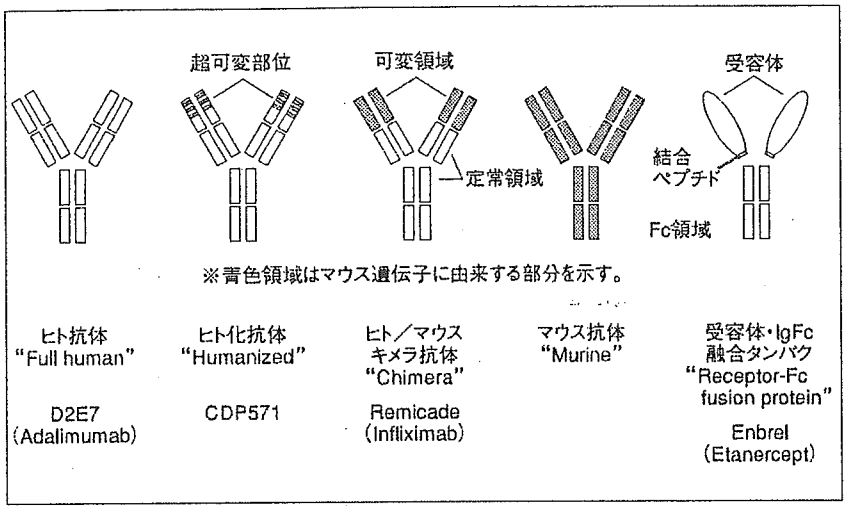

図2　各種TNF-α抗体の分子構造（文献4より改変引用）

	プラセボ群	infliximab投与群		
		5mg/kg	10mg/kg	20mg/kg
組み入れ患者数	25	27	28	28
CDAI				
治療前	288±54	312±56	318±59	307±50
第2週	272±75	182±79*	238±92*	217±90*
第4週	271±82	166±76*	226±115*	211±107*
IBDQ				
治療前	128±29	122±29	116±23	118±28
第4週	133±28	168±36*	146±41*	149±35*
CRP (mg/L)				
治療前	12.8±13.9	22.1±23.6	23.2±34.2	22.4±23.9
第2週	16.4±16.9	4.2±3.0*	6.7±7.3*	8.7±13.8*
第4週	14.8±18.6	5.7±9.3*	12.1±18.6*	6.9±11.6*

CDAI；Crohn's disease activity index, IBDQ；Inflammatry Bowel Disease Questionnaire
*；$p<0.05$

表1　クローン病の緩解導入に対するInfliximabの効果（文献8より改変引用）

ジの細胞表面上の膜結合型TNF-αに作用して、活性型マクロファージをアポトーシスに陥らせることにより、Th1系のサイトカイン産生を抑制する、などのメカニズムが考えられている。Infliximabの構造は、75％がヒト、25％はマウス由来で、humicadeが95％がヒト、5％がマウス由来で構成されている。実際にこの両抗体をヒトに投与すると、ヒト抗キメラ抗体（Human Antichimeric Antibody；HACAもしくはAntibodies to Infliximab；ATI）ができることが報告されたため、この解決策として100％がヒト由来であるetanerceptの導入が期待された。しかし米国での大規模なRCTで、その治療効果について否定的な成績が出たことより、現時点ではinfliximabが臨床の場で主として用いられている。しかしATIの問題もあり将来的にはヒト型抗TNF-αモノクローナル抗体（adalimumab）に期待が寄せられている。以下にクローン病に対する抗TNF-α抗体療法の治療成績につき概説する。

1．クローン病への応用

1993年オランダにてステロイドやその他の薬剤に抵抗性の大腸型クローン病の12歳の少女に抗TNF-αマウス・ヒトキメラ型モノクローナル抗体infliximabが投与されたのがクローン病に使用された最初の症例であり、その劇的な効果が世界で初めて報告された[5]。

2．緩解導入効果

この報告に続き、まず緩解導入を目的に、1995年にVan Dullemenら[6]は、ステロイド抵抗性の活動性クローン病の患者10人の内8人に10mg/kg、残りの2人に20mg/kgのinfliximabを1回静脈内投与するpilot studyを行った。その結果なんら副作用を認めることなく投与後4週以内に8例にCDAI（Crohn's disease activity index）の正常化（CDAI150以下）を認め、さらにその8例は4週後の内視鏡検査にてほとんどの潰瘍性病変の治癒が認められ、単回投与での臨床的効果は約4ヵ月間持続したと報告された。

最初のdose-responnse studyは1996年McCabeRPら[7]によって、infliximabの投与量を1、5、10、20mg/kgの群に分けた振り分け試験にて行われた。その結果1mg/kg投与では効果の持続が有意に短く、これをもとに

次のPhase Ⅱb/Ⅲtrialの投与量が決定された。

1997年Targanら[8]は、Phase Ⅱb/Ⅲtrialとして108例の中等症以上のクローン病の患者に現行治療(ステロイドなど)を継続したまま二重盲検法無作為化比較試験を行った。Infliximabを5、10、20mg/kg群に分けて1回静脈内投与しプラセボ群と比較したところ、投与後4週の時点で改善が認められた例は、プラセボ群で17%であったのに対し、5mgの群で81%、10mgの群で50%、20mgの群では64%に認められプラセボ群と比較し有意な改善を認めた。またCDAIが150以下の緩解に至った例は、infliximab投与群の33%に認められ、プラセボ群の4%に比べ有意に優れていた(表1)。

さらに改善のみられなかった群にinfliximab(10mg/kg)の再静脈内投与を施行したが、1回目にinfliximabを投与し効果が得られなかった群においては、改善率34%、緩解率17%と初回プラセボ投与群の成績と有意差を認めず、infliximabに抵抗性の群の存在が示唆された。また投与量に関しては5mg/kgと20mg/kgとの間に有意な差はなく、今後5mg/kgが投与量として適当ではないかとしている。

この結果infliximabの緩解導入効果に関する有用性は、ほぼ確定された。Infliximabの緩解導入効果の有用性は広く認知されるに至り米国においては、1998年に米国食品医薬品局(FDA)の認可を受け市販されている。またこれらの結果よりクローン病においてTNF-αが病因の中心的役割をなすという仮説がより強く裏付けられたかたちとなった。しかし単回投与では、その効果は比較的長期に認められるものの、そのほとんどは再燃が避けられないことも明らかとなった。

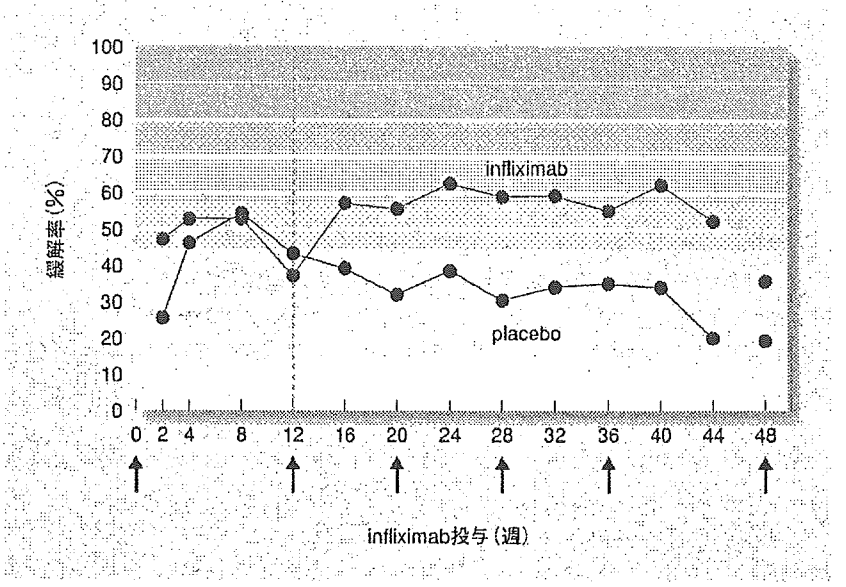

図3 Infliximab連続投与によるクローン病の効果(文献10より改変引用)

3. 反復投与による緩解維持効果

緩解維持を目的とした反復投与の安全性と効果についてRutgeertsら[9]がTarganら[8]のPhase Ⅱb/Ⅲrialの続きとして報告している。彼らはPhase Ⅱb/Ⅲtrialでinfliximabの投与にて有効であったクローン病患者73例を対象として、infliximabを8週ごとに10mg/kgを4回繰り返し静注し、投与終了後8週の段階で最終的な評価を行った。その結果、緩解維持率はinfliximab群で52.9%とプラセボ群の20%に比し有意に優れていた(図3)。また臨床効果維持率でもinfliximab群では62%であったのに対し、プラセボ群では37%であった。そしてinfliximab投与群のうち、6-MPやazathioprineなどの免疫抑制剤を同時投与(治験組み込み以前から)されたグループの臨床効果維持率は75%であり、免疫抑制剤の同時投与のないグループの50%に比べて良好な傾向を認めたとしている。

維持療法の有効性については、さらに大規模な試験(A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-Term Treatment Regimen Ⅰ:ACCENT Ⅰ)が実施された。ACCENT Ⅰ[10]では中等度～重度のクローン病患者573例(CDAI220以上)にinfliximab 5mg/kgを単回投与し、2週目に有効性が認められた患者を、プラセボを2および6週目とその後8週毎に46週目まで投与する群(Group Ⅰ)、2および6週目とその後8週毎にinfliximab 5mg/kgを46週目まで投与する群(Group Ⅱ)、2および6週目にinfliximab 5mg/kgを投与し、その後8週毎に46週目までinfliximab 10mg/kgを投与する群(Group Ⅲ)に無作為に振り分けた。有効性評価対象例335例の、54週後の有効率(CDAIが25%以上および70点以上低下した場合を有効と定義)および緩解

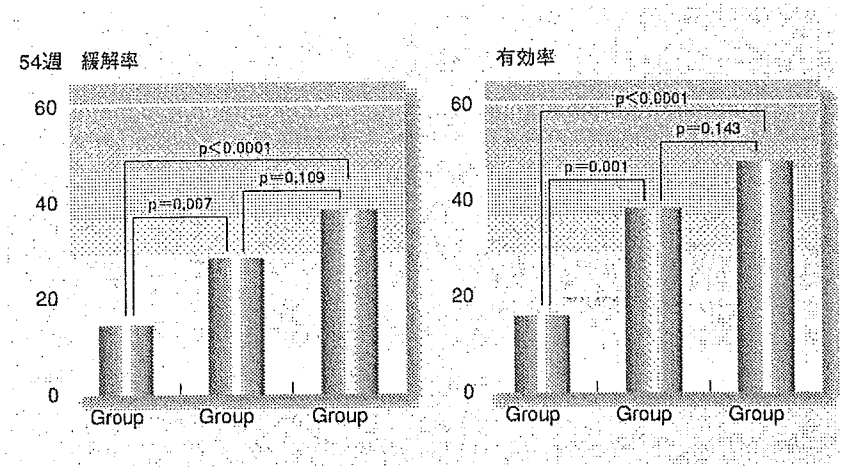

図4 Infliximab維持療法における有効率および緩解率(文献10より改変引用)

	プラセボ群	infliximab 5mg/kg群	infliximab 10mg/kg群
組み入れ患者数	31例	31例	32例
脱落患者数	4例	1例	1例
第一次目標到達率(排膿瘻孔50%以上減少)	26%	68%	56%
完全緩解率(排膿瘻孔の消失)	13%	55%	38%
CDAI(第18週)中央値	160	104	123
PDAI(第18週)中央値	7.0	4.0	5.0
infliximabの副反応(重症)	—	1例	4例

表2 クローン病の瘻孔に対するinfliximabの効果(文献12より改変引用)

率(CDAI150点未満を緩解と定義)は、infliximab 5mg/kgまたは10mg/kg投与群(Group Ⅱ、Ⅲ)の方がプラセボ投与(Group Ⅰ)に比べて有意に高かった(図4)。さらにACCENT Ⅰの別の解析からは、導入療法として単回投与よりも3回投与するほうが効果が高いことや、infliximabでの維持療法がステロイドの減量や中止に有効であることも判明している。また最近の報告では症状の有無によらず、8週おきに計画的に投与するほうが、症状再燃にあわせてepisodicに投与するよりもATIの産生も低く有効性が高いことが判明している[11]。

4. 瘻孔治療効果

クローン病の合併症としての瘻孔治療効果に関しても、優れた成績が1999年Presentら[13]により報告されている。その報告によれば、クローン病の合併症として3ヵ月以上にわたって排膿を有する腹壁瘻もしくは肛門周囲瘻を有する患者94例を対象としてプラセボおよびinfliximabを5mg/kg、10mg/kg投与する群に振り分け、0、2、6週の3回静脈注射とし、排膿を有する瘻孔の50%以上の減少で検討すると5mg/kg群で68%、10mg/kg群で38%であったのに対しプラセボ群では26%であった。さらにすべての瘻孔閉鎖率では5mg/kg群で55%、10mg/kg群で38%であったのに対しプラセボ群では13%であり有意差をもって効果を認めた(表2)。また瘻孔閉鎖期間の平均は約3ヵ月であった。瘻孔性クローン病患者におけるインフリキシマブの維持療法を検討したACCENT Ⅱ試験では、その維持効果が公表されている

5. 副作用

クローン病、関節リウマチの治験におけるinfliximabを投与された患者についてまとめると、infliximab投与に伴う一般的な副反応としては、頭痛、嘔気、上気道感染を76%に認めプラセボ投与群の57%と比べやや頻度が高い傾向があった[13]。Infliximabの投与時および投与後2時間以内の急性期に何らかの副反応が起こる率は、初回投与時は、わずか7%であるのに対し、2回目の投与では10%に認められた。やや頻度が高まるのは感作されることによるものと考えられ、それを裏付けるようにヒト抗キメラ抗体(Human Antichimeric Antibody;HACAもしくはAntibodies to Infliximab;ATI)陽性の群では投与早期の副反応を36%に認めるのに対し、HACA陰性の群では11%であった。しかしそのほとんどは非特異的かつ投与を中止するほどのものではなかった。またATI(Antibodies to Infliximab)に関しては、低値ではあるものの患者の13%に出現を認め、投与の継続に伴ってその出現頻度は高まり、反復投与では実に61%に出現を認め、臨床効果の減弱や効果

持続期間の短縮などが報告されている[14]。最近infliximab投与前にhydrocortisoneを点滴投与することによってATIのlevelを有意に低下できることが報告され、投与方法の改善などが期待されている[15]。そのほか、患者の9%に二本鎖DNAに対する抗体の出現を認めたが、その他のSLEに特徴的な抗核抗体の出現や低補体血症は認めず、SLEの発症は認められなかった[14]。これまでの結果においては、infliximabは重大な副作用がほとんどなく大変有用と考えられていたが、最近infliximabと結核発症の関連が報告され注意を促されている[16]。さらに心不全を悪化させる可能性や治癒過程でのイレウスの発症の報告、多発性硬化症の発生や悪化の報告などもあり、長期投与に伴う抗キメラ抗体の出現や悪性疾患の発生、感染症などを含め安全性に関しては、今後さらなるデータの蓄積と検討が不可欠と考えられる。

おわりに

TNF-α阻害によるクローン病の治療は米国ではすでに一般的な治療法となっている。わが国でもinfliximabのクローン病への保健適応が認可され、その効果が期待されている。今後は、医療経済や栄養療法など他の治療法とのかかわりを含めinfliximabの適応とその位置付けをより明確にしていくことが大切と考えられる。Infliximabはクローン病の慢性炎症機序の中心にせまる治療法と考えられるが、いまだ不明な点も多く、病因の解明と根本的な治療法の開発が待たれる。

文献

1) Podolsky DK. Inflammatory bowel disease. N Engl J Med 1990; 324: 928.
2) Pizarro TT, et al. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. J Immunol 1999; 162: 6829.
3) Kanai T, et al. IL-18 is a potent proliferative factor for intestinal mucosal lymphocytes in Crohn's disease. Gastroenterology1 2000; 19: 1514.
4) Sandborn W, et al. Antitumor necrosis factor therapy for inflammatory bowel disease: review of agents, pharmacology, clinical results, and safety. Inflammatory Bowel Disease 1999; 5: 119.
5) Derkx B, et al. Tumor-necrosis factor antibody treatment in Crohn's disease. Lancet 1993; 342: 173.
6) Van Dullemen HM, et al. Treatment of Crohn's disease with antitumor necrosis factor chimeric monoclonal antibody(cA2). Gastroenterorogy 1995; 109: 129.
7) McCabe RP, et al. A multicenter trial of cA2 anti-TNF chimeric monoclonal antibody in patient with active Crohn's disease. Gastroenterorogy 1996; 110: A962.
8) Targan SR, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor α for Crohn's disease. N Engl J Med 1997; 337: 1029.
9) Rutgeert P, et al. Efficacy and safety of retreatment with anti-tumor necrosis factor antibody (Infliximab) to maintain remission in Crohn's disease. Gastroenterorogy 1999; 117: 761.
10) Rutgeert P, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomized trial. Lancet 2002; 359: 1541.
11) Ghosh S. Maintenance therapy versus episodic therapy with infliximab for Crohn's disease. Nature Clinical Practice Gastroenterology & Hepatplogy 2004; 1: 80-1.
12) Presents P, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med 1999; 340: 1398.
13) Remicade(infliximab). Prescribing Information. Physician's desk reference 1999.
14) Baert F, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003; 348(7): 601-8.
15) Farrell RJ, et al. Intravenous hydrocortisone premedication reduces antibodies to infliximab in Crohn's disease: a randomized controlled trial. Gastroenterology 2003; 124(4): 917-24.
16) Keane J, et al. Tuberculosis associated with infliximab, a tumor necrosis factor α-neutralization agent. N Engl J Med 2001; 345: 1098.

【炎症性腸疾患の病態と粘膜免疫ー最近の動向ー】

Current Topics in Inflammatory Bowel Disease and Mucosal Immunology

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Key words
inflammatory bowel disease, GALT,
innate immunity, cytokine

要　約

腸管は消化吸収・排泄のみでなく常に外来抗原や腸内細菌にさらされながらも複雑な免疫システムを有することによって恒常性を維持している特殊な臓器である。炎症性腸疾患は大きく潰瘍性大腸炎 (UC)とCrohn病(CD)に分類される。現在我が国でも患者数は増加傾向にあり特定疾患登録患者数でみてもUCで7万人を超え，CDも2万人を超えた。いずれも腸管の慢性持続性炎症が特徴で原因究明には至っていない。しかしながら，腸管局所の免疫制御異常が病態に関与しているのは間違いなく，最近では腸内細菌との関係など自然免疫機構が発症メカニズムに関与しているのではないかと注目されている。またいくつかの疾患関連遺伝子も報告され，治療においても抗サイトカイン抗体などの分子標的治療が開発され実用化されつつある。

1．特殊な免疫装置としての腸管 (Gut Associated Lymphoid Tissue, GALT)

消化管は消化，吸収，排泄を司るだけでなく，複雑なGut Associated Lymphoid Tissue, GALTと呼ばれる免疫担当装置を形成している（図1）。さらに豊富な血管網や神経組織が迷路のように存在し，消化管ホルモンや神経ペプチドなどが生理機能を調節している。全消化管粘膜の表面積はテニスコート1.5面にも及び，そこに10^{14}個以上の腸内細菌が常在している。さらに，病原体や食餌抗原などの外来抗原に曝露される。つまり消化管は体内にありながら常に外界と接している特殊な臓器ということが言える。通常，免疫装置は外来からの侵入者に対して防御的に働き生体を守っている。例えば肺には肺胞マクロファージが存在し侵入してきた病原体に対して速やかに反応，処理し感染を防いでいる。ところが常に食餌抗原や腸内細菌にさらされている腸管粘膜では過剰な免疫応答は好ましくない。むしろ恒常性を保つための抑制的機構が存在すると考えられる。腸管上皮は構造的に微生物や抗原の侵入を防いでおり，さらにムチン，trefoil factorや抗菌ペプチドなどの分泌蛋白を産生し粘膜表面を守っている。しかし，これらの上皮細胞による防御に留まらず，抑制性の免疫学的機序が存在していると思われる。実際，大腸粘膜をポリペクトミーで切除し粘膜を破壊しても我々は腸炎を発症することはない。また一過性に食あたりや感染性腸炎にかかることはあってもほとんどの場合は慢性化せず自然に沈静化する。この腸管の低反応性を説明する機序として腸管の自然免疫を司るマクロファージや樹状細胞の特殊性が明らかとなってきた。我々は，マウスの腸管マクロファージは細菌刺激に対してTNF-αやIL-6などの急性反応性のサイトカインは産生するものの，決してTh1型免疫応答を引き起こすIL-12やIL-23を産生せずむしろ抑制性サイトカインであるIL-10を高産生することを明らかにした。さらに自然発症腸炎モデルマウスであるIL-10 KOマウスではこの腸管マクロファージの抑制性の機能が喪失しており細菌刺激によりTh1反応が誘導されることがわかった[1]。

またSmythiesらはヒトの腸管マクロファージは細菌に対し貪食能を保ったままサイトカイン産生に関しては低応答となっていることを報告している[2]。このように消化管は非常に複雑で精密な仕組みでホ

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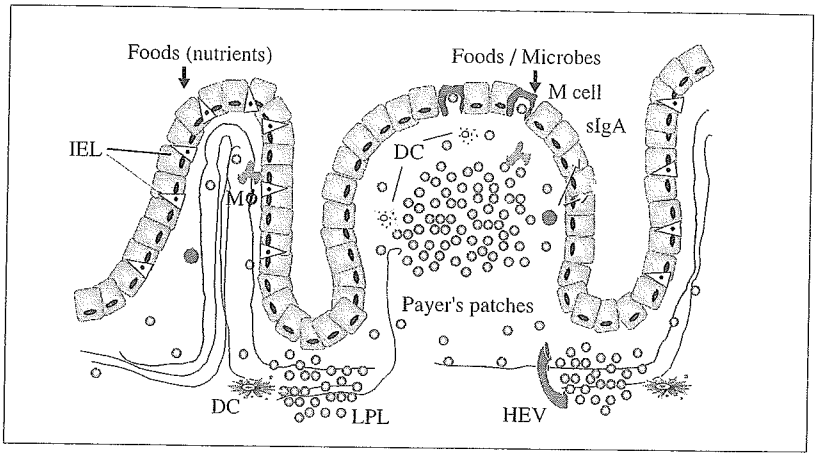

図1　GALT (gut associated lymphoid tissue)

メオスターシスを保っており，その破綻が炎症性腸疾患という特殊な慢性持続炎症を引き起こすものと考えられる。

2．炎症性腸疾患 (Inflammatory Bowel Disease, IBD)の病態

IBDは大きく潰瘍性大腸炎 (UC)とCrohn病 (CD)の二大疾患に区別される。これら二つの疾患は基本的に独立した疾患概念と考えられている。UCでは標的臓器は大腸のみであるのに対し，CDでは小腸，大腸を含めた全消化管が標的となり，しばしば瘻孔を形成する。内視鏡所見や病理像も大きく異なり，UCでは直腸からびまん性，連続性の病変分布を呈し炎症の主座は大腸粘膜表層にある。一方，CDの病変はskip lesionと呼ばれ非連続性で縦走潰瘍と呼ばれる特徴的な所見を呈する。病理所見では炎症は全層性で非乾酪性肉芽腫が特徴的な所見とされる。CDでは腸管局所の免疫応答はTh1型にシフトしていることが分かっており，エフェクター細胞は腸管局所のCD4陽性T細胞である。一方，UCでの局所の免疫応答の状態は報告により異なっておりTh2型にシフトしていると報告しているグループもあるがコンセンサスは得られていない。

IBDが純然たる自己免疫疾患であるというエビデンスはなく，むしろ遺伝素因，環境因子，免疫応答の異常が複雑に関与した多因子疾患であると考えられている（図2）。しかしながら，IBD患者ではしばしば腸管外に虹彩炎，皮膚症状，関節炎などの多彩な全身症状を合併することが知られており，また逆にSLEやシェーグレン症候群などの膠原病患者にIBD様の慢性腸炎が認められることもある。さらにUCでは大腸上皮やムチンに対する抗体産生が亢進していることやpANCA陽性例が多いことが知られている。一方CDではASCAs (anti-Saccharomyces cerevisiae antibodies)，Omp C, I2, flagellinに対する抗体CBir1など酵母や腸内細菌に対する抗体価の上昇が認められる[3,4]。これらのことから食餌や腸内細菌などの何らかの外来抗原に対する異常な免疫応答が背景にあり，時として腸管局所のみならず全身の免疫系が活性化し自己抗原とも交差反応することで自己免疫疾患に類似した症状を呈するのではないかとも考えられる。

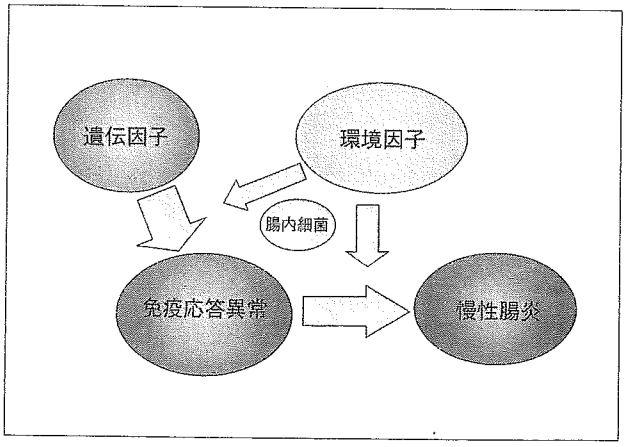

図2　IBDの病因

3．遺伝的素因

先に述べたように，以前より遺伝的素因が発症に重要な要素であることは疫学的研究から強く疑われていた。IBDの発症率は人種間で大きく異なっており，例えば居住地域が異なっていてもユダヤ系白人での発症が非ユダヤ系白人よりも高いことが報告されている。また家族内発症例が以前より報告されており，一卵性双生児間の発症率は二卵性双生児間の発症率より高いことが判明している。特に遺伝因子の関与はUCと比較しCDでより高いと考えられている。Linkage解析によりいくつかのIBDのsusceptibility locusが同定されていたが，ヒトゲノム解析が進むのと平行していくつかの疾患関連遺伝子が同定された。

1）NOD2/CARD15

NOD2/CARD15は第16染色体の*IBD1 locus*に位置するCD疾患関連遺伝子として2001年に二つのグループから報告された[5,6]。NOD2/CARD15は細菌の菌体構成成分であるペプチドグリカンのムラミルジペプチド (MDP) 部分を認識する細胞質内受容体であると考えられている。*In vitro*の研究ではNOD2/CARD15のC末端側に存在するleucine rich repeat (LRR)がMDPの結合部位でN末端側のCARDドメインを介して細胞内シグナルを活性化し最終的にNF-κBを活性化することがわかっている。白人のCD患者ではこのLRRおよびその近傍の3箇所の遺伝子多型が発症に相関を示している。これらの変異型ではMDPによるNF-κBの活性化能が低下していることが*in vitro*で示されている。同遺伝子のCDとの相関は人種によって大きく異なっており日本人を含めたアジア人種では相関が確認されていない[7]。また白人CD患者においても変異の保有率は約20～30%であり同遺伝子だけですべてのCDを説明することはできない。

さらにNOD2/CARD15がいかに病態に関与しているかはまだ解明されてはいないが，NOD2/CARD15は単球系細胞であるマクロファージや樹状細胞，腸管上皮細胞，特にPaneth細胞に発現しており，おそらくは侵入してきた細菌の認識と生体防御に働いていると考えられている。一方でMDP-NOD2のシグナルがペプチドグリカン-TLR2のシグナルに対し抑制的に働いている可能性も報告されている[8]。

2）OCTN (SLC22A4), OCTN2 (SLC22A5)

OCTN (organic cation/carnitine transporter)は第5染色体の*IBD 5 locus*に位置する疾患関連遺伝子として2004年に報告された[9]。しかしながら，日本人でのOCTNとCDとの相関は確認されていない。

3）DLG5

第10染色体に位置するDLG5のsingle nucleotide polymorphism (SNP)がIBDおよびCDと相関を示すことが報告された[10]。DLG5は腸管上皮細胞のintegrityの維持に働くscaffolding proteinをcodeしており，SNP多型によりこの機能が変化するのではないかと考えられているが病態への関与は解明されていない。またDLG5も日本人のCDやIBDでの相関は明らかとなっていない。

4）TNFSF15

これまで述べてきたようにNOD2/CARD15, OCTN, DLG5など疾患関連遺伝子として同定された報告はいずれも欧米を中心としたデータで，これまで日本人におけるCDでは相関が確認されていなかった。一方，Yamazakiらは第9染色体上に位置する炎症性サイトカインであるTNFSF 15 (Tumor necrosis factor family member 15)のSNPが日本人CD感受性と相関していることを報告した[11]。今後の機能解析の結果が待たれる。

4．免疫関与分子をターゲットとした新たな治療

近年の研究により詳細な病態が解明され，それに伴い特定の分子を標的とした治療の開発が進んでいる。

1）TNF-αをターゲットとした治療薬

キメラ型TNF-α抗体Infliximab（商品名：レミケード）は1998年に米国で承認され，我が国でも2002年から使用可能となっている。現在我が国では活動性CDに対する単回投与もしくは外瘻を有する症例に対する3回投与が認められている。すでに欧米ではACCENT Ⅰ試験の結果からレミケード継続投与による緩解維持治療の有効性が報告されており[12]，さらに早期からレミケードによる治療介入を行うべきであるというTop-down therapyを推奨する意見もあり今後CD治療の中心にレミケードが位置

してくるのは間違いない。またレミケードの有効性が報告されて以来，様々なTNF-α阻害剤が開発されており，その中でcertolizumab pegol (CDP870)はPEG化された抗TNF-α抗体のFab fragmentであり，Schreiberらはcertolizumab pegol (CDP870)のCDに対するrandomized, placebo-controlled studyの結果を報告している[13]。

2）抗IL-12抗体

完全ヒト型抗IL-12p40抗体の活動性CDに対する有効性が2004年に報告された[14]。IL-12はTh1型免疫応答を誘導するkeyサイトカインであり，CDの病態形成に重要な役割を果たしていると考えられている。さらに，活性型IL-12はp35とp40のヘテロダイマーからなっており，新たに発見されたTh1誘導サイトカインであるIL-23はp40とp19のヘテロダイマーの形をとる。したがってp40に対する抗体はこの両者を抑えている可能性がある。

3）抗IL-6抗体

ヒト型抗IL-6抗体 (MRA)の活動性CDに対するプラセボ対照多施設二重盲検試験が我が国で行われ有効性が報告された[15]。特にレミケードに見られる抗核抗体や中和抗体の出現が認められず今後が期待されている。

4）接着因子を標的とした治療薬

リンパ球の腸管へのホーミングには接着因子であるα4β7インテグリンが必要であるが，α4インテグリンに対するヒト化モノクローナル抗体であるNatalizumabのCDに対する大規模試験の結果が報告された[16]。Natalizumabは緩解導入についてはそれほど高い効果は期待できないものの有効例では維持療法としての効果は期待できる可能性がある。しかしながら投与については重篤な副作用である進行性多発性白質脳症 (progressive multifocal leukoencephalopathy, PML) の発症のリスクを考慮しなければならず汎用化には課題が残されている。

文　献

1) Kamada N, *et al.*: J Immunol. 175: 6900-6908, 2005.
2) Smythies L E, *et al.*: J Clin Invest. 115: 66-75, 2005.
3) Vernier G, *et al.*: Curr Gastroenterol Rep. 6: 482-487, 2004.
4) Targan S R, *et al.*: Gastroenterology. 128: 2020-2028, 2005.
5) Hugot J P, *et al.*: Nature. 411: 599-603, 2001.
6) Ogura Y, *et al.*: Nature. 411: 603-606, 2001.
7) Inoue N, *et al.*: Gastroenterology. 123: 86-91, 2002.
8) Watanabe T, *et al.*: Nat Immunol. 5: 800-808, 2004.
9) Peltekova V D, *et al.*: Nat Genet. 36: 471-475, 2004.
10) Stoll M, *et al.*: Nat Genet. 36: 476-480, 2004.
11) Yamazaki K, *et al.*: Hum Mol Genet. 14: 3499-3506, 2005.
12) Hanauer S B, *et al.*: Lancet. 359: 1541-1549, 2002.
13) Schreiber S, *et al.*: Gastroenterology. 129: 807-818, 2005.
14) Mannon P J, *et al.*: N Engl J Med. 351: 2069-2079, 2004.
15) Ito H, *et al.*: Gastroenterology. 126: 989-996, discussion 947, 2004.
16) Sandborn W J, *et al.*: N Engl J Med. 353: 1912-1925, 2005.

<細　胞ニュース>

第40回　日本成人病（生活習慣病）学会

日本成人病（生活習慣病）学会では下記の日程にて第40回学術集会を開催いたします。

会　期：2006年1月14日（土）～15日（日）
会　場：日本都市センター会館（東京 千代田区）
会　長：堀　正二（大阪大学大学院医学系研究科循環器内科学）
テーマ：個人に還元される医療のあり方を探る
　　ーテーラーメイド医療をめざしてー

ープログラムー

プレナリーセッション テーラーメイド医療の実現に向けて：心疾患とテーラーメイド医療，肥満治療成功の秘訣ーテーラーメイド型食事指導の実践，他／教育講演：生活習慣と腎移植成績，医療情報と個人情報保護／ランチョンセミナー：糖尿病発症早期の治療戦略，他／シンポジウム：メタボリックシンドロームの徹底解明ー職域から防ぐメタボリックシンドロームー，メタボリックシンドロームと高血圧ー職場高血圧を含めて，糖代謝異常とメタボリックシンドローム，他／特別講演：大腸癌治療ガイドラインの解説

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Bone Marrow Transplantation (2005) 36, 1109–1110

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Human herpesvirus 6 variant A infection with fever, skin rash, and liver dysfunction in a patient after unrelated cord blood transplantation

Bone Marrow Transplantation (2005) **36,** 1109–1110. doi:10.1038/sj.bmt.1705184; published online 10 October 2005

Human herpesvirus 6 (HHV-6) causes significant complications after hematopoietic stem cell transplantation (SCT) including skin rash, encephalitis, pneumonia, and bone marrow suppression.[1,2] HHV-6 is classified into two variants, A (HHV-6A) and B (HHV-6B). The two variants are thought to have different epidemiologies. Most symptomatic HHV-6 infections after SCT are caused by HHV-6B.[3–5] Although HHV-6A has occasionally been isolated after SCT,[6,7] the pathogenicity of HHV-6A remains largely uncertain. Here, we describe the first case of HHV-6A infection after umbilical cord blood transplantation (CBT).

In April 1999, a 49-year-old woman with overt leukemia from myelodysplastic syndrome underwent CBT from an HLA-one-antigen-mismatched donor. The grafts contained 2.1×10^7/kg total nucleated cells and 0.2×10^5/kg CD34-positive cells. The conditioning regimen included 12 Gy total body irradiation, 120 mg cyclophosphamide, and 12 g/m^2 cytarabine with the concomitant administration of recombinant human granulocyte colony-stimulating factor.[8] Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin and methotrexate. She received 1000 mg/day acyclovir orally from day −3 to day 35 to prevent herpes simplex virus reactivation. From day 12 to 17 after CBT, she developed fever higher than 38.0°C with the maximum temperature of 39.6°C on day 16 (Figure 1). On day 13, an erythematous skin rash occurred in the forearms and hands. On day 16, the serum aspartate aminotransferase (ALT) and alanine aminotransferase (AST) levels were mildly elevated to 36 and 56 IU/l, respectively (normal, within 30 IU/l). The maximum levels of AST and ALT were 36 IU/l on day 16 and 85 IU/l on day 18, respectively. No jaundice, body weight gain, edema, or pulmonary symptoms were observed. These symptoms resolved spontaneously without additional antiviral therapy. On day 26, myeloid engraftment with an absolute neutrophil count of more than 500/μl was achieved. No acute or chronic GVHD were observed. She did not developed cytomegalovirus infection. No bacterial or fungal infections were documented. By a real-time quantitative polymerase chain reaction (PCR) method,[9] the presence of HHV-6A DNA in serum was retrospectively examined. HHV-6A DNA was detected in serum samples on days 14, 21, and 28 after CBT with levels of 1×10^3, 6×10^3, and 2×10^2 copies/ml, respectively (lower limit of detection, 2×10^2 copies/ml). However, HHV-6A DNA was not detected in serum samples on days 7 or 35 after CBT. HHV-6B DNA was not detected in any serum samples obtained from day 7–35 after CBT. After a post-CBT follow-up of 72 months, she is currently alive without disease progression.

We have previously examined the incidences of HHV-6B infection in adults after CBT and bone marrow transplantation (BMT) by a real-time quantitative PCR method on

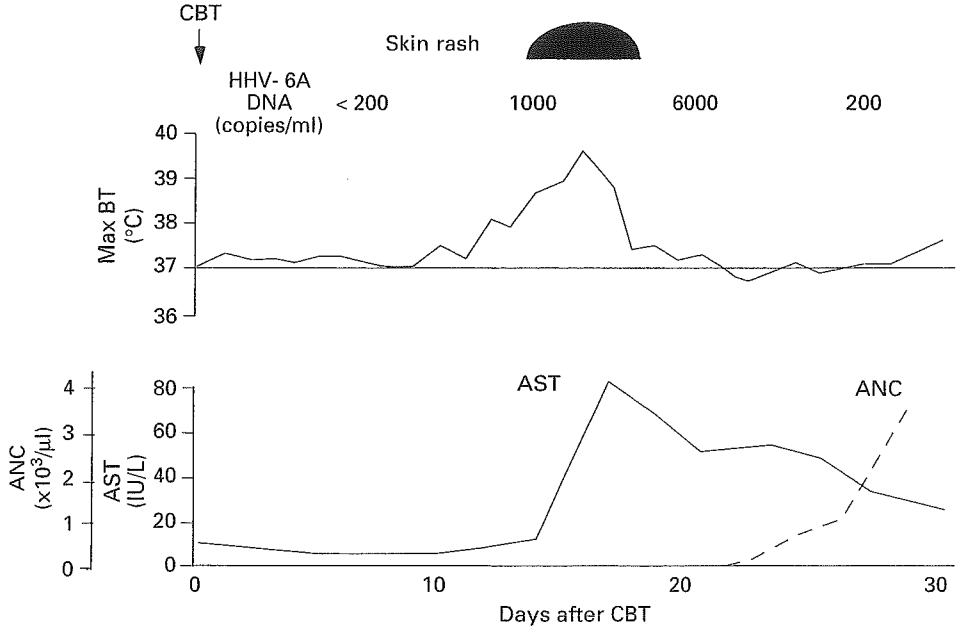

Figure 1 Clinical course of the patient with HHV-6A DNAemia.

serum samples.[10] HHV-6B DNA was more frequently detected in serum samples at weeks 2 or 3 after CBT than BMT (20 of 23 patients, 87% *vs* 4 of 21 patients, 19%, $P<0.0001$). By using the same methodology, we studied the incidence of HHV-6A infection in 50 adults after CBT. In one patient (2%), as described above, HHV-6A DNA was detected in serum samples on days 14, 21, and 28 after CBT. In the remaining 49 patients, HHV-6A DNA was not detected. The presence of HHV-6 DNA in peripheral blood or other samples does not necessarily indicate clinical disease.[1] In addition, further examination for other pathogenic viruses were not undertaken in our patient. However, previous studies have shown that detection of HHV-6 DNA in serum or plasma is a useful marker of active infection.[2,5,6] Thus, fever, skin rash, and liver dysfunction in our patient were considered to be associated with HHV-6A infection. This is the first report studying the incidence and the clinical features of HHV-6A infection in the early period after CBT.

The majority of HHV-6 infections after SCT are caused by HHV-6B.[3–5] However, Secchiero *et al*[6] examined infections by HHV-6A and HHV-6B differentially by a PCR method, on serum samples after BMT. In three of 13 patients (23%), HHV-6A DNA was transiently detected during episodes of fever and pulmonary symptoms after BMT. In these three patients, HHV-6A DNA in serum was first detected on days −3, 88, and 123 after BMT. However, HHV-6B DNA was not detected in the 13 patients. The incidence of symptomatic HHV-6A infections in the study seemed to be higher than in other studies. In Japanese adults, most HHV-6 infections within 4 weeks after CBT are caused by HHV-6B. Despite the relatively low incidence of HHV-6A infection after CBT, the pathogenesis of HHV-6A needs to be examined further.

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References

1 Hentrich M, Oruzio D, Jager G *et al.* Impact of human herpesvirus-6 after haematopoietic stem cell transplantation. *Br J Haematol* 2004; **128**: 66–72.
2 Yoshikawa T. Human herpesvirus 6 infection in hematopoietic stem cell transplant patients. *Br J Haematol* 2004; **124**: 421–432.
3 Drobyski WR, Eberle M, Majewski D, Baxter-Lowe LA. Prevalence of human herpesvirus 6 variant A and B infections in bone marrow transplant recipients as determined by polymerase chain reaction and sequence specific oligonucleotide probe hybridization. *J Clin Microbiol* 1993; **31**: 1515–1520.
4 Maeda Y, Teshima T, Yamada M *et al.* Monitoring of human herpesviruses after allogeneic peripheral blood stem cell transplantation and bone marrow transplantation. *Br J Haematol* 1999; **105**: 295–302.
5 Zerr DM, Corey L, Kim HW *et al.* Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. *Clin Infect Dis* 2005; **40**: 932–940.
6 Secchiero P, Carrigan DR, Asano Y *et al.* Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. *J Infect Dis* 1995; **171**: 273–280.
7 Nitsche A, Muller CW, Radonic A *et al.* Human herpesvirus 6A DNA is detected frequently in plasma but rarely in peripheral blood leukocytes of patients after bone marrow transplantation. *J Infect Dis* 2001; **183**: 130–133.
8 Ooi J, Iseki T, Nagayama H *et al.* Unrelated cord blood transplantation for adult patients with myelodysplastic syndrome-related secondary acute myeloid leukaemia. *Br J Haematol* 2001; **114**: 834–836.
9 Sashihara J, Tanaka-Taya K, Tanaka S *et al.* High incidence of human herpesvirus 6 infection with a high viral load in cord blood stem cell transplant recipients. *Blood* 2002; **100**: 2005–2011.
10 Tomonari A, Takahashi S, Ooi J *et al.* Human herpesvirus 6 variant B infection in adult patients after unrelated cord blood transplantation. *Int J Hematol* 2005; **81**: 352–355.

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Case Report

Unrelated Cord Blood Transplantation after Myeloablative Conditioning for Adult Patients with Refractory Anemia

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Abstract

We report the results of unrelated cord blood transplantation (CBT) after myeloablative conditioning in 3 patients with myelodysplastic syndrome–refractory anemia (MDS-RA). All patients were treated with total body irradiation, cytosine arabinoside (Ara-C), and cyclophosphamide, followed by unrelated HLA-mismatched CBT. Granulocyte colony-stimulating factor was infused continuously, starting 12 hours before Ara-C therapy and continuing until the end of Ara-C therapy. All patients received standard cyclosporine and methotrexate therapy as graft-versus-host disease prophylaxis. All patients had myeloid reconstitution, and the times to reach an absolute neutrophil count >0.5 × 10^9/L were 23, 20, and 26 days. All patients showed full donor chimerism at the time of the first bone marrow examination (on day +42, +43, and +62) after CBT. All patients are alive and free of disease at between 17 and 39 months after CBT. These results suggest that adult MDS-RA patients without suitable related or unrelated bone marrow donors should be considered as candidates for CBT.
Int J Hematol. 2005;81:424-427. doi: 10.1532/IJH97.05014

Key words: MDS; Refractory anemia; Cord blood transplantation; Adult

1. Introduction

Allogeneic stem cell transplantation is considered the only curative therapy for myelodysplastic syndrome (MDS) patients. Recently, alternative donor sources other than human leukocyte antigen (HLA)-identical siblings have been used as allogeneic stem cell sources [1-5]. We have previously reported the results for a group of adult patients with advanced MDS who underwent unrelated cord blood transplantation (CBT) [6]. However, there have been no reports detailing the results of adult MDS–refractory anemia (MDS-RA) patients treated with CBT after conventional myeloablative conditioning. Here, we report our clinical experience with 3 adult patients with MDS-RA treated with unrelated CBT after myeloablative conditioning.

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2. Case Reports

Between October 2001 and August 2003, 3 adult patients with MDS-RA were treated with unrelated CBT at the Institute of Medical Science, University of Tokyo. MDS was defined by French-American-British Cooperative Group criteria [7]. All patients received 12 Gy total body irradiation as 4 fractions on days –8 and –7. Cytosine arabinoside (Ara-C) was administered intravenously over 2 hours at a dosage of 3 g/m^2 every 12 hours on days –5 and –4 (total dose, 12 g/m^2). Recombinant human granulocyte colony-stimulating factor (G-CSF) was administered by continuous infusion at a dosage of 5 μg/kg per day. Infusion of G-CSF was started 12 hours before the first dose of Ara-C and stopped at the completion of the last dose. Cyclophosphamide was administered intravenously over 2 hours at a dosage of 60 mg/kg once daily on days –3 and –2 (total dose, 120 mg/kg). Two days after the completion of conditioning, the patients received a CBT. All patients received standard cyclosporine and methotrexate therapy as graft-versus-host disease (GVHD) prophylaxis. Cyclosporine was given every day, starting on day –1 at a dosage of 3 mg/kg per day. Methotrexate (15 mg/m^2 intravenously) was given on day 1, and 10 mg/m^2 was given on

days 3 and 6. Both acute and chronic GVHD were graded according to previously published criteria [8-10]. All patients received G-CSF (5 μg/kg per day) by intravenous infusion, starting on day 1 and continuing until durable granulocyte recovery was achieved. The cord blood unit was selected according to the number of nucleated cells per the recipient's body weight and HLA compatibility (HLA-A and HLA-B determined by serology and HLA-DRB1 determined by high-resolution DNA typing). Chimerism status after CBT was determined either by fluorescence in situ hybridization with a Y-chromosome probe for sex-mismatched CBT or by polymerase chain reaction DNA typing of HLA antigens for HLA-mismatched CBT. One patient (case 1) was included in our previous study [11], and all 3 patients were included in another study [12]. All patients were transfusion dependent before transplantation. No patient had a related or unrelated bone marrow donor available at the time of transplantation. Written informed consent for treatment was obtained from all patients. The characteristics of the 3 patients and the cord blood units are shown in Table 1. All patients had myeloid reconstitution, and the times to reach an absolute neutrophil count greater than 0.5×10^9/L were 23, 20, and 26 days. All patients showed full donor chimerism at the time of the first bone marrow examination (on day +42, +43, and +62) after CBT. Acute and chronic GVHD occurred in all patients. All patients are alive and free of disease at between 17 and 39 months after transplantation (Table 2).

3. Discussion

Although allogeneic stem cell transplantation from an HLA-identical related donor offers a potential cure for MDS-RA patients, a suitably matched related donor is unavailable for approximately two thirds of patients. In addition, the optimal timing of transplantation for low-risk MDS is unknown, and transplantation with the use of unrelated donors has remained controversial. A recent analysis by the International Bone Marrow Transplant Registry and the Fred Hutchinson Cancer Research Center group showed that delayed transplantation for the International Prognostic Scoring System (IPSS) low and intermediate-1 risk groups is associated with maximized overall survival [13]. They hypothesized that the optimal timing of transplantation for this cohort is at the time of a clinically important cytopenia or the progression from one IPSS group to a higher-risk group. Because all patients described in this report showed a clinically important cytopenia and transfusion dependence, we made the decision that all patients should receive a transplant. None of our patients had any related or unrelated bone marrow donors. Therefore, unrelated cord blood, which has the advantage of rapid availability, was used as an alternative stem cell source. Several studies that have included MDS patients have suggested promising results for unrelated CBT after myeloablative conditioning for adult patients [11,12,14-18]. In the study by Laughlin et al [14], 68 adult patients received a CBT. Of these 68 patients, 2 had MDS. In a report by the Eurocord group [16], 12 of the 108 adult patients who received a CBT had MDS. However, these 2 reports did not detail the preparative regimens, the prophylaxis therapy against

Table 1.
Characteristics of Patients and Cord Blood Units*

Case No.	Age, y/Sex	Body Weight, kg	HLA-A, HLA-B, DRB1 Mismatches, n	Cord Blood Cell Dose (Cryopreserved), $\times 10^7$/kg	Recipient CMV Status	Time from Diagnosis to Transplantation, mo	Treatment before Transplantation	Blood Counts at Transplantation†			Cytogenetics‡	IPSS Score at CBT
								Neutrophils, $\times 10^9$/L	Hemoglobin, g/dL	Platelets, $\times 10^9$/L		
1	48/M	61.6	1 (DRB1)	2.45	Positive	23	ATG, CyA, Pred, mPred	0.04	8.0	18	Poor (−7)	Int-2
2	31/M	59.0	3 (HLA-A, HLA-B, DRB1)	2.31	Negative	146	Pred, mPred, anabolic steroid	1.94	6.3	30	Poor (complex§)	Int-2
3	28/M	49.8	2 (HLA-B, DRB1)	2.43	Positive	27	ATG, CyA, Pred, mPred, anabolic steroid	0.46	7.9	19	Good (normal)	Int-1

*CMV indicates cytomegalovirus; IPSS, International Prognostic Scoring System; CBT, cord blood transplantation; ATG, antithymocyte globulin; CyA, cyclosporin A; Pred, prednisolone; mPred, methylprednisolone; Int-2, intermediate-2.
†Blood counts were performed immediately before the conditioning regimen.
‡Cytogenetic analyses were performed before transplantation and according to IPSS classification [21].
§48,XY,+9,+15,der(18)t(1;18)(q21;p11) (1/20); 48,XY,+9,+15,der(18)t(1;18)(q21;p11),inv(12)(p13q24) (1/20); 46,XY,del(11)(q?) (9/20); 46,XY,ins(1;?)(q21;?),del(11)(q?),add(18)(p11) (3/20); 46,XY (6/20)

Table 2.
Transplantation Outcomes*

Case No.	Neutrophils >0.5 × 10^9/L, d	Reticulocytes >1%, d	Hemoglobin >8.5 g/dL, d	Platelets >50 × 10^9/L, d	Acute GVHD Grade (Organ Involvement and Stage)	Chronic GVHD	Immunosuppressive Treatment at Last Follow-up Day	Blood Counts and Performance Status at Last Follow-up Day				Survival, mo
								Neutrophils, ×10^9/L	Hemoglobin, g/dL	Platelets, ×10^9/L	PS	
1	23	32	55	48	I (skin 1, liver 0, gut 0)	Limited	None (Jan 13, 2005)	3292	12.9	213	0	39
2	20	32	125	131	II (skin 3, liver 0, gut 0)	Extensive	Prednisolone, 7.5 mg/d (Jan 20, 2005)	9143	13.7	209	1	30
3	26	46	126	109	I (skin 1, liver 0, gut 0)	Limited	None (Jan 13, 2005)	5001	13.9	141	0	17

*GVHD indicates graft-versus-host disease; PS, Eastern Cooperative Oncology Group performance status.

GVHD, or the results of transplantation for the MDS patients. Sanz et al [15] reported the results with 22 adult patients who received a CBT following a standardized preparative and GVHD regimen. Of the 22 patients, only 1 had MDS. Long et al [17] reported the results with 57 adult patients who underwent CBT at Duke University. Although 3 of the 57 patients had MDS, the details of the outcomes for the MDS patients were not described. Recently, Laughlin et al [18] reported the results with 150 adult patients who received a CBT. Although 10 of the 150 patients had MDS, the details of the outcomes for the MDS patients also were not described. At present, therefore, the role of unrelated cord blood as an alternative stem cell source is not well defined for adult MDS-RA patients eligible for conventional conditioning regimens. Because the safety of the preparative regimen including G-CSF has been confirmed for the treatment of myeloid malignancies [19,20], we have used the same regimen for CBT patients with myeloid malignancies, including those with MDS-RA. Because unrelated CBT can be performed without severe regimen-related toxicities and because all 3 patients are alive and free of disease after transplantation, we suggest that unrelated CBT may be beneficial for adult MDS-RA patients without a suitable related or unrelated bone marrow donor.

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References

1. Anderson JE, Anasetti E, Appelbaum FR, et al. Unrelated donor transplantation for myelodysplasia (MDS) and MDS-related acute myeloid leukaemia. *Br J Haematol.* 1996;93:59-67.
2. Arnold R, de Witte T, van Biezen A, et al. Unrelated bone marrow transplantation in patients with myelodysplastic syndromes and secondary acute myeloid leukemia: an EBMT survey. European Blood and Marrow Transplantation Group. *Bone Marrow Transplant.* 1998;21:1213-1216.
3. de Witte T, Hermans J, Vossen J, et al. Haematopoietic stem cell transplantation for patients with myelo-dysplastic syndromes and secondary acute myeloid leukaemias: a report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Br J Haematol.* 2000; 110:620-630.
4. de Witte T, Pikkemaat F, Hermans J, et al. Genotypically nonidentical related donors for transplantation of patients with myelodysplastic syndromes: comparison with unrelated donor transplantation and autologous stem cell transplantation. *Leukemia.* 2001;15: 1878-1884.
5. Deeg HJ, Storer B, Slattery JT, et al. Conditioning with targeted busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. *Blood.* 2002;100:1201-1207.
6. Ooi J, Iseki T, Takahashi S, et al. Unrelated cord blood transplantation for adult patients with advanced myelodysplastic syndrome. *Blood.* 2003;101:4711-4713.
7. Bennett JM, Catovsky D, Daniel MD, et al. Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol.* 1982; 51:189-199.
8. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation.* 1974;18:295-304.

9. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995;15:825-828.
10. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. *Am J Med.* 1980;69:204-217.
11. Ooi J, Iseki T, Takahashi S, et al. Unrelated cord blood transplantation after myeloablative conditioning in patients over the age of 45 years. *Br J Haematol.* 2004;126:711-714.
12. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. *Blood.* 2004;104:3813-3820.
13. Cutler CS, Lee SJ, Greenberg P, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. *Blood.* 2004;104:579-585.
14. Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. *N Engl J Med.* 2001;344:1815-1822.
15. Sanz GF, Saavedra S, Planelles D, et al. Standardized, unrelated donor cord blood transplantation in adults with hematologic malignancies. *Blood.* 2001;98:2332-2338.
16. Gluckman E. Current status of umbilical cord blood hematopoietic stem cell transplantation. *Exp Hematol.* 2000;28:1197-1205.
17. Long GD, Laughlin M, Madan B, et al. Unrelated umbilical cord blood transplantation in adult patients. *Biol Blood Marrow Transplant.* 2003;9:772-780.
18. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med.* 2004;351:2265-2275.
19. Takahashi S, Okamoto SI, Shirafuji N, et al. Recombinant human glycosylated granulocyte colony-stimulating factor (rhG-CSF)-combined regimen for allogeneic bone marrow transplantation in refractory acute myeloid leukemia. *Bone Marrow Transplant.* 1994; 13:239-245.
20. Takahashi S, Oshima Y, Okamoto S, et al. Recombinant human granulocyte colony-stimulating factor (G-CSF) combined conditioning regimen for allogeneic bone marrow transplantation (BMT) in standard-risk myeloid leukemia. *Am J Hematol.* 1998;57: 303-308.
21. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood.* 1997;89:2079-2088.

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Unrelated cord blood transplantation for a human immunodeficiency virus-1-seropositive patient with acute lymphoblastic leukemia

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The concurrent use of highly active antiretroviral therapy (HAART) improves results of high-dose chemotherapy with autologous stem cell transplantation (SCT) for human immunodeficiency virus-1 (HIV)-associated lymphomas.[1] Recently, successful allogeneic SCT from HLA-matched sibling donors was reported in HIV-infected patients.[2–4] Here, we describe the first case of an HIV-infected patient with acute lymphoblastic leukemia (ALL) who underwent umbilical cord blood transplantation (CBT).

In July 1996, a 23-year-old Japanese woman presented with fever and genital herpes. She was confirmed as seropositive for HIV, probably transmitted from her boyfriend. In March 2001, a real-time quantitative polymerase chain reaction (PCR) analysis showed that the HIV-RNA level was elevated to 25 000 copies/ml (lower limit of detection, 50). The CD4 count decreased to 28/μl. Therefore, HAART consisting of 60 mg stavudine, 300 mg lamivudine, and 600 mg efavirenz was initiated. In July 2001, the HIV-RNA level decreased to 220 copies/ml, and the CD4 count increased to 129/μl. In May 2003, her complete blood count tests showed a white blood cell count (WBC) of 3990/μl with 29% lymphoblasts. Bone marrow (BM) examination showed hypercellularity with 96% lymphoblasts, which were positive for CD4, CD10, CD13, CD19, CD33, CD34, and HLA-DR. Cytogenetic analysis disclosed the presence of t(9;22)(q34;q11) in 12 of 20 metaphases. The p190$^{BCR-ABL}$ transcript was shown by a reverse transcriptase (RT)-PCR analysis. She was diagnosed as Philadelphia chromosome-positive ALL. She achieved hematological complete remission after two courses of chemotherapy. She has been taking HAART during and after the chemotherapy and her HIV-RNA level continued to be below detectable levels. She was negative for hepatitis B virus surface antigen and anti-hepatitis C virus antibody, and positive for anti-cytomegalovirus antibody. As she had no HLA-matched related or unrelated BM donors, the patient underwent CBT from an unrelated donor with mismatches at two loci (HLA-B and DR) in September 2003 (Figure 1). The numbers of total nucleated cells and CD34-positive cells in the cord

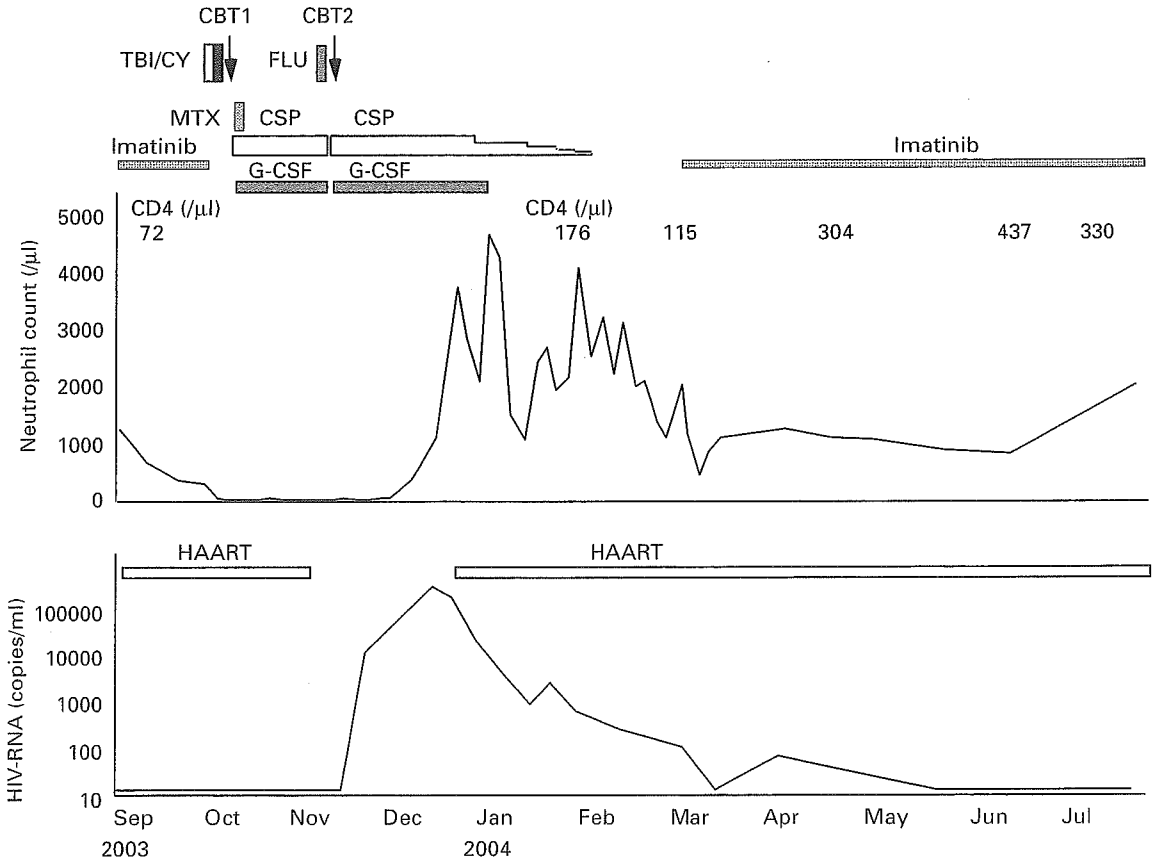

Figure 1 Clinical course of the patient.

blood (CB) unit were 2.9×10^7/kg and 0.76×10^5/kg, respectively. The conditioning regimen included 12 Gy total body irradiation and 120 mg/kg cyclophosphamide. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate. The patient tolerated the procedure well with minimal regimen-related toxicity. Owing to possible myelosuppression, HAART was discontinued on day +28. On day +33, her WBC remained below 100/μl and all of the BM cells were shown to be derived from the recipient. At 40 days after the first CBT, second CBT was performed from an unrelated donor with a one-locus mismatch at HLA-DR. The numbers of total nucleated cells and CD34-positive cells in the CB unit were 2.1×10^7/kg and 0.46×10^5/kg, respectively. The conditioning regimen included 40 mg/m^2 fludarabine for 3 days. Cyclosporine was administered for GVHD prophylaxis. A neutrophil count consistently greater than 500/μl was achieved on day +27. Full donor chimerism of BM cells was shown on day +28. The HIV-RNA level increased to 3×10^6 copies/ml on day +31. After the administration of HAART from day +38, the HIV-RNA levels returned to below detectable levels from day +195, and the CD4 count increased to above 300/μl from day +170. No bacterial or fungal infections were documented during the first and second CBT processes and cytomegalovirus reactivation was successfully treated with ganciclovir and foscarnet. Grade I acute GVHD occurred, but resolved without any additional immunosuppressants. No chronic GVHD was observed. An RT-PCR analysis showed continuous negative test results for the $p190^{BCR\text{-}ABL}$ transcript until the last follow-up evaluation at 15 months post-CBT.

CBT for adults has been associated with a high rate of early transplantation-related mortality (TRM).[5,6] However, our single-institution experience showed a 1-year TRM of 9% and 2-year disease-free survival of 74% in 68 adults after CBT.[7] Both CB donors and the patient in the present study were Japanese. The lesser genetic diversity in a single ethnic population in our studies might be associated with the favorable outcomes of CBT, such as the lower rates of severe acute GVHD. Although our results suggest that CBT is feasible for HIV-infected patients on HAART, the safety and efficacy should be further examined by prospective studies.

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References

1 Krishnan A, Zaia J, Forman SJ. Should HIV-positive patients with lymphoma be offered stem cell transplants? *Bone Marrow Transplant* 2003; **32**: 741–748.
2 Schlegel P, Beatty P, Halvorsen R, McCune J. Successful allogeneic bone marrow transplant in an HIV-1-positive man with chronic myelogenous leukemia. *J Acquir Immune Defic Syndr* 2000; **24**: 289–290.
3 Sora F, Antinori A, Piccirillo N *et al.* Highly active antiretroviral therapy and allogeneic CD34(+) peripheral blood progenitor cells transplantation in an HIV/HCV coinfected patient with acute myeloid leukemia. *Exp Hematol* 2002; **30**: 279–284.
4 Kang EM, de Witte M, Malech H *et al.* Nonmyeloablative conditioning followed by transplantation of genetically modified HLA-matched peripheral blood progenitor cells for hematologic malignancies in patients with acquired immunodeficiency syndrome. *Blood* 2002; **99**: 698–701.
5 Laughlin MJ, Eapen M, Rubinstein P *et al.* Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med* 2004; **351**: 2265–2275.
6 Rocha V, Labopin M, Sanz G *et al.* Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med* 2004; **351**: 2276–2285.
7 Takahashi S, Iseki T, Ooi J *et al.* Single institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematological malignancies. *Blood* 2004; **104**: 3813–3820.

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Human Herpesvirus 6 Variant B Infection in Adult Patients after Unrelated Cord Blood Transplantation

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Abstract

Human herpesvirus 6 variant B (HHV-6B) infection was studied in 23 adult patients who underwent cord blood transplantation (CBT). HHV-6B DNA was detected by quantitative polymerase chain reaction analysis after CBT in the sera from 15 patients (65%) at day 14 or 15 (week 2), from 16 patients (70%) at day 21 or 22 (week 3), and from 3 patients (13%) at day 28 or 29 (week 4). HHV-6B DNAemia was found in none of the 20 patients examined at day 7 or 8 (week 1). The overall incidence of HHV-6B DNAemia reached 87% (20 of 23 patients). This incidence was much higher than after unrelated bone marrow transplantation (19%, $P < .0001$). In CBT patients, positive HHV-6B DNAemia at week 3 was significantly associated with early skin rash (88% versus 14%, $P < .005$) and grade II-IV acute graft-versus-host disease (aGVHD) (69% versus 14%, $P < .05$). In contrast, positive HHV-6B DNAemia at week 2 was associated with neither skin rash nor aGVHD. Prospective large-scale studies are needed to determine the role of HHV-6 infection in CBT patients.
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Key words: Human herpesvirus 6; Cord blood transplantation; Graft-versus-host disease

1. Introduction

Human herpesvirus 6 (HHV-6) is classified into variants A and B. HHV-6B causes significant complications after hematopoietic stem cell transplantation (SCT), including encephalitis, pneumonia, and bone marrow suppression [1,2]. An association between HHV-6 infection and early skin rash or acute graft-versus-host disease (aGVHD) in SCT patients has also been reported [3-5]. The probability of HHV-6 infection after bone marrow transplantation (BMT) or peripheral blood SCT (PBSCT) ranges from 30% to 80% and may vary according to the method of detection and the type of SCT [1,2]. In previous studies, the detection of HHV-6 DNA in peripheral blood mononuclear cells (PBMCs) by polymerase chain reaction (PCR) analysis was mainly used to examine HHV-6 infection in SCT patients. However, this method may detect latent infection by viral genomes in PBMCs. In contrast, several studies have shown that the detection of HHV-6 DNA in serum or plasma is a marker of active infection [6-8]. Using a real-time quantitative PCR method on serum samples, we compared the HHV-6 DNA levels in adults after cord blood transplantation (CBT) and after BMT.

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2. Patients and Methods

2.1. Patients

Twenty-three adults who underwent CBT from HLA-mismatched unrelated donors between October 2000 and May 2003 were studied (Table 1). Transplantation procedures and supportive care were described previously [9,10]. The severity of aGVHD was graded according to the standard criteria [11]. All patients received 1000 mg/day acyclovir orally from day –3 to day 35 to prevent herpes simplex virus reactivation. After neutrophil engraftment, cytomegalovirus infection was monitored with an antigenemia assay. Preemptive ganciclovir therapy was initiated as described previously [10]. Patient no. 20 developed positive cytomegalovirus antigenemia and received 5 mg/kg ganciclovir from day 22. The remaining patients did not receive ganciclovir therapy within

Table 1.
Patient Characteristics*

	CBT (n = 23)	BMT (n = 21)
Median age (range), y	36 (18-53)	28 (17-50)
Sex (male/female), n	16/7	14/7
Disease, n		
AML	16	7
ALL	5	4
CML	1	8
MDS	1	2
Disease status, n		
Low risk	15	8
High risk	8	13
HLA matching, n†		
6/6	0	21
5/6 or 4/6	14	0
3/6 or 2/6	9	0
Preparative regimen, n		
TBI + CY + Ara-C	17	17
TBI + CY	4	4
TBI + FLU + Ara-C	2	0
GVHD prophylaxis, n		
CSP + MTX	23	9
FK + MTX	0	12
Acute GVHD, n		
0-I	11	8
II-IV	12	13

*CBT indicates cord blood transplantation; BMT, bone marrow transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; TBI, total body irradiation (12 Gy); CY, cyclophosphamide; Ara-C, cytarabine; FLU, fludarabine; CSP, cyclosporine; MTX, methotrexate; FK, tacrolimus; GVHD, graft-versus-host disease.

†The matching of HLA-A and HLA-B was confirmed by serologic typing methods, and the matching of DRB1 was confirmed by genomic typing methods.

28 days after CBT. One patient (no. 21) developed neurologic symptoms that might be attributable to HHV-6B infection. The patient died of fungal infection on day 48. Three patients (nos. 12, 14, and 18) developed leukemia relapse on days 96, 89, and 368, respectively. The remaining 19 CBT patients were alive at a median follow-up time of 30 months (range, 17-48 months). The control patients were 21 adults who underwent BMT from HLA-matched unrelated donors between March 1997 and February 2003 (Table 1). No patients developed positive cytomegalovirus antigenemia or received ganciclovir therapy within 28 days after BMT. Ten BMT patients developed leukemia relapse at a median of 236 days after BMT (range, 35-1539 days). Four patients died of transplantation-related complications on days 75, 79, 110, and 1523. The remaining 7 BMT patients were alive at a median follow-up time of 50 months (range, 21-90 months).

2.2. Real-Time Quantitative PCR

Serum samples were obtained on day 7 or 8 (week 1), day 14 or 15 (week 2), day 21 or 22 (week 3), and day 28 or 29 (week 4). Real-time quantitative PCR for HHV-6B DNA was performed with the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) as described previously [12]. In brief, the reaction mixture consisted of the TaqMan Universal PCR Master Mix (Applied Biosystems) with 300 nM of each primer, 200 nM TaqMan probe, and sample DNA that had been purified with a QIAamp DNA Minikit (Qiagen, Hilden, Germany) from 200 μL serum. After 50 cycles of PCR amplification, the data were analyzed with Sequence Detection System software, version 1.6.3 (Applied Biosystems). The numbers of HHV-6B DNA copies in the samples were determined by comparing the data with the standard curves obtained with serially diluted plasmids (10^1-10^7 copies) containing the sequence of the HHV-6B immediate-early-1 gene. The forward and reverse primers were 5′-GGTCATACAAGGAAGCGTT TCG-3′ and 5′-GTACAGCCTCAGTGACAGATCTG-3′, respectively. The probe was 5′-CAGCCCCGATAAAAG GTCACAGACAAAAGA-3′ and was labeled with a fluorescent dye, 6-carboxyfluorescein (FAM) (Applied Biosystems). The assay could distinguish HHV-6B from other herpesviruses, including HHV-6A, cytomegalovirus, and Epstein-Barr virus. The minimum detectable level of this assay was 2×10^2 copies/mL.

2.3. Statistical Methods

The frequencies of categorical variables were compared with the Fisher exact test. The levels of HHV-6B DNA were compared by means of the Mann-Whitney *U* test.

3. Results

3.1. Detection of HHV-6B DNA in Sera after CBT

The presence of HHV-6B DNA in sera from 23 CBT patients at weeks 2, 3, and 4 was examined by the quantitative PCR method (Table 2). In 20 (87%) of 23 CBT patients, HHV-6B DNA was found in at least 1 serum sample. HHV-6B DNAemia was found in 15 patients (65%) at week 2, 16 patients (70%) at week 3, and 3 patients (13%) at week 4. However, HHV-6B DNAemia was found in none of the 20 patients examined at week 1. The median HHV-6B DNA level was 6×10^2 copies/mL (range, $<2 \times 10^2$ to 6×10^4 copies/mL) at week 2 and 1×10^3 copies/mL (range, $<2 \times 10^2$ to 2×10^4 copies/mL) at week 3. The difference between the HHV-6B DNA levels at weeks 2 and 3 was not statistically significant ($P = .73$).

As a control, HHV-6B DNAemia was examined in 21 BMT patients at weeks 2, 3, and 4. In 4 (19%) of 21 BMT patients, HHV-6B DNAemia was found in the serum sample. One patient developed HHV-6B DNAemia with 2×10^2 copies/mL at week 2 and 1×10^3 copies/mL at week 3, 2 patients developed HHV-6B DNAemia with 7×10^3 copies/mL and 1×10^4 copies/mL only at week 2, and the fourth patient developed DNAemia with 4×10^3 copies/mL only at week 3. HHV-6B DNAemia was not found at week 4. The probabilities for HHV-6B DNAemia at week 2, week 3, and week 2 or 3 after CBT were significantly higher than after BMT ($P < .001$, $P < .0001$, and $P < .0001$, respectively). The HHV-6B DNA levels at weeks 2 and 3 after CBT were also higher than after BMT ($P < .01$, and $P < .001$, respectively).

Table 2.
Human Herpesvirus 6 Variant B (HHV-6B) DNA Levels, Skin Rash, and Grade II-IV Acute Graft-versus-Host Disease (aGVHD) in Cord Blood Transplantation Patients*

Patient No.	HHV-6B DNA, copies/mL				Rash, d	aGVHD, grade
	Week 1†	Week 2	Week 3	Week 4		
1	—	ND	1×10^4	ND	13	II
2	ND	2×10^4	ND	ND	No	I
3	ND	ND	1×10^4	ND	13	II
4	ND	ND	ND	ND	13	I
5	ND	4×10^4	1×10^4	ND	11	II
6	ND	2×10^4	2×10^4	3×10^2	13	IV
7	ND	2×10^2	2×10^2	ND	No	I
8	ND	ND	5×10^3	ND	No	0
9	—	1×10^4	9×10^3	ND	11	I
10	ND	ND	ND	ND	No	II
11	ND	ND	ND	ND	No	0
12	ND	2×10^3	1×10^3	ND	12	I
13	ND	1×10^4	3×10^2	ND	12	II
14	ND	ND	4×10^3	ND	15	I
15	ND	1×10^4	1×10^3	ND	11	II
16	ND	2×10^4	2×10^3	ND	11	II
17	ND	ND	2×10^3	ND	16	III
18	ND	4×10^2	5×10^3	ND	8	II
19	ND	2×10^4	ND	ND	No	I
20	ND	2×10^3	2×10^4	2×10^2	10	II
21	ND	2×10^2	5×10^3	6×10^5	10	III
22	—	6×10^2	ND	ND	No	0
23	ND	6×10^4	ND	ND	No	I

*ND indicates not detected.
†The HHV-6B DNA level at week 1 was not examined in 3 patients (nos. 1, 9, and 22).

3.2. Associations of HHV-6B Infection after CBT

3.2.1. Early Skin Rash

Within 21 days after CBT, 15 (65%) of 23 patients developed an erythematous maculopapular skin rash (Table 2). The onset was a median of 12 days (range, 8-16 days) after CBT. In all of the 15 patients, the skin rash was accompanied by a high-grade fever higher than 39.0°C. A skin biopsy was performed on the onset day for 2 patients (nos. 13 and 16) and 3 days after the onset of the skin rash for 1 patient (no. 12). There was a sparse lymphocytic infiltration within the epidermis in all patients, and apoptotic figures were observed in 1 patient (no. 12). The skin rash was clinically and histologically indistinguishable from that of aGVHD. A skin rash was observed in 14 (70%) of 20 patients with HHV-6B DNAemia at any week and in 1 (33%) of the 3 patients without it. The association of skin rash with HHV-6B DNAemia at any week was not statistically significant ($P = .27$). Next, the association of skin rash with HHV-6B DNAemia at week 2 or at week 3 was examined separately. A skin rash was observed in 10 (67%) of 15 patients with HHV-6B DNAemia at week 2 and in 5 (63%) of 8 patients without it ($P = .60$). In contrast, a skin rash was observed in 14 (88%) of 16 patients with HHV-6B DNAemia at week 3 and in 1 (14%) of 7 patients without it ($P < .005$). The median HHV-6B DNA level at week 3 in patients with skin rash was 4×10^3 copies/mL (range, $<2 \times 10^2$ to 2×10^4 copies/mL), which was higher than the median level ($<2 \times 10^2$ copies/mL; range, $<2 \times 10^2$ to 5×10^3 copies/mL) found in those without a skin rash ($P < .005$).

3.2.2. Grade II-IV aGVHD

Because skin rash occurring within 21 days after CBT was not distinguishable from that of aGVHD, even by histologic examination, a skin rash was considered to be the skin manifestation of aGVHD in this study. Twelve (52%) of 23 patients developed grade II-IV aGVHD at a median of 32 days after CBT (range, 10-42 days). Grade II-IV aGVHD was observed in 11 (55%) of 20 patients with HHV-6B DNAemia at any week and in 1 (33%) of 3 patients without it. The association of grade II-IV aGVHD with HHV-6B DNAemia at any week was not statistically significant ($P = .47$). Next, the association of HHV-6B DNAemia at week 2 or at week 3 was examined separately. Grade II-IV aGVHD was observed in 8 (53%) of 15 patients with HHV-6B DNAemia at week 2 and in 4 (50%) of 8 patients without it ($P = .61$). In contrast, grade II-IV aGVHD was observed in 11 (69%) of 16 patients with HHV-6B DNAemia at week 3 and in 1 (14%) of 7 patients without it ($P < .05$). The median HHV-6B DNA level at week 3 in patients with grade II-IV aGVHD was 5×10^3 copies/mL (range, $<2 \times 10^2$ to 2×10^4 copies/mL), which was higher than the median level ($<2 \times 10^2$ copies/mL; range, $<2 \times 10^2$ to 5×10^3 copies/mL) of those with grade 0 to I aGVHD ($P < .05$).

4. Discussion

HHV-6B infection in adults after CBT was studied by means of quantitative PCR on serum samples. In 20 (87%) of 23 CBT patients, positive HHV-6 DNAemia was found in at

least 1 sample. This incidence was much higher than after BMT (19%). This report is the first to compare the incidences of HHV-6B DNAemia and the viral loads after CBT and BMT in adults. In children, Sashihara et al [12] detected HHV-6 DNA in PBMCs more frequently in CBT patients than in BMT or PBSCT patients (100% [7 of 7] versus 56% [9 of 16]). A lack of primed HHV-6–specific T-cells in the infused CB units and the immunologic immaturity of CB lymphocytes may be associated with the higher rate of HHV-6 infection after CBT.

We used a real-time quantitative PCR method on serum samples to study HHV-6 infection. In previous studies, detection of HHV-6 DNA in PBMCs by PCR had been used to examine HHV-6 infection in SCT patients. However, this method may detect both latent and active infections. In contrast, several studies have shown that the detection of viral DNA in serum or plasma is a marker of active infection [6,7]. In addition, reverse transcriptase–PCR for detecting both immediate-early and late gene transcripts can distinguish active and latent infection and may be a useful method for monitoring active viral infection [13]. For most CBT patients, however, obtaining sufficient RNA samples from PBMCs is difficult because most patients do not attain neutrophil engraftment within 3 weeks after CBT. Therefore, the measurement of viral DNA in serum or plasma by real-time PCR methods may give a clinical benefit to CBT patients.

The association between HHV-6 infection and skin rash or aGVHD has been reported [3-5]. There are 2 possible explanations for this association. First, HHV-6 infection in the skin or other organs may trigger or enhance aGVHD. Yoshikawa et al [14] suggested that the alterations in surface molecule expression caused by HHV-6 infection might enhance the infiltration of inflammatory cells into epidermal tissues and result in the skin rash. The second possible explanation is that the presence of severe aGVHD and the effects of immunosuppressive therapy may trigger or enhance HHV-6 infection. We found that HHV-6B DNAemia at week 3 was associated with skin rash and grade II-IV aGVHD after CBT. All 4 CBT patients with positive HHV-6B DNAemia only at week 2 (nos. 2, 19, 22, and 23) did not develop skin rash or grade II-IV aGVHD. These results suggest that transient HHV-6B infection prior to sufficient lymphocyte recovery may not induce strong lymphocyte reactions leading to skin rash or severe aGVHD. However, the reason for the association of skin rash and grade II-IV aGVHD with HHV-6B DNAemia only at week 3 was unclear. Because our study included only a small number of patients, prospective large-scale studies are needed to determine the role of HHV-6 infection in CBT patients.

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References

1. Clark DA, Griffiths PD. Human herpesvirus 6: relevance of infection in the immunocompromised host. *Br J Haematol.* 2003;120: 384-395.
2. Yoshikawa T. Human herpesvirus 6 infection in hematopoietic stem cell transplant patients. *Br J Haematol.* 2004;124:421-432.
3. Yoshikawa T, Ihira M, Ohashi M, et al. Correlation between HHV-6 infection and skin rash after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 2001;28:77-81.
4. Wilborn F, Brinkmann V, Schmidt CA, Neipel F, Gelderblom H, Siegert W. Herpesvirus type 6 in patients undergoing bone marrow transplantation: serologic features and detection by polymerase chain reaction. *Blood.* 1994;83:3052-3058.
5. Boutolleau D, Fernandez C, Andre E, et al. Human herpesvirus (HHV)-6 and HHV-7: two closely related viruses with different infection profiles in stem cell transplantation recipients. *J Infect Dis.* 2003;187:179-186.
6. Imbert-Marcille BM, Tang XW, Lepelletier D, et al. Human herpesvirus 6 infection after autologous or allogeneic stem cell transplantation: a single-center prospective longitudinal study of 92 patients. *Clin Infect Dis.* 2000;31:881-886.
7. Secchiero P, Carrigan DR, Asano Y, et al. Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. *J Infect Dis.* 1995;171:273-280.
8. Zerr DM, Gupta D, Huang ML, Carter R, Corey L. Effect of antivirals on human herpesvirus 6 replication in hematopoietic stem cell transplant recipients. *Clin Infect Dis.* 2002;34:309-317.
9. Ooi J, Iseki T, Takahashi S, et al. Unrelated cord blood transplantation for adult patients with de novo acute myeloid leukemia. *Blood.* 2004;103:489-491.
10. Tomonari A, Iseki T, Ooi J, et al. Cytomegalovirus infection following unrelated cord blood transplantation for adult patients: a single institute experience in Japan. *Br J Haematol.* 2003;121: 304-311.
11. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995;15: 825-828.
12. Sashihara J, Tanaka-Taya K, Tanaka S, et al. High incidence of human herpesvirus 6 infection with a high viral load in cord blood stem cell transplant recipients. *Blood.* 2002;100:2005-2011.
13. Yoshikawa T, Akimoto S, Nishimura N, et al. Evaluation of active human herpesvirus 6 infection by reverse transcription-PCR. *J Med Virol.* 2003;70:267-272.
14. Yoshikawa T, Goshima F, Akimoto S, et al. Human herpesvirus 6 infection of human epidermal cell line: pathogenesis of skin manifestations. *J Med Virol.* 2003;71:62-68.