, Ser9C-Tyr9C and Phe99C-Tyr99C, were strongly linked in our sample. We were therefore unable to determine which substitutions are the main contributors to the effect of interest (Table 5). These amino acid positions, 9, 99, and 156, were identical to those we elucidated in our previous study as responsible for severe acute GVHD.[8] These findings suggest that these 3 amino acid positions are important determinants of alloreactivity. Although position 156 of the HLA molecule has been shown to modify T-cell alloreactivity in vitro in HLA-A2,[22-24] -B35,[25] and -B44,[26] to our knowledge, the present study is the first to identify positions 9 and 99. On the other hand, substituted amino acids were not necessarily identical. In Ser9C-Tyr9C and Phe99C-Tyr99C substitutions, for example, the substituted amino acid position was identical with that responsible for severe acute GVHD, whereas the substituted amino acids were inverse between donor and recipient, even though both substituted position and amino acids were identical in the Arg156C-Leu156C substitution. These findings suggest that Ser9C-Tyr9C and Phe99C-Tyr99C might play an important role in separating GVL from acute GVHD in HLA-Cw mismatch, although the mechanism requires further molecular clarification.

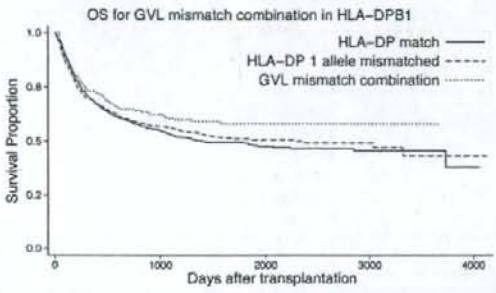

Figure 2. Clinical importance of GVL mismatch combinations in HLA-DP mismatch. Kaplan-Meier estimates of survival according to HLA-DPB1 mismatch status. The solid line indicates HLA-DPB1 matched pairs; the short broken line, HLA-DPB1 1 allele mismatched but not GVL mismatch combinations; and the dotted line, HLA-DPB1 1 allele mismatched (GVL mismatch combinations). All groups are HLA-A, -B, -C, -DRB1, and -DQB1 matched pairs.

Table 5. Impact of position and type of amino acid substitution of HLA molecules on relapse

Position and amino acid substitution in HLA-C (donor-recipient)	n	HR (95% CI)	P
Ser9C-Tyr9C	152	0.53 (0.30-0.92)	.024
Phe99C-Tyr99C	153	0.52 (0.30-0.91)	.022
Arg156C-Leu156C*	225	0.59 (0.37-0.92)	.020

The impact of position and type of amino acid substitution in HLA molecules was evaluated in pairs with HLA one-locus mismatch in HLA-C and -DPB1 separately. For example, Tyr9C-Ser9C indicated amino acid substitutions of position 9 in the HLA-C molecule in which the donor had tyrosine and the patient serine. The impact of position and kind of amino acid substitution in each HLA molecule was evaluated in pairs with HLA one locus mismatch in each HLA locus separately. Pairs that substituted a specific amino acid at each position were compared with amino acid matched pairs at that position.

No significant amino acid substitutions were found in HLA-DPB1.

All indicated results were concurrently significant in both base analysis and validation analysis using bootstrap resampling.

The 2 specific amino acid substitutions Tyr9C-Ser9C and Tyr99C-Phe99C were strongly linked in our sample.

*An amino acid substitution that was significantly associated with a higher occurrence of severe acute GVHD in our previous study.[8]