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H. 知的財産権の出願・登録状況

該当事項なし

厚生労働科学研究費補助金（医療技術実用化総合研究事業）

分担研究報告書

「再生不良性貧血に対するアレムツズマブを用いた同種造血幹細胞移植法の開発を意図した多施設共同医師主導治験に関する研究」

研究分担者　宮本　敏浩　九州大学病院　血液腫瘍内科　講師

研究要旨：同種造血幹細胞移植の長期予後を改善するためには、慢性移植片対宿主反応病のコントロールが重要課題である。我々はステロイド難反応性の慢性移植片対宿主反応病症例に対してリツキシマブ療法の安全性と有効性を実証した。

A. 研究目的

再生不良性貧血に代表される造血不全や急性白血病など造血器腫瘍に対する根治的治療法の一つとして同種造血幹細胞移植療法が確立されている。移植片対宿主反応病（graft-versus-host disease：GVHD）は、同種造血幹細胞移植後の非再発死亡の原因として最も重要な合併症の一つであり、その治療法の確立が急務である。中でも慢性GVHDは、同種造血幹細胞移植における長期生存者の非再発死亡の重要な原因のみならず、患者の生活機能やQOLにも重大な影響を及ぼす。治療法としては、限局された慢性GVHDに対してはステロイド外用等で対処可能な場合もある。しかしながら広範型の場合には、ステロイドおよびカルシュニューリン阻害薬などの免疫抑制剤の全身的な投与が必要となる。しかしながら、未だ確立された有効な治療法はなく、また、免疫抑制に伴う感染症発症の頻度・重症度も問題となる。

我々は、同種造血幹細胞移植後のステロイド抵抗性の慢性GVHD全身型の症例に対して、抗CD20抗体のリツキシマブ療法の安全性と有効性を検討した。

B. 研究方法

同種造血幹細胞移植後100日以降の慢性GVHD全身型の症例で、ステロイド治療無効例、もしくはステロイド減量中の再燃症例を対象とした。リツキシマブ375mg/sqmを一週間間隔で合計4回投与した。リツキシマブ4回投与終了後に臓器毎に効果判定を行った。免疫抑制剤の併用は、リツキシマブ導入時に既に投与しているステロイド、カルシュニューリン阻害薬の継続は可とするが、免疫抑制剤の追加あるいは増量は行わないこととした。また、免疫抑制剤の減量は可能とした。評価方法は、NIH consensus development projectによる基準に従った。

C. 研究結果

対象となった7例の患者は、3例が急性骨髄性白血病、1例が骨髄異形成症候群、1例が急性リンパ性白血病、2例が慢性骨髄性白血病であった。移植ソースは、3例がHLA一致血縁末梢血幹細胞、3例がHLA一致非血縁骨髄、1例がHLA一座不一致非血縁骨髄移植であった。GVHD予防は2例がFK/MTX、4例がCSP/MTX、1例がFK単独で施行した。7例全例とも全身型でquiescent型の慢性GVHDであり、リツキシマブ投与時は移植後中央値42カ月、慢性GVHD発症後中央値37ヶ月であった。GVHDに対する前治療としは、ステロイドおよびカルシュニューリン阻害薬の投与が行われていた。

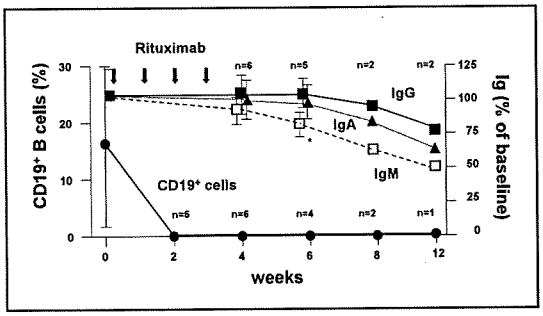
7例全例においてリツキシマブ4回投与は完遂可能であった。観察期間中にリツキシマブによる重篤な合併症は認めなかった。慢性GVHDに対するリツキシマブ療法の効果は、7例中3例がPR、3例がSD、1例がPDであった。PRおよびSDの6例中4例でステロイドの減量および中止が可能であ

UPN	Age / sex	Diagnosis	Donors	HLA	Stem cell source	GVHD prophylaxis
1	24/F	CML	sibling	identical	PBSC	CSP+MTX
2	39/M	MDS	unrelated	identical	BM	TAC+MTX
3	48/M	AML	unrelated	identical	BM	TAC
4	51/M	CML	unrelated	DR mismatch	BM	TAC+MTX
5	55/F	AML	unrelated	identical	BM	CSP+MTX
6	55/M	AML	sibling	identical	PBSC	CSP+MTX
7	29/M	ALL	sibling	identical	PBSC	CSP+MTX

UPN	Type of onset	Prior therapy	Interval from transplantation to Rituximab (mo)	Interval from onset of chronic GVHD to Rituximab (mo)
1	quiescent	PSL, CSP	19	8
2	quiescent	PSL, pulse mPSL, CSP, TAC	42	39
3	quiescent	PSL, TAC	46	43
4	quiescent	PSL, CSP, TAC	112	109
5	quiescent	PSL, CSP	34	30
6	quiescent	PSL, CSP	47	37
7	quiescent	PSL, mPSL, CSP	27	25

った。また、リツキシマブ投与後は、免疫抑制剤の追加は必要なかった。臓器別の評価では、口腔病変および血小板減少・貧血にはリツキシマブは有効であった。一方では、硬化性皮膚病変、関節拘縮、筋膜炎には効果が乏しかった。

7例において末梢血中のCD19陽性成熟B細胞数と免疫グロブリンの推移を検討した。リツキシマブ投与後に成熟B細胞は著減した。免疫グロブリンはIgM, IgA, IgGの順に、リツキシマブ投与終了後12週までに緩やかに減少した。



D. 考察

同種造血幹細胞移植後のステロイド抵抗性の全身型慢性 GVHD に対するリツキシマブ療法は安全に施行可能である。しかしながら、その効果は症状の固定していない軽度・中等度の慢性GVHD、特に口腔病変や血球減少には期待できる結果であった。

E. 結論

本研究により、同種造血幹細胞移植後ステロイド抵抗性の慢性 GVHD に対するリツキシマブ療法の安全性と有効性を評価した。症状の固定していない軽度・中等度の慢性GVHD患者に有用であると考えられる。今後の保険適応拡大を含めて、対応を検討する必要がある。

F. 健康危険情報

なし。

G. 研究発表

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H. 知的財産権の出願・登録状況

なし。

厚生労働科学研究費補助金（医療技術実用化総合研究事業）

分担研究報告書

「東海地区における対象症例リクルートと治験の実施」
－当院における同種造血幹細胞移植後の早期心合併症の臨床的検討－

研究分担者　宮村　耕一　名古屋第一赤十字病院　血液内科部長

研究要旨：名古屋第一赤十字病院においては1977年に本邦におけるはじめての再生不良性貧血の移植をおこなっていらい70例余りの移植を施行しているが、そのほとんどがエンドキサン200mg/kgを使用している。長期生存率はおよそ80%であるが、問題点としてはエンドキサンによる心毒性がある。当院による白血病も含む移植の解析ではエンドキサン200mg/kgは心不全のリスクファクターとして同定された。このことよりエンドキサンの量を下げることを可能とする「再生不良性貧血に対するアレムツズマブを用いた同種造血幹細胞移植療法の安全性及び有効性の検討（多施設共同医師主導治験）」は有望な前処置と考えられ、当地区におけるリクルートの正当性を裏付けるものである。

A. 研究目的

当院で用いているエンドキサン 200mg/kg の前処置が移植後の心合併症において、どのような影響を与えるのか、他の疾患も含め同種移植後早期に発症する心合併症の頻度を後方視的に検討を行い、心合併症発症の危険因子および心合併症発症例の予後について検討する。

B. 研究方法

2001年1月から2009年8月までに当院で行われた同種造血幹細胞移植（317例）を対象とした。前処置開始からday28までの心不全について検討した。心不全については「day28までにFramingham criteria（表 1）を満たしたもの」と定義した。心不全発症の危険因子について Fisher's exact testを用いて解析した。患者背景は表２に示す。

C. 研究結果

心不全の発症のリスクファクターとして再生不良性貧血、エンドキサン 200mg/kg 以上、EF<60%が単変量解析であげられた。（表3）心不全を発症した再生不良性貧血の3例はいずれも心不全発症後の心エコーで 大量の心嚢水貯留を認めた。うち1例の心嚢水は自然に消失した。残りの2例は心嚢水貯留が改善せず心不全状態が持続した。心タンポナーデを来すことはなかったが、2例とも徐々に腎機能低下を認めたため心嚢穿刺（day35, day55）を施行した。その後心不全、腎機能低下は改善した。

D. 考察

移植前の EF 低下例(EF<50%[1]あるいは EF<55%[2])では 心不全が多いという報告があり、今回の結果はこれを支持するものであった。心不全を発症したAML症例では進行期例が多くEFが低い傾向にあり、前治療の影響が示唆された。アントラサイクリン総投与量などの検討を要する。再生不良性貧血に対するエンドキサン 200mg/kg の使用は大量の心嚢水貯留に伴う心不全を惹起しうると考えられた。心不全に伴って臓器障害が進行する例では心嚢穿刺が必要である。

E. 結論

移植後早期心合併症の頻度は低いが、心不全を発症すると予後不良である。移植前 EF の低い例（EF<60%）は心不全のリスクが高かった。再生不良性貧血例に対しエンドキサン 200mg/kg を使用すると心嚢水貯留に伴う心不全を起こしうるた

め、エンドキサンを減量した前処置の開発が望まれる。

F. 健康危篤情報

該当なし

G. 研究発表

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2) 当院における同種造血幹細胞移植後早期心合併症の検討

3) 第 32 回日本造血細胞移植学会総会　浜松　2010.2

H. 知的財産権の出願・登録状況

該当なし

表1 Framingham criteria

- Major criteria

Paroxysmal nocturnal dyspnea or orthopnea,
Neck-vein distention, Rales, Cardiomegaly,
Acute pulmonary edema, S3 gallop,
Increased venous pressure≧16cm of water,
Circuration time≧2.5sec, Hepatojugular reflux

- Minor criteria

Ankle edema, Night cough, Dyspnea on exertion, Hepatomegaly,
Pleural effusion, Tachycardia(rate of ≧120/min)
(ただしこれらは明らかな他疾患によらないものとする。)

「Major criteria 2項目以上 または major criteria 1項目＋minor criteria 2項目以上」をdefinite congestive heart failureとする。

The New England Journal of Medicine 1971(285) 1441-1446

表2 患者背景(n=317)

- 年齢 16～65歳(中央値41歳)
- 男性193例 / 女性124例
- 疾患 AML 98/ALL 50/acute bilineage leukemia 1/ MDS 63/CML 33/CMMoL 7/NHL 21/HL 1/ATL 6/MM 10/ AA 21/PNH 5/原発性免疫不全 1
- 疾患リスク standard 144/high 173
- 幹細胞ソース BM 221/PB 39/CB 57
- 前処置 full 177/RIC 140
  CYなし=152 CY2g/m²=3 CY120mg/kg=141
  CY100mg/kg=3 CY200mg/kg=18
  TBI 165/non TBI 152
- GVHD予防 CyA (±MTX) 144/FK (±MTX) 172/none 1
- 移植前EF 36～89%(中央値70%)

表3

		心不全あり		
		n	%	
年齢	50歳未満 (n=214)	8	3.3	
	50歳以上 (n=103)	0	0.0	p=0.057
疾患	AA以外 (n=296)	5	1.7	
	AA (n=21)	3	14	p=0.011
疾患リスク	Standard (n=144)	4	2.9	
	High (n=173)	4	2.3	p>0.99
TBIの有無	non TBI (n=152)	2	1.3	
	TBI (n=165)	6	3.6	p=0.29
CY投与量	CYなし (n=152)	1	0.66	
	CYあり (n=165)	7	4.2	p=0.069
	CY<200mg/kg (n=299)	5	1.7	
	CY=200mg/kg (n=18)	3	17	p=0.0072
EF	≧60 (n=285)	5	1.8	
	<60 (n=27)	3	11	p=0.024

発症頻度8/317=2.5% CY使用例のみに限ると7/163=4.3%

厚生労働科学研究費補助金（医療技術実用化総合研究事業）

分担研究報告書

「造血幹細胞増幅技術の開発的研究」

研究分担者　千葉　　滋　筑波大学大学院　人間総合科学研究科　教授

研究要旨：現行の非血縁者間造血幹細胞移植はボランティアドナーの篤志に支えられて成立しているが、この体制への依存度を低減するための技術開発が求められる。移植片の安定確保を目指して我々が取り組んでいる造血幹細胞増幅技術開発は、その一端である。我々はこれまでに、免疫不全マウスへの移植実験などにより、臍帯血中の造血幹細胞および未分化造血前駆細胞の試験管内増幅が可能であることを示してきた。今年度は、この知見に基づいて臨床試験を行うために必要な周辺技術の確立および標準作業手順書（SOP）の作成を行っている。周辺技術とは、患者への移植用に保存されたものの研究使用に供されることが決定し、つくば理研細胞バンクで管理されている臍帯血を用いた融解、幹細胞分離、培養の手順確認である。また SOP は、筑波大学附属病院内に設置されている cell processing factory（CPF）で臍帯血幹細胞の分離と培養を行うためのもので、CPF の SOP と一体化して使用するものである。

A. 研究目的

現行の非血縁者間造血幹細胞移植はボランティアドナーの篤志に支えられて成立している。この体制への依存度を低減することは、医療体制の安全弁強化につながる。本研究では、移植片の安定確保を目指して、造血幹細胞増幅技術の開発を行う。

B. 研究方法

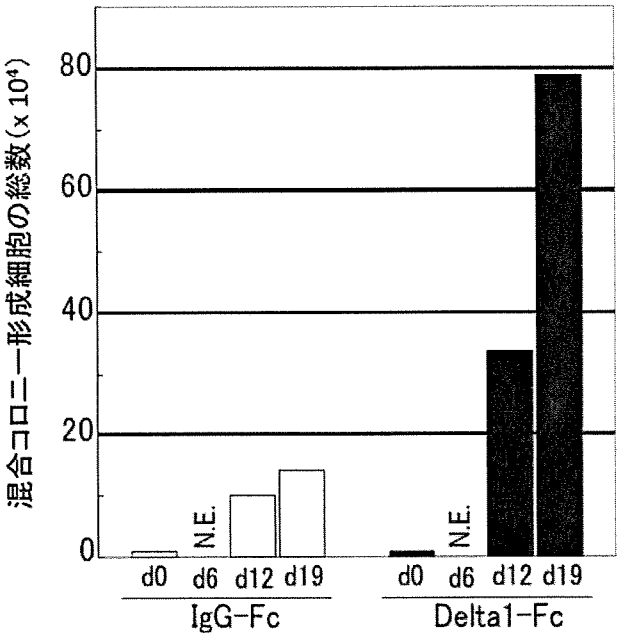
臍帯血造血幹細胞増幅についての基盤技術はすでに開発しており（Suzuki, et al., Chiba. Stem Cells, 2006）、この技術を用いて臨床研究を開始するための周辺技術の確立および標準作業手順書（SOP）の作成を行う。周辺技術としては、理化学研究所バイオリソースセンター（RBRC；つくば市）に保存されている、「患者への移植を目的として採取されたものの移植利用適応外となり研究用に保存されている」臍帯血を用い、これまでの培養法により未分化造血前駆細胞増幅の再現性を確認する。一方、筑波大学附属病院内の屋内屋型 cell processing factory（CPF）で臨床試験用の培養を行うため、SOP を作成する。

（本研究における倫理面への配慮）

RBRC における臍帯血の保管は、文部科学省再生医療実現化プロジェクトとして行われているもので、厳密な倫理的な配慮が行われている。RBRC から我々が臍帯血の供給を受けるにあたっては、筑波大学人間総合科学研究科に設置された倫理委員会で審査を受け承認されている。

C. 研究結果

臍帯血造血幹細胞増幅についての基盤技術はすでに開発済みであるが、再現性についてチェックを行った。その結果を図に示す。



図：凍結ヒト臍帯血 CD133 陽性細胞由来の混合コロニー形成細胞総数の経時的変化。融解した CD133 陽性細胞を 3 週間培養し、培養 0 日、12 日、19 日の時点で回収した細胞をメチルセルロース培地に播種した。播種から 2 週間後の混合コロニー数をカウントし、それぞれのポイントにおける総細胞数から算出される総混合コロニー形成細胞数をプロットした。Delta1-Fc を用いた培養では、12 日目および 19 日目の細胞を用いた混合コロニー形成細胞の総数は、それぞれ約 39 倍および 92 倍に増加していた。

この結果はこれまで同様に、脂質などを特殊に調整した無血清培地にウシアルブミンを加えた条件で培養したものであるが、ウシアルブミンをヒトアルブミンで置き換えても培養結果に悪影響がなければ、ヒトアルブミンに置き換える予定である。このため、新たにウシアルブミン入りおよびヒトアルブミン入りの 2 種類の特殊無血清培地の調整を専門業者に委託して入手し、現在両者の比較を行っている。

一方、RBRC から入手した臍帯血では、融解による細胞喪失により十分な結果が得られていない。このため、保存臍帯血を培養する臨床研究で先行している先端医療センター病院におけるプロトコールについて、同病院の母体である先端医療情報センター（神戸市）と筑波大学との間で秘密保持契約を結び、プロトコールの提供を受けた。今後これに基づいて RBRC から入手する臍帯血の融解と細胞調整を行う。

先端医療情報センターからは、秘密保持契約に基づいて GMP 対応の SOP の提供も受けた。これを参考にして、筑波大学附属病院内 CPF における作業について SOP 作成を進めている。

D. 考察

臨床試験を開始するために残された課題は、1) GMP 準拠の Delta1-Fc 作製、2) ウシアルブミンをヒトアルブミンに置き換えるべきかどうかの検討、3) 凍結保存された臍帯血を用いた培養技術の確立（先行施設における成功例の踏襲）、4) SOP の完成、の 4 点である。

これらを完成させた後、「ヒト幹細胞を用いる臨床研究に関する指針」（平成 18 年 7 月 3 日、厚生労働省）に則って筑波大学内に設置される倫理審査委員会での審議を経て、厚生労働大臣に意見を求め、臨床試験開始を是とする回答を得る、というプロセスを経て、臨床試験を開始する。

E. 結論

我々が開発した臍帯血造血幹細胞増幅についての基盤技術に基づいて、臨床研究を行う準備を行っている。技術の再現性確認、および周辺技術の確立および標準作業手順書（SOP）の作成を行っている。

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該当なし

G. 知的財産権の出願・登録状況

該当なし

Ⅲ．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

雑誌

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Ⅳ．研究成果の刊行物・別刷

Possible graft-versus-host disease involving the central nervous system soon after cord blood transplantation

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The concept that central nervous system (CNS) could be a target of graft-versus-host disease (GVHD) is controversial. There are a few case reports which support the possibility of CNS-GVHD [1,2]. Here, we describe a patient who developed unique CNS symptoms soon after cord blood transplantation with reduced-intensity conditioning (RI-CBT). On Day 7 post-transplant, a high fever, slight skin eruption, moderate diarrhea, and liver damage suddenly developed. Three days later, her white blood cell (WBC) count rapidly increased to 1,700 μl^{-1} and consisted mostly of mature lymphocytes. Generalized convulsions developed on the same day. An analysis of the cerebrospinal fluid (CSF) revealed elevated proteins and pleocytosis comprising mostly mature lymphocytes. The lymphocytes found in the peripheral blood (PB) and CSF were phenotypically polyclonal T-cells that were donor derived. Extensive investigations did not detect any microorganisms or other causes for the T-cell proliferation and CNS symptoms. Considering the coexistence of CNS and systemic GVHD-like symptoms, proliferation of donor-derived polyclonal T-cells in the CSF and PB, and no microorganisms or other factors detected, CNS GVHD seems to be the most likely explanation for her clinical course.

Cord blood (CB) has been increasingly applied as a viable source of stem cells for allogeneic hematopoietic stem cell transplantation (allo-SCT) [3,4]. The incidence and severity of GVHD following cord blood transplantation (CBT) are lower than those after allo-SCT using bone marrow or peripheral blood stem cells from either matched siblings or unrelated donors [5–7]. On the other hand, unique immune-mediated complications, such as pre-engraftment immune reaction (PIR) and hemophagocytic syndrome (HPS), have been observed early after RI-CBT [8,9]. Thus, the spectrum of immune-mediated reactions after RI-CBT has not yet been fully clarified.

CNS complications have been described following allo-SCT [10]. Infections, drug toxicity, and metabolic and cerebrovascular disorders are the major causes, and there have been rare cases of apparent immune-mediated reaction to CNS [1,2].

Here, we present an interesting case of a patient who developed unique CNS symptoms soon after RI-CBT. A 40-year-old woman with follicular lymphoma that was refractory to chemotherapy was admitted to our hospital in September 2006. Her clinical stage was IV B at diagnosis in 2002. Six cycles of rituximab (R)-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) resulted in complete remission, and rituximab therapy was maintained for 1 year. A relapse occurred in 2005 and was treated with R-ACES (high-dose Ara C, carboplatin, etoposide, and steroids), R-ICE (ifosfamide, carboplatin, etoposide), cladribine, and R-COP (cyclophosphamide, vincristine, and prednisone), which resulted in a partial response at each cycle. However, the disease gradually progressed thereafter, with the development of systemic lymphadenopathy, pleural effusion, and ascites. Since no suitable related or unrelated donors from the Japan Marrow Donor Program were available, unrelated CB was considered as an alternative graft, and she was referred to our hospital. The patient and graft were sex-mismatched and phenotypically two and genotypically three-loci mismatches in HLA-A, HLA-B, and DRB1 loci. The types of the HLA-A, HLA-B, and DRB1 loci were *A01* (*0101*)/A31 (3101), B35 (3501)/*B48* (*4801*), and DRB1*04

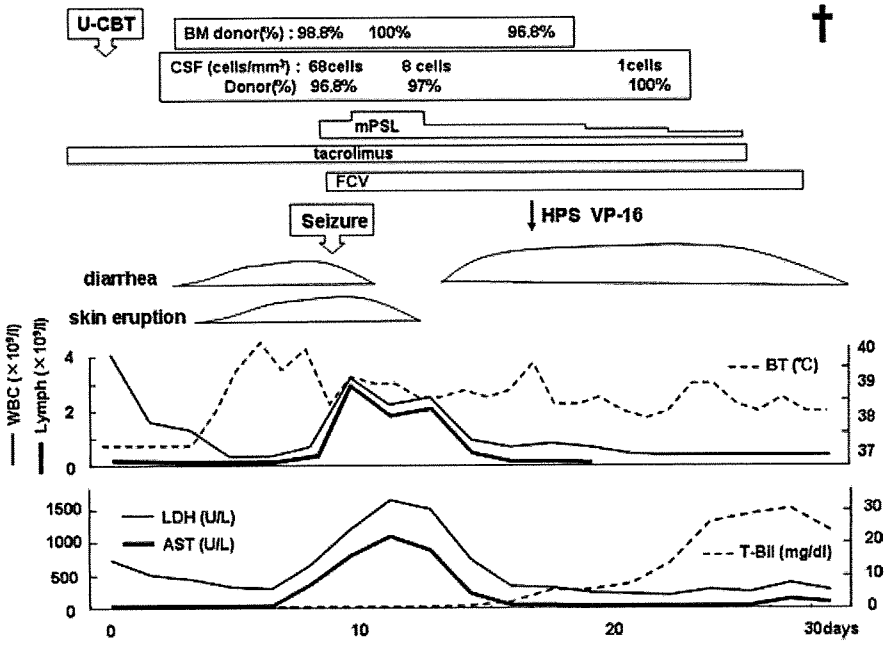

Figure 1. Clinical course of the patient. Abbreviations: U-CBT, unrelated cord blood transplantation; BM, bone marrow; CSF, cerebrospinal fluid; mPSL, methylprednisolone; FCV, foscarnet; HPS, hemophagocytic syndrome; VP-16, etoposide; WBC, white blood cell; BT, body temperature; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; T-bil, total bilirubin.

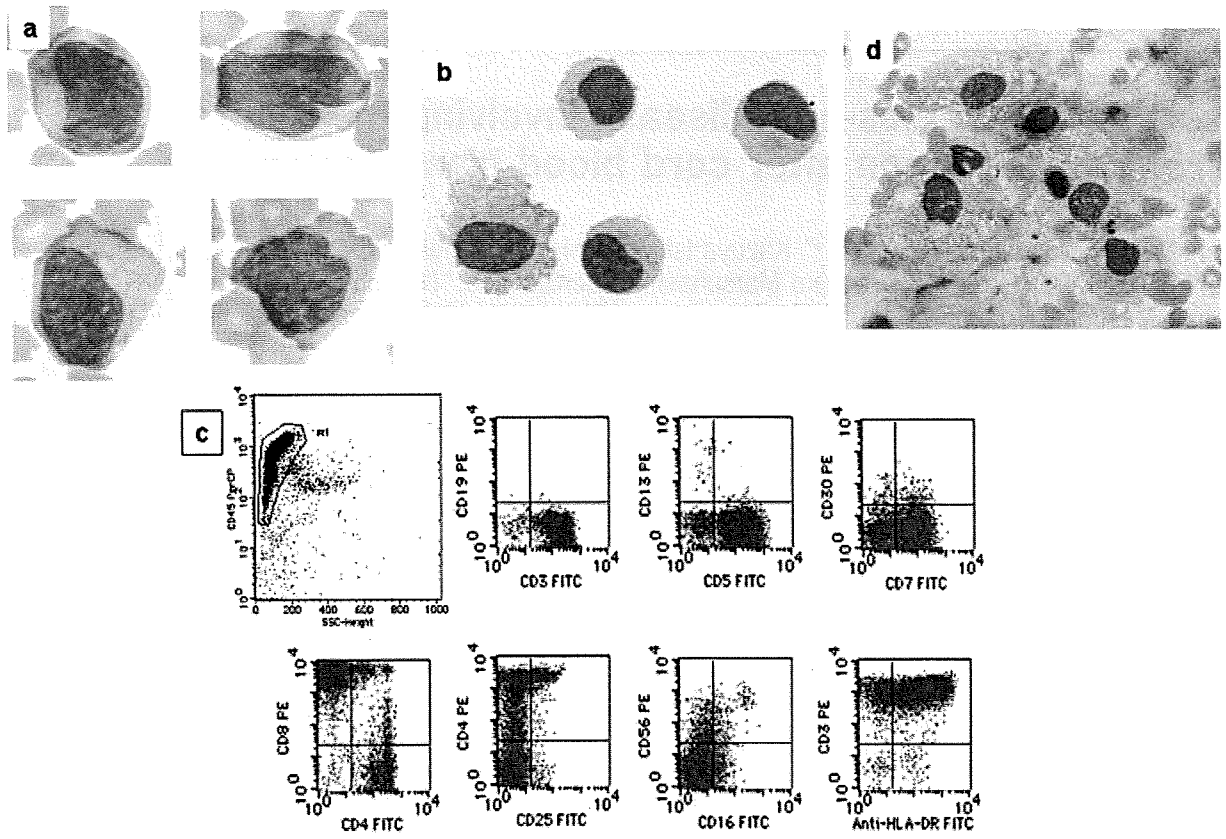

Figure 2. Activated lymphocytes in peripheral blood (a) and in cerebrospinal fluid (b) on Day 10 post-transplant. Flow cytometry of peripheral blood on Day 10 post-transplant (c). Activated macrophages in bone marrow on Day 17 post-transplant (d). May-Giemsa staining ×1000 (a, b) ×400 (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(*0404*)/DRB1*09 (0901), respectively, in the recipient, and *A26* (*2601*)/A31 (3101), B35 (3501)/*B51* (*5101*), and DRB1*04 (*0401*)/DRB1*09 (0901), respectively, in the donor. The graft contained 2.4 × 10^7/kg total nucleated cells and 0.92 × 10^5/kg $CD34^+$ cells. The pretransplant conditioning regimen consisted of fludarabine (25 mg/m^2/day) for 5 days, melphalan (40 mg/m^2/day) for 2 days, and 4 Gy of total body irradiation. Tacrolimus alone was administered as GVHD prophylaxis. Granulocyte colony-stimulating factor was started from Day 1. Pretransplant viral serology was positive for HSV, HVZ, CMV, and EBV, and negative for HIV and HTLV-1. She received 600 mg/day of oral acyclovir, 400 mg/day of oral tosufloxacin, 200 mg/day of oral itraconazole, and trimethoprim-sulfamethoxazole (160 mg/day of the trimethoprim component) as for antimicrobial prophylaxis. Figure 1 shows her entire clinical course following RI-CBT. On Day 7 post-transplant, a high fever, slight skin eruption, and moderate diarrhea developed with a slightly increased WBC count (from 10 μl^{-1} on Day 6 to 30 μl^{-1} on Day 7). Her WBC count rapidly increased on Day 10 to 1,700 μl^{-1} and comprised 90% lymphocytes (Fig. 2a). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels increased to 715 and 359 IU/l, respectively, and serum lactate dehydrogenase (LDH) levels increased to 1,101 IU/l. The patient suddenly lost consciousness along with generalized convulsions on the same day and required mechanical ventilation. Cerebrospinal fluid (CSF) analysis revealed an extremely elevated protein level of 675 mg/dl (normal range: 15–40 mg/dl) and pleocytosis (68 cells/μl), consisting mainly of lymphocytes (98%) (Fig. 2b). Magnetic resonance imaging scans of the brain revealed no specific abnormalities typically seen in cerebrovascular disorders, tacrolimus encephalopathy, thrombotic microangiopathy, or other CNS complications, and schistocytes were undetectable in the PB. Flow cytometry revealed that the excessive lymphocytes in both PB and CSF comprised polyclonal mature T-lymphocytes expressing CD3, CD4, CD5, CD8, and HLA-DR. The expression of CD4 and CD8 was variable, in which $CD4^-CD8^+$, $CD4^+CD8^-$, and $CD4^+CD8^+$ cells accounted for 65, 25, and 9%, respectively, of the cells in PB, and 38, 56, and 6%, respectively, of those in the CSF (Fig. 2c). Y chromosome-based fluorescence in situ hybridization analysis showed that most of these cells were derived from the donor (98.8% in PB and 96.8% in CSF). Furthermore, 98% of BM cells obtained on Day 10 were also donor derived. Routine cultures of PB and CSF for bacteria and fungi were negative. Analyses by real-time polymerase chain reactions were negative for HSV-1, HHV-6, VZV, CMV, and EBV in PB and CSF, and, for HSV-2, HSV-7, HSV-8, JCV, BKV, ADV, Parvovirus B19, HBV, and HCV in PB. Southern blotting of cells from the PB showed that the genes for both T-cell receptor Cβ1 and Jδ1 were in germ-line configuration, and EBV genome clonality was undetectable. Methylprednisolone (500 mg/day) was administered for 3 days, and acyclovir was switched to foscarnet, considering the possibility of acute GVHD and viral infection insensitive to acyclovir. After the initiation of these therapies, the numbers of lymphocytes in PB and CSF gradually decreased, and her clinical symptoms and laboratory data improved, so methylprednisolone was carefully tapered. However, high fever, diarrhea, and CNS symptoms recurred around Day 17, and then pancytopenia and cholestatic liver damage rapidly progressed. On Day 17, BM aspiration revealed an increase of activated macrophages (35%) with massive hemophagocytosis (Fig. 2d). The chimeric status of the BM cells revealed sustained donor cell dominance (96.8%), indicating that the hematopoietic cells and macrophages in the BM were both donor derived. Despite the administration of etoposide (50 mg/m^2) to control the hemophagocytosis, pancytopenia and cholestatic liver damage progressed and the patient died of bacterial sepsis 32 days after transplantation. An autopsy was not performed.

Polyclonal T-cell proliferation is the principal mechanism of the antigen-specific immune response that generally occurs upon infection and/or inflammation. GVHD is also primarily a T-cell-mediated event, and the subsequent expansion of donor T-cell clones-recognizing antigens causes tissue damage either directly through T-cells encountering recipient MHC-bearing cells in target tissues or indirectly through cytokine production [11].

We previously reported higher incidence of immune-mediated complications, such as PIR, characterized by high-grade fever, skin eruption, diarrhea, jaundice, and body weight gain developing before engraftment, and HPS early after RI-CBT [8,9]. Despite the known immunological naïvety of CB cells, the exceptionally high incidence of PIR and HPS suggests that the properties of CB cells are unique and distinctly different from adult donor cells.

The most striking features of our patient were the remarkable polyclonal T-cell proliferation both in PB and CSF, followed by sudden generalized convulsions and loss of consciousness. As the coexistent CNS and systemic GVHD-like symptoms, proliferating donor-derived polyclonal T-cells in the CSF and PB, and microorganisms or other factors that might be responsible for these symptoms or T-cell proliferation were undetectable. We therefore postulated that an alloimmune reaction of the CB graft against the CNS caused the CNS symptoms in our patient.

The concept that CNS could be a target of GVHD is controversial. Some case reports support the possibility of CNS-GVHD [1,2]. All of the patients in these reports were diagnosed with CNS-GVHD only when they responded to immunosuppressive therapy and had histologically and immunophenotypically documented perivascular T-cell infiltration without evidence of other CNS diseases with overlapping features. However, uniform diagnostic approaches or criteria have not been established. Most of the reported CNS-GVHD was diagnosed at the time of chronic GVHD development. Powles et al. [12] reported that convulsions, possibly due to cerebral edema, could develop as a manifestation of severe acute GVHD after haploidentical transplantation. This could explain the events in our patient, although information about the CSF, the presence or absence of T-cell proliferation, or detectable infectious organisms was not provided in the literature. We reported that early CNS complications are more frequent after RI-CBT than after transplantation with other stem cell sources and that hypercytokinemia associated with PIR could influence the development of CNS complications [13]. T-cell proliferation in CSF along with the severe systemic symptoms in our patient might have resulted from a type of hypercytokinemia that is unique to RI-CBT.

Moreover, severe HPS developed around 10 days after T-cell proliferation, and the activated macrophages in the BM were donor derived. Although HPS is a rare complication following allo-SCT, some investigators have suggested that a severe alloimmune response could result in HPS after PB transplantation [14,15]. Furthermore, we recently reported that the incidence of HPS following RI-CBT is higher than was previously reported and that HPS is a significant risk factor for engraftment failure [9]. Hypercytokinemia associated with engrafted T-cell proliferation may have played an important role in donor-derived macrophage activation and in the development of HPS in our patient.

In conclusion, we described a patient who developed sudden generalized convulsions and lost consciousness at the same time as polyclonal T-cell proliferation soon after RI-CBT. The findings of extensive investigations indicated that the CNS can be a target of GVHD. Further accumulation of clinical and laboratory data with the awareness of this devastating complication soon after RI-CBT is warranted to precisely understand the underlying basic mechanisms and to develop optimal intervention strategies.

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Treatment with hydroxyurea in a patient compound heterozygote for a high oxygen affinity hemoglobin and β-thalassemia minor

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Compound heterozygotes for β-thalassemia and high oxygen affinity hemoglobin (Hb) have been documented, but experience in the management of such rare cases is minimal. Although hydroxyurea (HU) has never been used in a heterozygote with high oxygen affinity Hb and β-thalassemia, we hypothesized that it would decrease erythrocytosis through a lowered production of abnormal cells and increase of P_{50} by induction of fetal hemoglobin (HbF). We present the case of a patient with compound high oxygen affinity Hb mutation with β-thalassemia. PCR analysis revealed combined Hb Regina and IVS1-110 G/A mutations. Treatment with HU caused a decrease in Ht (61.1% to 38.6%) and erythrocyte volume (74.87 mL/kg to 40.65 mL/kg), as well as an increase in P_{50} (6 mmHg to 10 mmHg) and HbF level (3.6% to

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High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults

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Summary

Umbilical cord blood transplantation (CBT) is widely accepted, but one critical issue for adult patients is a low engraftment rate, of which one cause is haemophagocytic syndrome (HPS). We aimed to identify the contribution of HPS to engraftment failure after CBT, following preparative regimens containing fludarabine phosphate, in 119 patients (median age, 55 years; range; 17–69 years) with haematological diseases. Graft-*versus*-host disease prophylaxis comprised continuous infusion of a calcineurin inhibitor with or without mycophenolate mofetil. Of the 119 patients, 20 developed HPS within a median of 15 d (cumulative incidence; 16·8%) and 17 of them did so before engraftment. Donor-dominant chimaerism was confirmed in 16 of 18 evaluable patients with HPS. Despite aggressive interventions including corticosteroid, ciclosporin, high-dose immunoglobulin and/or etoposide, engraftment failed in 14 of 18 patients. Of these 14 patients, four received second rescue transplantation and all resulted in successful engraftment. Overall survival rates significantly differed between patients with and without HPS (15·0% vs. 35·4%; $P < 0·01$). Univariate and multivariate analysis identified having fewer infused $CD34^+$ cells as a significant risk factor for the development of HPS ($P = 0·01$ and 0·006, respectively). We concluded that engraftment failure closely correlated with HPS in our cohort, which negatively impacted overall survival after CBT.

Keywords: cord blood transplantation, reduced-intensity chemotherapy, haemophagocytic syndrome, engraftment failure.

Umbilical cord blood transplantation (CBT) is an alternative allogeneic haematopoietic stem cell transplantation (HSCT) strategy for patients with haematological diseases who do not have a matched related or unrelated donor and who need urgent transplantation. The value of CBT using myeloablative preparative regimens has already been confirmed among paediatric and adult patients (Laughlin *et al*, 2004; Rocha *et al*, 2004; Takahashi *et al*, 2004). However, conventional myeloablative preparative regimens are associated with significant morbidity and mortality, particularly in older patients or in those who have experienced extensive prior therapy or organ dysfunction associated with transplantation-related mortality. Various reduced-intensity preparative regimens that have been applied to such patients by several groups, including the authors of the present study, have proven feasible (Barker *et al*, 2003, 2005; Chao *et al*, 2004; Jacobsohn *et al*, 2004; Miyakoshi *et al*, 2004, 2007; Yuji *et al*, 2005; Misawa *et al*, 2006; Ballen *et al*, 2007; Brunstein *et al*, 2007; Komatsu *et al*, 2007; Uchida *et al*, 2008).

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Engraftment failure is a critical problem that can arise after CBT. The limited doses of infused total nucleated and $CD34^+$ cells contained in umbilical cord blood are thought to influence the rate and kinetics of haematopoietic recovery. In order to overcome engraftment failure, various kinds of strategies, such as multiple unit or *ex-vivo* expanded CBT, and co-infusion of peripheral blood stem cells, have been employed (Shpall *et al*, 2002; Fernandez *et al*, 2003; Barker *et al*, 2005).

Several recent reports have described HPS that arose after autologous and allogeneic HSCT followed by engraftment failure (Sokal *et al*, 1987; Levy *et al*, 1990; Reardon *et al*, 1991; Nagafuji *et al*, 1998; Sato *et al*, 1998; Takahashi *et al*, 1998; Ishikawa *et al*, 2000; Fukuno *et al*, 2001; Abe *et al*, 2002; Tanaka *et al*, 2004, 2007; Kishi *et al*, 2005a; Boelens *et al*, 2006; Ostronoff *et al*, 2006; Ishida *et al*, 2007; Koyama *et al*, 2007). In a reduced-intensity conditioned CBT (RI-CBT) setting, we experienced one patient who failed to achieve engraftment due to HPS following HSCT (HSCT–HPS). Following this case, several similar cases were observed in our institute. We postulated that HPS could play a critical role in engraftment failure after CBT. This report describes the characteristics of 20 patients with HSCT–HPS among 119 who underwent CBT.

Materials and methods

Patients

The study population consisted of 119 adult patients with haematological diseases, who underwent CBT as the first allogeneic HSCT at Toranomon Hospital, Tokyo, Japan between January 2004 and December 2006. All the patients were incurable using conventional approaches, lacked a human leucocyte antigen (HLA)-identical sibling or a suitable unrelated donor from Japan Marrow Donor Program. Most of the patients were considered inappropriate for conventional myeloablative allogeneic HSCT due to being >50 years and/or having organ dysfunction (cardiac ejection fraction <50%, forced expiratory volume 1·0 s % <80%, or serum creatinine > 1·5× upper limit of normal range). Written informed consent was provided by all patients in accordance with the Declaration of Hersinki. The Institutional Review Board of Toranomon Hospital approved the study.

Transplantation procedures

Cord blood units that were serologically matched for ≥4 of six HLA antigens and which contained at least $1{\cdot}8 \times 10^7$ nucleated cells/kg of recipient body weight before freezing were obtained from the cord blood bank at the Japan Cord Blood Bank Network (Nishihira *et al*, 2003). The units were not depleted of T lymphocytes. All patients received purine analogue-based preparative regimens comprising fludarabine phosphate (125–180 mg/m^2), melphalan (80–140 mg/m^2) or busulfan (BU; 8–16 mg/kg) and 0–8 Gy of total body irradiation (TBI), as decided by the treating physician. Graft-versus-host disease (GVHD) prophylaxis comprised a continuous intravenous infusion of either 0·03 mg/kg of tacrolimus (TAC) or 3 mg/kg of ciclosporin (CsA), starting on day-1, except eight patients who received 2 g/d of mycophenolate mofetil (MMF) starting on day-1 in addition to TAC.

Supportive cares

All the patients were treated in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis. Fluoroquinolone and azole and acyclovir were administered to prevent bacterial, fungal and herpes virus infection, respectively. Neutropenic fever was managed according to the guidelines (Hughes *et al*, 2002). Cytomegalovirus pp65 antigenaemia was monitored weekly and preemptive therapy with foscarnet was initiated in the event of a positive result (Matsumura *et al*, 2007; Narimatsu *et al*, 2007a). Haemoglobin and platelet counts were maintained at >70 g/l and 10×10^9/l, respectively. Granulocyte colony-stimulating factor was administered intravenously from day 1 until neutrophil recovery became durable.

Assessment of engraftment, chimaerism, pre-engraftment immune reactions, disease risk and survival

Engraftment was defined as the first of three consecutive days in which white blood cell counts were $>1{\cdot}0 \times 10^9$/l or the absolute neutrophil counts were $>0{\cdot}5 \times 10^9$/l. When the above definition was not met by day 28 without subsequent neutrophil recovery, the patient was considered to have primary engraftment failure. Delayed engraftment was defined as neutrophil engraftment after day 29. Secondary engraftment failure was defined as a decrease in the neutrophil count to $<0{\cdot}5 \times 10^9$/l for three consecutive days after successful engraftment. The date of platelet recovery was defined as the first of seven consecutive days during which the non-transfused platelet count was at least 20×10^9/l.

Chimaerism was assessed using fluorescent *in situ* hybridization (FISH) in sex-mismatched donor–recipient pairs. In sex-matched pairs, chimaerism was assessed using the polymerase chain reaction for variable numbers of tandem repeats with donor cells detected at 10% sensitivity (Thiede *et al*, 1999).

Pre-engraftment immune reactions (PIR) were diagnosed when febrile patients (body temperature ≥38·0°C) developed skin eruption, diarrhoea, jaundice (serum total bilirubin >34·2 μmol/l) or body weight gain of >10% of baseline, with no direct evidence of infection or adverse effects of medication, developing ≥6 d before engraftment (Kishi *et al*, 2005b).

Patients with acute myeloid leukaemia in first or second complete remission (CR) at the time of transplant, with acute lymphoblastic leukaemia in first or second CR, with chronic myeloid leukaemia in the chronic phase, with refractory