表 1　単球より誘導されたGM，M型マクロファージの特徴

	GM-Mφ	M-Mφ
growth factor	GM-CSF	M-CSF
phagocytic/bacteriocidal function	weak	strong
antigen presenting function	strong	weak
cytokine production	IL-12(+)	IL-12(−)
	IL-23(++)	IL-23(−)
	IL-10(+/−)	IL-10(++)
in vivo localization	lung	intestine

学的改善を認めたと報告している[9]．通過菌である *Lactobacillus* は一定期間で腸管より排除されるため，治療に必要なときにのみ投与すればよく，安全性にも優れているといえる．その後，Steidler らは，Crohn 病患者に対して IL-10 遺伝子導入 *Lactobacillus lactis*(LL-Thy12)の phase I trial を行い，安全性と有効性を報告した[10]．

2. プロバイオティクスの炎症性腸疾患への応用

プロバイオティクスのヒト炎症性腸疾患への応用は，腸炎を惹起しない安全な *Lactobacillus* と *Bifidobactrerium* の両菌属を用いておもに行われている．*Lactobacillus* は代謝産物である酪酸が大腸上皮のエネルギーバランスを改善し，またサイトカイン産生を亢進させる転写因子である NF-κB を抑制することが報告されている[11]．潰瘍性大腸炎術後の回腸嚢炎が，*Lactobacillus* と *Bifidobactrerium* など 13 株を組み合わせた VSL#3 というプロバイオティクス製品により改善したことが報告されている[12]．潰瘍性大腸炎患者に非病原性 *E. coli* を投与し，5-ASA と同等の緩解維持効果が認められたとする報告や[13]，ビフィズス菌発酵乳が再発を抑制した成績も報告されている[14]．

一方，2006 年のアメリカ消化器病学会では，ステロイドによる緩解導入後の潰瘍性大腸炎患者で *Lactobacillus salivarius* および *Bifidobactrerium infantis* の緩解維持効果を検討したが，プラセボと比較して有意差を認めないことが報告された．

前述の VSL#3 は活動性潰瘍性大腸炎患者に投与されて，77%で有効性が報告されて[15]，現在，わが国，欧米を含めてプラセボを用いた二重盲検試験が進行中である．今後は免疫調節作用をはじめとしたプロバイオティクスの作用機序を解明し，病態に応じてそれぞれの腸内細菌を使い分けることが期待される．

腸管マクロファージと腸管ホメオスタシス

マクロファージは細菌などの外来抗原に対する自然免疫のおもな担当細胞であり，感染防御において重要な役割を果たしている．しかし，腸管局所ではつねに多数の腸内細菌が存在しているため，マクロファージはそれら腸内細菌に対して過剰な免疫反応を引き起こさないように，何らかの機構によって免疫反応を制御していると考えられる．近年，マクロファージは分化誘導因子の違いにより，相反する機能をもち異なる形態を示す 2 つのサブセットに分化することが明らかになった[16,17]．GM-CSF により分化誘導される GM 型マクロファージは，Th1 型免疫応答を誘導する炎症性サイトカイン IL-12，IL-23 を高産生する炎症性マクロファージで，一方，M-CSF により分化誘導される M 型マクロファージは抑制性サイトカインである IL-10 を産生し抗炎症的に働く抑制性マクロファージである．さらに，著者らの研究によりマウス正常腸管では M-CSF が優位に発現していることが明らかになり，実際にマウス腸管マクロファージは腸内細菌抗原刺激に対し 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，けっして Th1 型免疫応答を引きこす 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 を高産生する抑制性のマクロファージであった[18]．このように正常な腸管マクロファージは腸内細菌に対し抑制性の免疫反応を誘導し，ホメオスタシスの維持にかかわっていると考えられる(表 1)．

炎症性腸疾患と腸管マクロファージ

炎症性腸疾患，とくにCrohn病においてその病態にマクロファージが重要な役割を果たしていることはいくつもの報告がある[19-21]．Crohn病の腸管局所にはインターフェロン(IFN)-γやIL-2産生に特徴づけられるTh1型のCD4$^+$T細胞が集積している．

IL-10 KOマウスはTh1型の慢性腸炎を自然発症するモデルであり，Crohn病の実験腸炎モデルとして広く用いられている[22]．前述のようにIL-10 KOマウスの腸炎発症，進展には腸内細菌の存在が必須であるが，これまで詳細なメカニズムについては不明であった．

著者らはマクロファージの腸内細菌認識に着目し，IL-10 KOマウスの骨髄単球由来GM型，M型マクロファージ，および腸管マクロファージの反応性について検討を行った．その結果，本来抑制性に働いている骨髄由来M型マクロファージ，腸管マクロファージにおいて，腸内細菌である*Eschelichia coli*や*Enterococcus faecalis*加熱死菌抗原刺激により過剰なIL-12p70の産生が認められた[18]．IL-12の過剰産生は転写レベルでも同様に確認された．

つぎにM-CSFにより分化誘導される抑制性のマクロファージ(腸管マクロファージも含めて)の機能的成熟には，分化時にM-CSFにより誘導される内因性IL-10の必要性が推測される．これを証明するために，IL-10 KO M型マクロファージの分化時にIL-10を加え分化実験を行った．結果，IL-10を分化時に添加したマクロファージは，IL-10産生能がないにもかかわらずIL-12，IL-23の過剰産生は抑制された[18]．本結果より著者らは，通常下では分化段階で内因性のIL-10によりマクロファージによるIL-12誘導機構は負に制御されているが，IL-10欠損下ではIL-12の抑制機構が破綻し，その結果，腸内細菌認識により過剰なIL-12が産生され，Th1優位な腸炎を引き起こすことを明らかにした．

おわりに

以上より定量的PCRを用いた検討では，炎症性腸疾患患者では腸内細菌全体の構成が乱れており，炎症性腸疾患の病因に関与していることが示唆された．さらに，腸管局所ではマクロファージは貪食，殺菌能は有しているが，IL-12，IL-23などのTh1誘導性サイトカインを産生しない抑制性の性質に分化することで，外来の病原体に対する防御能を維持しながら食餌抗原や腸内細菌に対する過剰な免疫反応を制御していると考えられる．このように消化管は非常に複雑で精密な仕組みでホメオスタシスを保っており，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．このように腸管マクロファージと腸内細菌の関係を解明していくことで炎症性腸疾患における腸内細菌の役割を明確にし，本疾患の病態解明，プロバイオティクスを用いた治療法の開発につながっていくと考えられる．

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Molecular
Gastrointestinal
Medicine

腸管粘膜マクロファージによる腸管ホメオスターシスとその破綻

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KEY WORDS

炎症性腸疾患，腸管マクロファージ，Interleukin-10，Interleukin-12，Interleukin-23

SUMMARY

腸管局所ではつねに多数の腸内細菌が存在しているため，腸管マクロファージ(Mφ)はそれら腸内細菌に対して過剰な免疫反応を引き起こさないよう，何らかの機構によって制御されていると考えられる．本研究により正常腸管 Mφ は IL-10 高産生の抑制性 Mφ であり，腸内細菌への過剰な免疫反応を制御していることが明らかになった．一方，炎症性腸疾患モデルである *IL-10* 遺伝子欠損マウスでは内因性 IL-10 の欠損のため腸管 Mφ が異常な分化を遂げ，腸内細菌に対し IL-12 や IL-23 といった Th1 誘導性のサイトカインを過剰産生することが明らかになった．このように，腸管 Mφ の腸内細菌に対する免疫制御機構の破綻が，腸内細菌に対する過剰な免疫応答を誘導し，炎症性腸疾患の病態に寄与していることが示唆された．

はじめに

消化管は消化，吸収，排泄をつかさどるだけでなく，複雑な gut associated lymphoid tissue (GALT) とよばれる免疫担当装置を形成している．さらに消化管には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 面分にも及び，そこに 10^{14} 個以上の腸内細菌が常在している．さらに，消化管は病原体や食餌抗原などの外来抗原にも恒常的に曝露されている．つまり，消化管は体内にありながらつねに外界と接している特殊な臓器といえる．

通常，免疫装置は外界からの侵入者に対して免疫反応を誘導することで外来抗原に対しすみやかに反応，処理することで生体を守っている．しかしながら，つねに食餌抗原や腸内細菌に曝されている腸管粘膜では，それらの抗原に対して過剰な免疫反応を誘導するのは好ましくなく，むしろ恒常性を保つため過剰な免疫反応を抑制的に制御する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，

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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とどまらず，抑制性の免疫学的機構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われわれ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つかさどるマクロファージの特殊性が明らかとなってきた．

炎症性腸疾患の病態に innate immunity は関与している

炎症性腸疾患（inflammatory bowel disease：IBD）は大きく潰瘍性大腸炎とクローン病の二疾患に区別される．これら2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．潰瘍性大腸炎では標的臓器は大腸のみであるのに対し，クローン病では小腸，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．クローン病では腸管局所の免疫応答は type 1 helper T cell（Th 1）型にシフトしていることがわかっており，エフェクター細胞は腸管局所の $CD4^+$T 細胞である．一方，潰瘍性大腸炎での局所の免疫応答の状態は報告により異なっており type 2 helper T cell（Th 2）型にシフトしているという報告もあるがコンセンサスは得られていない．潰瘍性大腸炎，クローン病ともにその病因はいまだ明らかにはなっていないが，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．

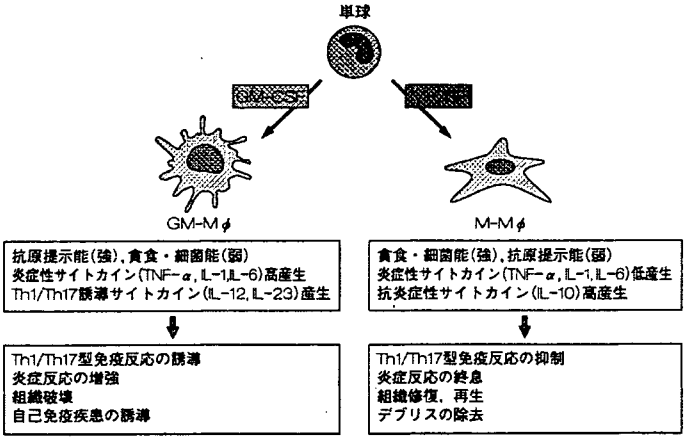
IBD の病因因子として近年最も注目されているのが腸内細菌の役割である．先に述べたように腸管では常在する腸内細菌に対してある種の免疫寛容が成立していると考えられるが，IBD ではおそらくこのバランスが破綻していると考えられる．実際にクローン病患者では anti-Saccharomyces cerevisiae antibodies（ASCAs），Omp C，I2，flagellin に対する抗体 CBir 1 など酵母や腸内細菌に対する抗体価の上昇が認められ[1)2)]，これらのことから食餌や腸内細菌などの何らかの外来抗原に対する異常な免疫応答が背景にあり，時として腸管局所のみならず全身の免疫系が活性化し自己抗原と交差反応することで関節炎などの自己免疫疾患に類似した症状も呈するのではないかとも考えられる．一方，遺伝的素因としては疾患関連遺伝子として *NOD 2* や *OCTN* などが同定されている．とくに NOD 2 は細胞質内 pathogen 認識分子として自然免疫（innate immunity）と IBD を関連づけるあらたな証拠として注目を浴び，IBD の基礎研究において innate immunity を再認識させるきっかけとなった[3)4)]．

腸炎の発症には腸内細菌が関与する

これまで IBD モデルとして多くの遺伝子操作による自然発症腸炎モデルマウスが報告されてきた．これらのマウスは残念ながらヒト IBD を完全に反映しているとはいいがたいが，サイトカインやシグナル伝達に関与する分子など免疫に関与する分子の発現異常が慢性腸炎を引き起こすという事実は腸管における免疫機構の重要性を明らかにした点で重要な知見であった．しかしながらいずれのマウスモデルにおいてもなぜ発症するのか？という疑問は解決されないままである．ただ重要な点は多くのこれらマウスモデルは無菌状態（germ free）では発症しないということである．すなわち，たとえ免疫異常を有していても“フローラとの相互作用”＝“innate immunity”がなければ腸炎は起こらないのである．

腸内細菌は善玉か悪玉か？

前述したように IBD，とくにクローン病の病態には腸内細菌に対する過剰な免疫反応が関与していると考えられている．では腸内細菌はわれわれにとって炎症を引き起こす悪者なのであろうか？　近年，この疑問の答えとなるいくつかの研究が報告された．それらの研究において，腸内細菌の認識にかかわる Toll 様レセプター（Toll-like receptors：TLRs）やそのシグナル伝達分子である MyD 88 の欠損マウスではデキストラン硫酸ナトリウム（DSS）誘発腸炎が増悪すること，同様に腸内細菌の存在しない無菌マウスでは通常マウスにくらべ DSS 腸炎が増悪することが明らかになった[5)6)]．すなわち正常の状態では腸内細菌と TLRs との相互作用は protective にはたらいている可能性が示唆される．このように腸内細菌の存在とそのバランスが腸の恒常性の維持にきわめて重



図❶ マクロファージの成長因子による機能の違い
マクロファージは成長因子の違いにより異なる2つのphenotypeへと分化する．GM-CSF誘導型のGM-Mφ（Mφ1ともよばれる）はIL-12, 23高産生な炎症惹起性マクロファージ，一方で，M-CSF誘導型のM-Mφ（Mφ2ともよばれる）はIL-10高産生でIL-12, 23は産生しない炎症抑制性のマクロファージである．腸管はM-CSF優位の組織であり，抑制性のM-Mφが分化し常在マクロファージを構築していると考えられる．

要であることは間違いがない．

腸管マクロファージは炎症抑制型マクロファージである

マクロファージは細菌などの外来抗原に対する自然免疫のおもな担当細胞であり感染防御において重要な役割を果たしている．しかしながら，腸管局所ではつねに多数の腸内細菌が存在しているため，マクロファージはそれら腸内細菌に対して過剰な免疫反応を引き起こさないように，何らかの機構によって免疫反応を制御していると考えられる．近年，マクロファージは分化誘導因子の違いにより相反する機能をもち異なる形態を示す2つのサブセットに分化することが明らかになった[7]．顆粒球マクロファージコロニー刺激因子（granulocyte-macrophage colony-stimulating factor：GM-CSF）により分化誘導されるGM型マクロファージは種々の炎症性サイトカインを高産生する炎症惹起型マクロファージであると考えられる．GM型マクロファージは強い抗原提示能を有し，獲得免疫の誘導に重要なインターロイキン(IL)-12やIL-23を産生することから，Th1/Th17型免疫反応の誘導に寄与していると考えられる．また，同時に腫瘍壊死因子（tumor necrosis factor：TNF)-αやIL-6といった炎症性サイトカインも強く産生し炎症反応を増強している．一方で，マクロファージコロニー刺激因子（macrophage colony-stimulating factor：M-CSF）により分化誘導されるM型マクロファージは抗原提示能が低く，逆に強い貪食・殺菌能をもつ．M型マクロファージはGM型と異なり細菌等の外来抗原刺激によりIL-12やIL-23などの炎症性サイトカインを産生せず，抑制性サイトカインであるIL-10を高産生する．すなわち，M型マクロファージはTh1, Th17型免疫反応に対して抑制的にはたらいていると考えられる．このように，これら異なる免疫応答を担うマクロファージサブセットがそれぞれの特性を発揮することで，生体の防御，そしてホメオスターシスの維持に重要な役割を果たしていると考えられる(図❶)．これらマクロファージサブセットの局在や腸炎における役割はこれまでまったくわかっていなかった．しかしながら，われわれ[8]の研究により，マウス正常腸管ではM-CSFが優位に発現していることが明らかになった．また，M-CSF欠損マウスである*op/op*マウスでは腸管マクロファージの分化が障害されているという事実からも，腸管はM-CSFの発

現が優位な組織であり，炎症抑制性であるM型マクロファージの分化の場であると考えられる[9]．実際にマウス腸管マクロファージは腸内細菌抗原刺激に対し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Th 1，Th 17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を高産生する抑制性のマクロファージであった[8]．またSmythiesら[10]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．最近，腸管マクロファージが炎症抑制的にはたらいていることを裏づける報告として腸管マクロファージ欠損マウスではDSS誘導腸炎が増悪することが明らかになった[11]．このように正常な腸管マクロファージは腸内細菌に対し抑制性の免疫反応を誘導し，ホメオスターシスの維持にかかわっていると考えられる．

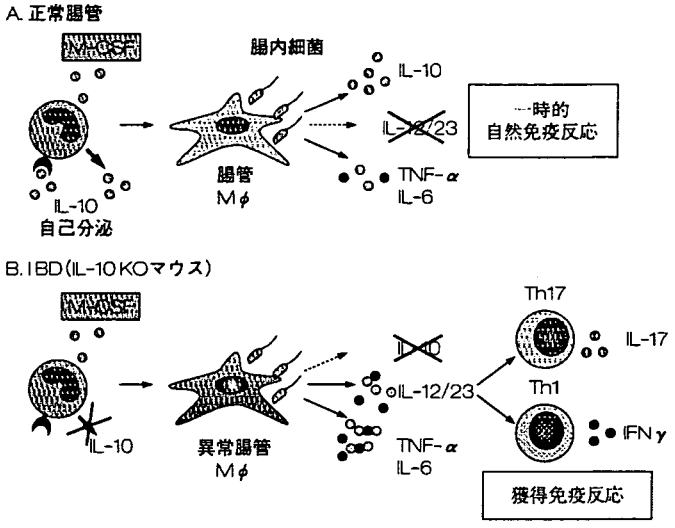
炎症性腸疾患（IBD）では腸管マクロファージ機能が破綻している

IBD，とくにクローン病においてその病態にマクロファージが重要な役割を果たしていることはいくつもの報告がある[12]．クローン病の腸管局所にはインターフェロン（IFN）γやIL-2産生に特徴づけられるTh 1型のCD 4$^+$T細胞が集積している．これらTh 1型のCD 4$^+$T細胞が産生するIFNγはマクロファージからのIL-12やIL-18などのサイトカインを産生し促し，IL-12，IL-18は逆にTh 1細胞を刺激することで炎症を持続させるサイクルが形成されると考えられる[13][14]．最近になり，いくつかのTh 1優位な疾患やそのモデルマウスにおいて，むしろIL-23/IL-17を主体とするTh 17免疫反応がより病的な意義が高いということが報告されはじめた[15]．実際にクローン病の腸管マクロファージからはIL-12のみならず，IL-23も高産生されるという報告もあり，クローン病の病態におけるIL-12/IL-23の役割が注目されている[16]．しかしながら，これらの報告は前述した腸管マクロファージ特有の炎症制御能とは一致しない．すなわちクローン病において腸管マクロファージは何らかの原因によりその免疫制御機能を失い，その結果，腸内細菌に対する過剰な免疫反応，IL-12/23産生に起因するTh 1/Th 17型獲得免疫反応の増強を引き起こしているのではないだろうか．

IBDモデル動物の1つである*IL-10*遺伝子欠損（knockout：KO）マウスはTh 1型の慢性腸炎を自然発症するモデルであり，クローン病の実験腸炎モデルとして広く用いられている[17]．IL-10 KOマウスのマクロファージや樹状細胞はナイーブT細胞やメモリーT細胞のTh 1反応を誘導する．また，腸管マクロファージを選択的に除去することで腸炎の発症が抑制されることから，本モデルにおいてマクロファージは炎症の主体をになっていると考えられる[18]．さらにIL-12 p 40とのダブルKOマウスやIL-12 p 40サブユニット抗体治療によりIL-10 KOマウスの腸炎発症が劇的に抑制されることから，クローン病と同様に，マクロファージからのIL-12/23産生が，本モデルの病態形成の鍵となっていると考えられる[19]．他のIBDモデル動物と同様，IL-10 KOマウスの腸炎発症，進展にも腸内細菌の存在が必須であることも報告されている[20]．しかしながら，これまで詳細なメカニズムについては不明であった．

われわれはマクロファージの腸内細菌認識機構に着目し，IL-10 KOマウスの骨髄単球由来GM型，M型マクロファージ，および腸管マクロファージの反応性について検討をおこなった．その結果，炎症性のGM型マクロファージではIL-12/23産生能に有意な差は認められなかったのに対し，本来抑制性にはたらいている骨髄由来M型マクロファージ，腸管マクロファージにおいて腸内細菌である*Escherichia coli*や*Enterococcus faecalis*加熱死菌抗原刺激により過剰なIL-12，IL-23の産生が認められた[8]．

つぎに，なぜIL-10の欠損はM-CSF誘導性のマクロファージにのみ強く影響するのだろうかという疑問がもちあがる．過去の研究により，単球をM-CSFで刺激するとIL-10が誘導されることがわかっている[21]．しかしながらGM-CSFにはこのようなIL-10誘導能は認められない．すなわち，M-CSFにより分化誘導される抑制性のマクロファージ（腸管マクロファージも含めて）の機能的成熟には分化時にM-CSFにより誘導されるIL-10の自己分泌刺激が必要なのではないだろうか．この仮説を証明するために，われわれはIL-10 KO M型マクロ



図❷ 腸管マクロファージ分化異常による免疫抑制能の破綻

A. 正常腸管マクロファージは腸管に存在するM-CSF依存的に分化する．この時M-CSFにより誘導されるIL-10の刺激を受け，腸管マクロファージは腸内細菌に対し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，獲得免疫応答を誘導する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を高産生する抑制性の機能を獲得すると考えられる．

B. IL-10 KOマウス由来腸管マクロファージはその抑制機能が破綻し，腸内細菌に対しTNF-αやIL-6のみならずIL-12やIL-23を過剰産生し，Th 1型やTh 17型の獲得免疫反応を誘導していると考えられる．

ファージの分化時にIL-10を加え分化実験をおこなった．刺激時にはIL-10は完全に除去し，検出できるレベルにないことを確認した．結果，IL-10を分化時に加えたマクロファージではIL-10産生能がないにもかかわらずIL-12, IL-23の過剰産生は抑制された．本結果よりわれわれ[8]は，通常下では分化段階で内因性のIL-10によりマクロファージによるIL-12誘導機構(つまりはTh 1優位な獲得免疫の誘導)は負に制御されているが，IL-10欠損下ではIL-12の抑制機構が破綻し，その結果，腸内細菌認識により過剰なIL-12が産生され，Th 1優位な腸炎を引き起こすことを明らかにした（図❷）．

興味深いことに，腸内細菌刺激によるIL-12/23の過剰産生はマクロファージによる貪食を阻害することで抑制された(未発表データ)．また，リポポリサッカライド(LPS)，ペプチドグリカンなどのpathogen associated molecular patterns (PAMPs) 刺激ではIL-10 KOマウスにおいても過剰なIL-12/23産生は認められなかった[8]．詳細なメカニズムは現在検討中であるが，貪食された細菌の認識にはTLR以外の細胞内認識機構を介している可能性がある．前述のクローン病疾患関連遺伝子である細胞内細菌認識レセプターNOD 2/CARD 15などの知見とあわせて考えても非常に興味深い．

おわりに

以上のように，腸管局所ではマクロファージは貪食，殺菌能は有しているがIL-12, IL-23などのTh 1, Th 17誘導性サイトカインを産生せず，抑制性サイトカインであるIL-10を高産生する抑制性の性質に分化している．つまり，正常腸管マクロファージは，外来の病原体に対する自然免疫反応を介した防御能を維持しながら，食餌抗原や腸内細菌に対する過剰な免疫反応を制御していると考えられる．このように消化管は非常に複雑で精密な

仕組みでホメオスターシスを保っており，その破綻がIBD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．

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ORIGINAL ARTICLE

Impact of cytomegalovirus serostatus on outcome of unrelated cord blood transplantation for adults: a single-institute experience in Japan

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Abstract

Cytomegalovirus (CMV) disease is one of the major infectious complications after allogeneic hematopoietic stem cell transplantation (SCT). Several studies have shown that CMV-seropositive patients have a substantial survival disadvantage after bone marrow transplantation (BMT) or peripheral blood SCT (PBSCT). Between August 1998 and February 2006, 101 adult patients underwent myeloablative cord blood transplantation (CBT) from unrelated donors at our institution. Sixteen and 85 patients were CMV-seronegative and CMV-seropositive, respectively, prior to CBT. Outcomes of CBT were compared between CMV-seronegative and CMV-seropositive patients. The cumulative incidences of neutrophil engraftment at 60 d after CBT did not differ between CMV-seronegative and CMV-seropositive patients (100% and 94%, $P = 0.09$); however, the cumulative incidence of platelet engraftment at 100 d was higher in CMV-seronegative patients than CMV-seropositive patients (100% vs. 86%, $P < 0.005$). The cumulative incidence of CMV antigenemia at 100 d was lower in CMV-seronegative patients than CMV-seropositive patients (0% vs. 77%, $P < 0.001$); however, the cumulative incidences of CMV disease did not differ between CMV-seronegative and CMV-seropositive patients (0% vs. 1%, $P = 0.84$). The probabilities of disease-free survival at 2 yr also did not differ between CMV-seronegative and CMV-seropositive patients (92% vs. 72%, $P = 0.16$). The outcomes of CBT for CMV-seropositive patients as well as CMV-seronegative patients in our series were favorable. This might be due to effective antiviral therapy for CMV infection. Large-scale studies are needed to determine the impact of recipient CMV serostatus on the outcome of CBT for adults.

Key words Cytomegalovirus; antigenemia; cord blood transplantation

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Cytomegalovirus (CMV) disease, particularly interstitial pneumonia, is one of the major complications after allogeneic hematopoietic stem cell transplantation (SCT) (1, 2). Ganciclovir therapy for preventing CMV disease has reduced the incidence of early CMV disease after SCT. In the recent era of effective antiviral therapy, the impact of recipient CMV serostatus on the outcome of SCT has been studied (3–5). Several studies have shown that CMV-seropositive patients have a substantial survival disadvantage after bone marrow transplantation (BMT) or peripheral blood SCT (PBSCT). The survival disadvantage has been suggested to be largely restricted to profoundly immunosuppressed SCT patients, such as those receiving T-cell-depleted grafts and/or grafts from human leukocyte antigen (HLA)-mismatched or unrelated donors. Increased mortality in CMV-seropositive recipients is probably due to both direct and indirect effects of CMV infection as well as drug toxicities.

Umbilical cord blood transplantation (CBT) from an unrelated donor has recently been utilized as an alternative therapy for patients who do not have suitable donors for BMT or PBSCT (6, 7). Cord blood (CB) lymphocytes are functionally and phenotypically immature when compared with adult blood lymphocytes (8); therefore, infectious complications including viral infections are suggested to occur frequently in patients after CBT. Our previous study showed that the probabilities of developing positive CMV antigenemia and requiring preemptive ganciclovir therapy after CBT were 79% and 67%, respectively, in 24 CMV-seropositive patients (9); however, no patients developed CMV disease. In four CMV-seronegative patients, none developed positive CMV antigenemia. In the present study, we compared the outcomes of CBT between 16 CMV-seronegative patients and 85 CMV-seropositive patients.

Patients and methods

Patients

Between August 1998 and February 2006, 101 adult patients underwent unrelated CBT following a myeloablative conditioning regimen including 12 Gy total body irradiation (TBI) at The Institute of Medical Science, The University of Tokyo. Sixteen and 85 patients were CMV-seronegative and CMV-seropositive, respectively, prior to CBT. Patient characteristics are shown in Table 1. CMV-seronegative patients were younger than CMV-seropositive patients (median age, 32 yr vs. 40 yr; $P = 0.014$). The other characteristics did not differ significantly between the two groups (data not shown).

Table 1 Patient characteristics

	Negative	Positive
No. patients	16	85
Age, yr		
Median	32	40
Range	16–49	16–55
Gender, *n*		
Male	9	46
Female	7	39
Disease, *n*		
AML	12	42
ALL	2	15
CML	1	3
MDS	1	7
NHL	0	8
Disease status, *n*		
Low risk	10	37
High risk	6	48
TNC, $\times 10^7$/kg		
Median	2.60	2.38
Range	2.04–3.98	1.16–5.29
CD34, $\times 10^5$/kg		
Median	0.88	0.94
Range	0.27–1.53	0.15–8.97
HLA matching, *n*		
5/6 or 4/6	8	65
3/6 or 2/6	8	20
ABO incompatibility, *n*		
Identical	4	23
Minor	5	28
Major	4	18
Bidirectional	3	16
Preparative regimen, *n*		
TBI + CY + AraC	14	63
TBI + CY	2	9
TBI + FLU + AraC	0	8
TBI + FLU + L-PAM	0	3
GVHD prophylaxis, *n*		
CSP + MTX	16	82
CSP	0	3

Negative indicates cytomegalovirus-seronegative; Positive, cytomegalovirus-seropositive; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; TNC, total nucleated cell; Identical, ABO-identical; Minor, minor ABO-incompatible; Major, major ABO-incompatible; Bidirect, bidirectional ABO-incompatible; TBI, total body irradiation; CY, cyclophosphamide; AraC, cytarabine; FLU, fludarabine; L-PAM, melphalan; CSP, cyclosporin; MTX, methotrexate.

Characteristics of the grafts

The median numbers of total nucleated cells (TNCs) before freezing were 2.60×10^7/kg (range, 2.04–3.98) in CMV-seronegative patients and 2.38×10^7/kg (range, 1.16–5.29) in CMV-seropositive patients ($P = 0.37$) (Table 1). The median numbers of CD34-positive cells were 0.86×10^5/kg (range, 0.27–1.53) in CMV-seronegative patients and 0.94×10^5/kg (range, 0.15–8.97) in CMV-seropositive patients ($P = 0.37$). The matching of HLA-A and -B was confirmed by low-resolution typing methods, and the matching of HLA-DRB1 was confirmed by high-resolution typing methods. All CB grafts were from HLA-mismatched unrelated donors.

Transplantation procedures and supportive care

Transplantation procedures and supportive care were described previously (10). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine (3 mg/kg/d) and a short course of methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days +3 and +6). To facilitate neutrophil recovery, recombinant human granulocyte colony-stimulating factor was administered intravenously at a dose of 5 μg/kg/d from day +1 after CBT. All patients received 1000 mg/d acyclovir

orally from day −3 to day +35 to prevent herpes simplex virus infection.

All platelet concentrates were obtained from single-donor apheresis. All packed red blood cells (RBCs) and platelets transfused were leukoreduced by filtration. In addition, four of 16 CMV-seronegative patients were transfused with only CMV-seronegative blood components.

Preemptive ganciclovir therapy

Cytomegalovirus infection was monitored using an antigenemia assay twice a week after engraftment during the hospital stay, and at each hospital visit after discharge. The antigenemia assay consisted of direct immunostaining of polymorphonuclear leukocytes with monoclonal antibodies C10/C11 (Clonab CMV; Biotest, Dreieich, Germany) directed against the lower matrix protein pp65. We used two preemptive strategies for preventing CMV disease. Prior to May 2002, we used risk-adapted preemptive ganciclovir therapy (9). In brief, ganciclovir was initiated when two or more positive cells per 3×10^5 cells on two slides were detected in patients at high-risk for CMV disease, defined as those developing grade II–IV acute GVHD and those receiving more than 0.5 mg/kg prednisolone. In patients other than the above, ganciclovir was initiated when four or more positive cells per 3×10^5 cells on two slides were detected. Ganciclovir was initially administered at a dose of 5 mg/kg twice daily for 14 d, and then continued at a dose of 5 mg/kg once daily. Ganciclovir therapy was discontinued when neutropenia with an absolute neutrophil count (ANC) of $< 1 \times 10^9$/l developed, or when negative results on two consecutive tests were obtained. From May 2002, we used another preemptive strategy with ganciclovir (11). Irrespective of the risk for CMV disease, preemptive ganciclovir therapy was initiated when any positive cells were detected. In addition, the initial dose of ganciclovir was reduced to 5 mg/kg once daily, which was half the dose in the previous method. Ganciclovir therapy was discontinued according to the same criteria as above.

Definition

Neutrophil engraftment was defined as an ANC exceeding 0.5×10^9/l for three consecutive days. Platelet engraftment was defined as a platelet count exceeding 20×10^9/l for three consecutive days without platelet transfusion. Acute GVHD was graded according to previously published criteria (12). Patients who survived more than 100 d after CBT with sustained donor hematopoiesis were considered at risk for the development of chronic GVHD (13).

Cytomegalovirus disease was diagnosed as described previously (14). Briefly, CMV pneumonia was defined by the presence of signs and symptoms of pulmonary disease together with the detection of CMV in bronchoalveolar lavage fluid or lung tissue samples. CMV disease of the central nervous system (CNS) is defined by the identification of CNS symptoms together with the detection of CMV in cerebrospinal fluid or brain biopsy specimens. CMV disease of other organs was diagnosed by biopsy with clinical signs and symptoms.

Low-risk diseases were defined as acute leukemia and lymphoma in the first and second complete remission, myelodysplastic syndrome in the early phase, and chronic myelogenous leukemia in the first chronic phase. High-risk diseases were defined as those other than the above.

Statistical methods

Patient characteristics in two groups were compared using the Mann–Whitney *U*-test or Fisher's exact test. Cumulative incidences were estimated in a competing risks setting, death being treated as a competing event (15). In multivariate analysis, a Cox proportional hazards model was used to assess the independent effect of risk factors on the end-points. We used the stepwise variable selection procedures at a significant level of 5%. The following factors were studied: age, gender, disease status, CMV serostatus, TNC dose, CD34-positive cell dose, HLA matching, and ABO incompatibility. Transplantation-related mortality (TRM) was evaluated using the Kaplan–Meier method as well as the cumulative incidence. Disease-free survival (DFS) was evaluated using the Kaplan–Meier method, and differences between curves were compared using the log-rank test. A two-sided P of < 0.05 was considered statistically significant.

Results

Engraftment

All 16 CMV-seronegative patients and 78 of 85 CMV-seropositive patients achieved donor-derived neutrophil engraftment within 60 d after CBT. The median days of neutrophil engraftment were day +21 (range, +19 to +26) and +22 (range, +16 to +46), respectively, after CBT. Of the remaining seven CMV-seropositive patients, three patients developed autologous hematopoietic recovery and four patients died before neutrophil engraftment. The cumulative incidences of neutrophil engraftment at 60 d after CBT were 100% and 94%, respectively [hazard ratio (HR), 1.62; 95% confidence interval (CI), 0.93–2.83; $P = 0.09$] (Fig. 1A). All CMV-seronegative

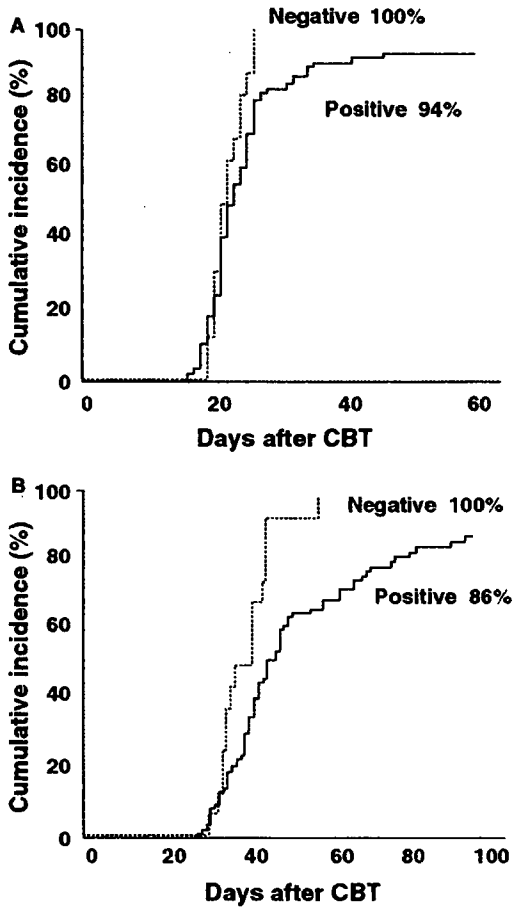


Figure 1 (A) Cumulative incidences of neutrophil engraftment at 60 d after CBT. (B) Cumulative incidences of platelet engraftment at 100 d after CBT. Positive, CMV-seropositive; Negative, CMV-seronegative.

patients and 73 CMV-seropositive patients achieved platelet engraftment within 100 d after CBT. The median days of platelet engraftment were day +37 (range, +29 to +54) and day +40 (range, +26 to +88), respectively. Of the remaining 12 CMV-seropositive patients, three patients developed autologous hematopoietic recovery, and four patients died before neutrophil and platelet engraftment, and five patients who achieved neutrophil engraftment died before platelet engraftment. The cumulative incidence of platelet engraftment at 100 d was significantly higher in CMV-seronegative patients than CMV-seropositive patients (100% vs. 86%; HR, 2.29; 95% CI, 1.29–4.08; $P < 0.005$) (Fig. 1B). In addition, multivariate analysis showed that the larger CD34-positive cell dose was also significantly associated with rapid neutrophil and platelet engraftment (HR, 1.80; 95% CI, 1.18–2.76; $P < 0.01$; and HR, 1.61; 95% CI, 1.03–2.52; $P < 0.05$, respectively).

GVHD

Seven CMV-seronegative patients and 46 CMV-seropositive patients developed grade II–IV acute GVHD after CBT. The cumulative incidences of grade II–IV acute GVHD at 100 d were 50% and 53%, respectively (HR, 0.98; 95% CI, 0.46–2.09; $P = 0.96$) (Fig. 2A). Twelve CMV-seronegative patients and 63 CMV-seropositive patients at risk developed chronic GVHD after CBT. The cumulative incidences of chronic GVHD at 2 yr were 79% and 75%, respectively (HR, 0.63; 95% CI, 0.33–1.19; $P = 0.15$) (Fig. 2B).

CMV infection

No CMV-seronegative patients developed positive CMV antigenemia after CBT. In contrast, 66 CMV-seropositive patients developed positive CMV antigenemia within 100 d after CBT. The median onset of antigenemia in

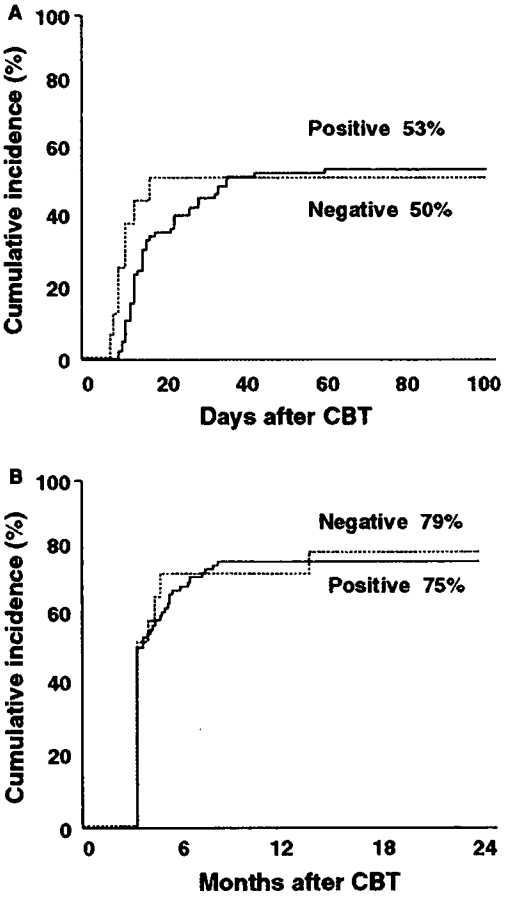
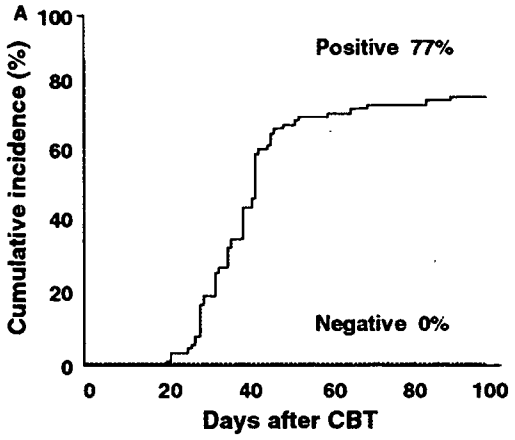


Figure 2 (A) Cumulative incidences of grade II–IV acute GVHD at 100 d after CBT. (B) Cumulative incidences of chronic GVHD at 2 yr after CBT.



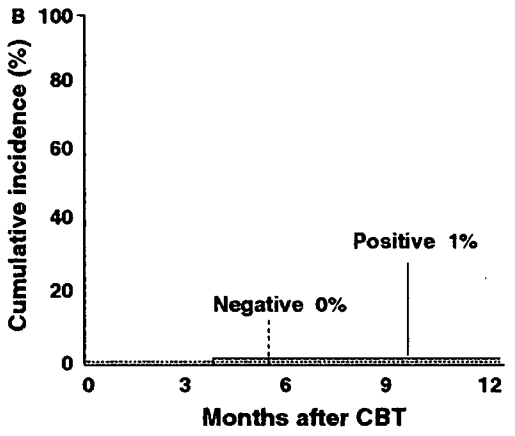
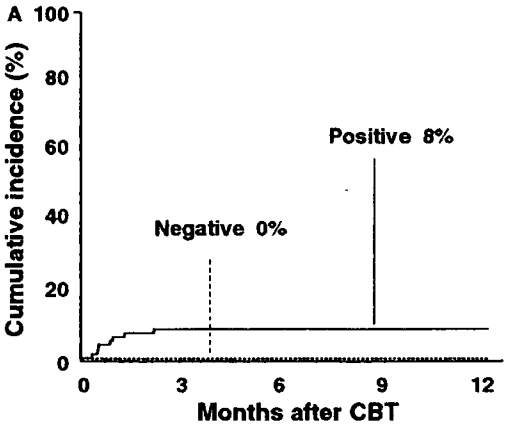


Figure 3 (A) Cumulative incidences of CMV antigenemia at 100 d after CBT. (B) Cumulative incidences of CMV disease at 1 yr after CBT.



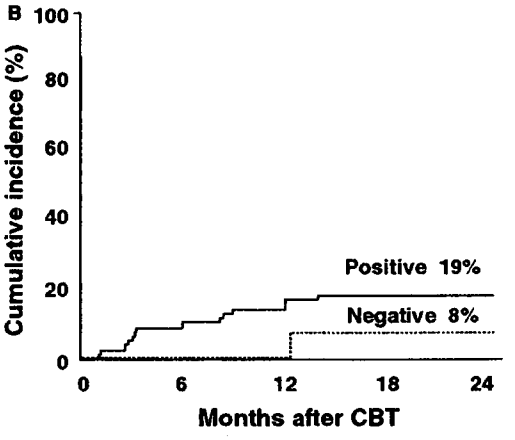


Figure 4 (A) Cumulative incidences of transplantation-related mortality at 1 yr after CBT. (B) Cumulative incidences of relapse at 2 yr after CBT.

CMV-seropositive patients was day +39 (range, +20 to +91) after CBT. The cumulative incidence of CMV antigenemia at 100 d was significantly lower in CMV-seronegative patients than CMV-seropositive patients (0% vs. 77%, $P < 0.001$) (Fig. 3A).

No CMV-seronegative patients developed CMV disease. One CMV-seropositive patient (1%) developed CMV disease of the CNS on day +111, which directly caused her death. The cumulative incidences of CMV disease at 1 yr did not differ significantly between CMV-seronegative and CMV-seropositive patients (0% vs. 1%, $P = 0.84$) (Fig. 3B).

TRM

Within 1 yr after CBT, no CMV-seronegative patients and seven CMV-seropositive patients died without leukemia relapse. CMV disease was considered to be the cause of death in one CMV-seropositive patient, as described above. The main causes of death in the remaining six patients were organ toxicity in three patients and infection in the remaining three patients. No patients died of GVHD within 1 yr after CBT. The cumulative incidences of TRM at 1 yr did not differ significantly between CMV-seronegative and CMV-seropositive patients (0% vs. 8%, $P = 0.23$) (Fig. 4A). The probabilities of TRM at 1 yr which were estimated using the Kaplan–Meier method also did not differ significantly between CMV-seronegative and CMV-seropositive patients (0% vs. 8%, $P = 0.24$).

Relapse

Leukemia relapse occurred in two CMV-seronegative patients at 12 and 54 months after CBT, and 16 CMV-seropositive patients at a median of 4 months (range, 1–36) after CBT. The cumulative incidences of relapse at 2 yr did not differ significantly between CMV-sero-

negative and CMV-seropositive patients (8% vs. 19%; HR, 0.28; 95% CI, 0.04–2.15; $P = 0.22$) (Fig. 4B).

Survival

The probabilities of DFS at 2 yr after CBT were 92% in CMV-seronegative patients and 72% in CMV-seropositive patients (Fig. 5A). The probabilities of DFS at 2 yr did not differ significantly between CMV-seronegative and CMV-seropositive patients (HR for death or relapse, 0.24; 95% CI, 0.03–1.79; $P = 0.16$). Multivariate analysis showed that high-risk disease status was significantly associated with a lower probability of DFS at 2 yr (HR, 4.92; 95% CI, 1.66–14.6; $P < 0.005$).

Discussion

In the present study, we compared the outcomes of CBT between CMV-seronegative and CMV-seropositive patients. The outcomes of CBT for CMV-seropositive patients as well as CMV-seronegative patients in our series were favorable; therefore, statistical analyses did not show significant differences for the cumulative incidences of neutrophil engraftment, CMV disease, and TRM at 1 yr, and the probability of DFS at 2 yr between CMV-seronegative and CMV-seropositive patients after CBT. In addition, the results showing no significant differences in TRM and DFS between two groups may be due to the small patient numbers, particularly of CMV-seronegative patients.

The most striking finding was a marked higher incidence of antigenemia after CBT in CMV-seropositive patients than CMV-seronegative patients (77% vs. 0%, $P < 0.001$). In BMT recipients, the infusion of bone marrow cells from a CMV-seropositive donor was associated with the early recovery of CMV-specific T-cell responses after BMT, which indicates the important role of primed CMV-specific T cells from the donor marrow inoculum for the early recovery of CMV-specific immunity of recipients (16). Therefore, the lack of CMV-specific T cells in the infused CB units as well as the immunological immaturity of CB lymphocytes might be associated with the delayed recovery of CMV-specific immunity after CBT. However, CMV disease occurred only in one CMV-seropositive patient after CBT. Preemptive ganciclovir therapy might effectively reduce the incidence of CMV disease in CMV-seropositive patients after CBT.

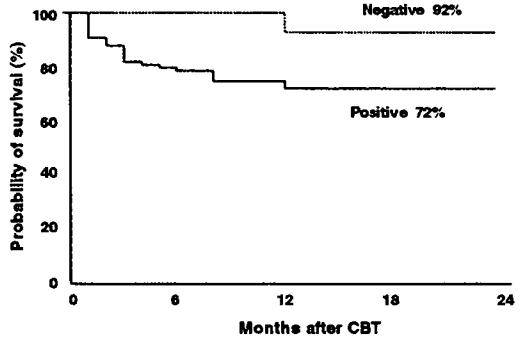


Figure 5 Probability of DFS at 2 yr after CBT.

Previous studies have suggested that CMV-seropositive BMT or PBSCT recipients, particularly those receiving T-cell-depleted grafts and/or grafts from HLA-mismatched or unrelated donors, have a substantial survival disadvantage after SCT (3–5). A higher incidence of infectious complications including CMV disease or severe acute GVHD is considered to be likely responsible for the poor outcome in CMV-seropositive recipients. In CBT recipients, the impact of CMV serostatus on various outcomes has not been studied in detail. In a recent study on CBT for adult patients (median age, 29 yr), the probabilities of DFS at 2 yr after CBT did not differ significantly between 55 CMV-seronegative and 106 CMV-seropositive patients (35% vs. 23%, $P = 0.17$) (17); however, the impact of CMV serostatus on outcomes other than survival was not shown. Other previous studies also did not clearly show the association between CMV serostatus and the outcomes of CBT for adult patients (6, 7).

The outcomes of CBT in CMV-seronegative patients in our series were considered to be excellent; however, the outcomes in CMV-seropositive patients, including the cumulative incidences of CMV disease and TRM at 1 yr, and the probability of DFS at 2 yr, were also favorable. This might be partly due to effective antiviral therapy for CMV infection. In addition, 99 of 101 recipient and donor pairs in the present study were Japanese. The lesser genetic diversity in a single ethnic population might be associated with the favorable outcomes of CBT for CMV-seropositive patients, such as a lower rate of fatal acute GVHD. As the CMV-seronegative population is small in Japanese adults, our study included only 16 CMV-seronegative patients. Large-scale studies are needed to determine the impact of CMV serostatus on the outcome of CBT for Japanese adults.

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Rapid Communication

Cytogenetic Remissions Induced by Interferon α and Imatinib Mesylate are Immunologically Distinct in Chronic Myeloid Leukemia

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Abstract

We compared immunologic parameters of chronic myeloid leukemia (CML) patients in cytogenetic remission receiving imatinib mesylate (STI) treatment, CML patients receiving interferon α (IFN-α), and healthy volunteers. Each group comprised 14 subjects. Median treatment dosages and durations were 6×10^6 IU/week and 174 months, respectively, for IFN-α and 400 mg/day and 54 months for STI. The numbers of T-cells were significantly lower in the 2 patient groups ($P = .0006$), whereas the 3 groups were comparable with respect to the numbers of natural killer cells. Not only the absolute numbers of monocytes and B-cells but also serum immunoglobulin G (IgG) and IgA titers were significantly lower in the STI group than in the IFN-α group ($P < .0001$). For T-cell subsets, the ratio of CD4 T-cells to CD8 T-cells was significantly lower in the IFN-α group than in the STI group, but the proportion of $CD26^{high}CD4^+$ T-cells among $CD4^+$ cells was significantly higher. Collectively, the 2 therapeutic agents induce a distinct immunologic status in CML patients whose hematopoiesis has returned to normal levels.
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Key words: CML; Cytogenetic remission; Interferon α; Imatinib mesylate

1. Introduction

Imatinib mesylate (STI), now the first-line therapy for chronic myeloid leukemia (CML) in the chronic phase, induces a complete cytogenetic response (CCyR) as well as a major molecular response in the vast majority of patients with newly diagnosed CML [1-3]. The long-term efficacy and feasibility of STI has also been confirmed in the IRIS trial [4], and its sustained administration is recommended for preventing disease progression and recurrence from minimal residual disease, which cannot readily be eradicated with STI alone [5-7]. On the other hand, recent articles have shown that STI inhibits not only the T-cell receptor–mediated proliferation and activation of effector T-cells [8,9] but also the development and function of dendritic cells [10,11], suggesting an immunosuppressive state in CML patients who have been treated continuously with STI. Moreover, a recent observation that interferon (IFN)-resistant or IFN-intolerant CML patients frequently showed hypogammaglobulinemia during the subsequent STI therapy suggests a therapy-related functional deficit in B-cells.

Prior to the STI era, IFN-α was the standard choice for the conventional treatment of CML in the chronic phase and was capable of inducing a CCyR in a sensitive but limited population (10%-20%) of patients with a new CML diagnosis [12,13]. Although the mechanisms involved in the selective inhibition of a Philadelphia chromosome–positive (Ph^+) clone by IFN-α have not yet been clarified, various clinical and laboratory observations strongly suggest that the activation of immunologic effector functions, including T-cell and dendritic cell responses, brought about by IFN-α may contribute to its life-prolonging effect [14].

These data taken together indicate that treatment with STI or IFN-α is likely to cause distinct immunologic alterations in CML patients, especially in therapy-sensitive long-term survivors. In the present study, we evaluated various immunologic

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parameters for CML patients in an STI-induced CCyR and for CML patients with an IFN-α–induced CCyR and detected significant differences between the 2 patient groups in the values for some of these parameters.

2. Patients, Materials, and Methods

2.1. Patients

Fourteen individuals were enrolled in each of 3 groups, the 2 patient groups plus a control group of healthy volunteers. Of the 14 patients in the STI group, 6 had a new CML diagnosis and had started STI treatment, and 8 patients had been treated with IFN-α but switched to STI at the beginning of the domestic release of STI in 2002. The latter 8 patients had already taken STI for approximately 5 years at the commencement of this study. All patients in the STI group have achieved both a CCyR [12] and a major molecular response. All patients in the IFN-α group had new diagnoses and had started IFN-α treatment. Ten of the patients in the IFN-α group are also in a CCyR, and the remaining 4 are very close to a CCyR (the incidence of the residual Ph^+ clone is between 3.0% and 6.7% by repeated fluorescence in situ hybridization analysis of 1000 interphase nuclei and a cutoff rate of 1.5%). The patients' profiles are summarized in Table 1. Peripheral blood cell counts and differential counts of leukocytes were measured with a Sysmex XE 2100 cell counter (Sysmex, Kobe, Japan).

2.2. Flow Cytometry

Peripheral blood was obtained after informed consent had been obtained and was directly stained with antibodies prior to the lysis of red blood cells with Lysing Solution (Beckman Coulter, Miami, FL, USA). Samples were analyzed by multicolor flow cytometry with an Epics XL instrument (Beckman Coulter) according to standard procedures, and results were analyzed with Expo32 ADC software provided with the Epics XL (Beckman Coulter). TruCount tubes (BD Biosciences, San Jose, CA, USA) were used in calculating the absolute number of blood cells staining for CD45, CD3, CD19, and CD56 markers. Fluorescein isothiocyanate (FITC)-conjugated anti-CD8α, anti-CD4–FITC, phycoerythrin (PE)-conjugated anti-CD4, ECD (PE–Texas red)-conjugated anti-CD3, RD1-conjugated anti-CD56, and PC5 (PE–cyanine 5)-conjugated anti-CD45 monoclonal antibodies were purchased from Beckman Coulter, and anti-CD4–PE, anti-CD25–FITC, and anti-CD19–FITC were from BD Biosciences. Anti-CD26–PE and anti-Foxp3–PE were obtained from eBioscience (San Diego, CA, USA).

2.3. Statistical Analysis

Flow cytometry results were analyzed with JMP 6.0.2 software (SAS Institute, Cary, NC, USA). Statistical analyses were performed with Wilcoxon and Kruskal-Wallis tests, with a P value of .05 regarded as statistically significant.

3. Results and Discussion

Prior to the approval of STI, all of the patients in the IFN-α group had already achieved major cytogenetic responses, and IFN-α has been gradually tapered to a minimal dose to maintain their responses. The standard dose has principally been continued in the STI group. Accordingly, the treatment durations for the 2 groups are markedly different, but the age distributions for the 2 groups are comparable. In both patient groups, white blood cell counts were well controlled at levels that were moderately lower than those in the control group of healthy volunteers (5.50×10^9/L in the control group, 4.18×10^9/L in the IFN-α group, and 3.95×10^9/L in the STI group). Red blood cell counts and hemoglobin levels were significantly reduced in the STI group compared with the other 2 groups ($P < .0001$), and platelet counts were lower in the IFN-α group than in the control group ($P < .01$) (Table 1).

TruCount tubes were used in the calculation of the absolute number of T-cell subsets and other immune cells. The total num-

Table 1.
Characteristics of the Patients and Healthy Control Individuals*

	Control (n = 14)	IFN-α (n = 14)	STI (n = 14)	P
Male/female sex, n	10/4	12/2	9/5	—
Age, y†	41 (30-63)	50 (39-73)	54 (29-65)	—
Treatment†				
Duration, mo	—	174 (61-235)	54 (7-61)	—
Dosage	—	6×10^6 IU/wk ($3\text{-}18 \times 10^6$ IU/wk)	400 mg/d (300-400 mg/d)	—
Blood data‡				
WBC, $\times 10^9$/L	5.50 ± 1.21	4.81 ± 1.36	3.95 ± 0.89	.0056
RBC, $\times 10^{10}$/L	479.7 ± 33.4	435.7 ± 55.1	357.9 ± 45.0	<. 0001
Hemoglobin, g/dL	14.4 ± 0.9	13.7 ± 1.5	11.8 ± 1.5	<. 0001
Platelets, $\times 10^9$/L	223 ± 59	153 ± 50	185 ± 23	.0057

*IFN-α indicates interferon α; STI, imatinib mesylate; WBC, white blood cells; RBC, red blood cells.
†Age and treatment data are presented as the median (range).
‡Blood data are presented as the mean ± SD. Asterisks indicate statistically significant difference.

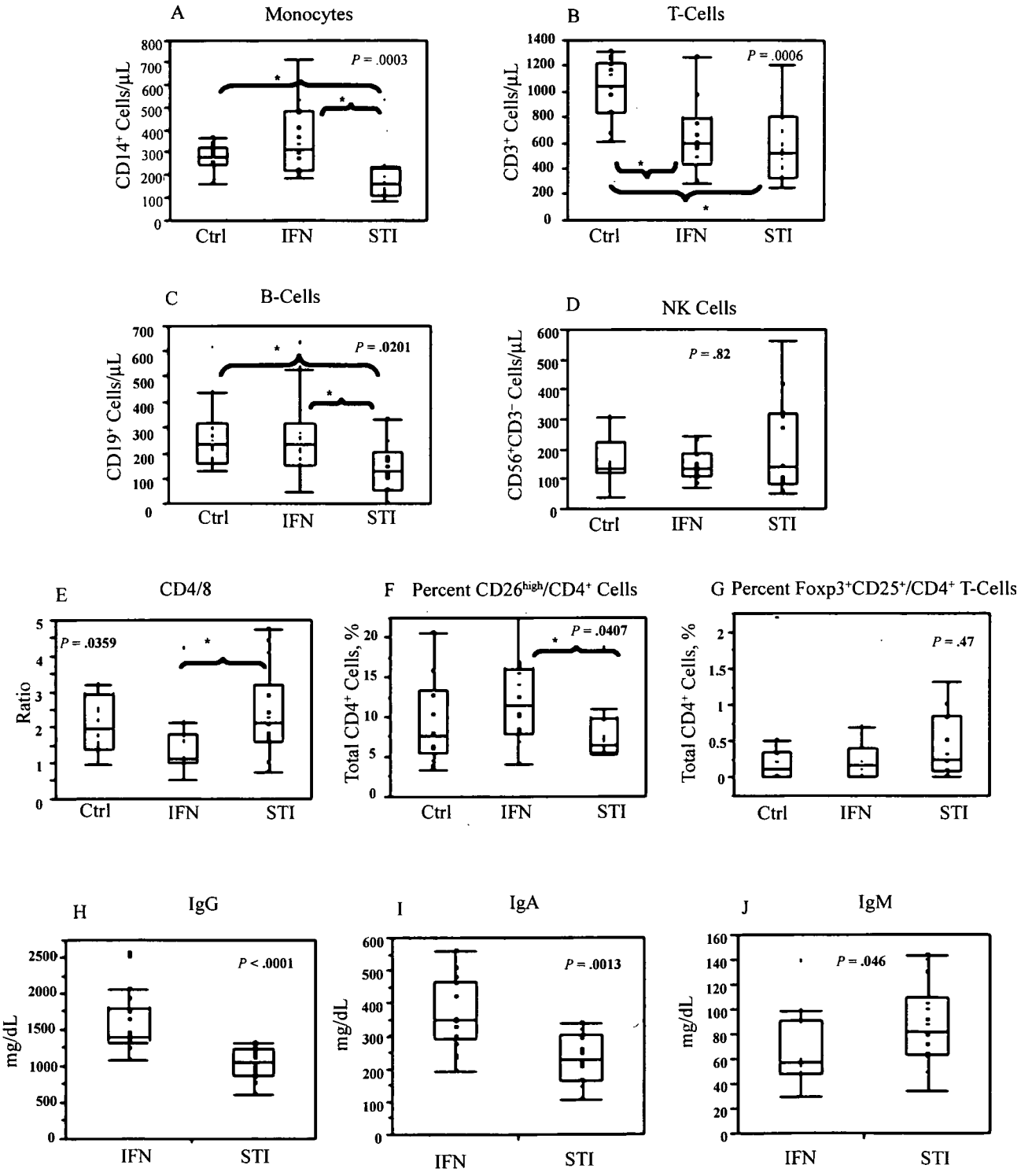

Figure 1. Immunologic parameters in chronic myeloid leukemia patients under interferon-α (IFN-α) or imatinib mesylate (STI) therapy. The absolute numbers of $CD14^+$ monocytes (A), $CD3^+$ T-cells (B), $CD19^+$ B-cells (C), and $CD56^+CD3^-$ natural killer (NK) cells (D) were measured by flow cytometry with TruCount tubes and were evaluated in these 2 groups and the control group of healthy volunteers (Ctrl). The CD4/CD8 T-cell ratio (CD4/8) (E), the proportion of $CD26^{high}$ cells among $CD4^+$ T-cells (F), and the proportion of $Foxp3^+CD25^+CD4^+$ T-cells among $CD4^+$ T-cells (G) were also obtained via flow cytometry. Serum titers for immunoglobulin G (IgG), IgA, and IgM (H-J) were measured simultaneously. Box-and-whisker plots show the median, interquartile range, and 95% confidence intervals. The sample numbers in the 3 groups are the same (n = 14). We used the Wilcoxon or Kruskal-Wallis test with JMP 6.0.2 software (SAS Institute) to calculate the statistical significance of differences between groups.

ber of T-cells was significantly suppressed in both patient groups compared with the control group ($P = .0006$), whereas the numbers of natural killer cells in the 3 groups were not different (Figures 1B and 1D). Only the STI group showed significantly reduced numbers of monocytes and B-cells. In particular, the absolute mean (±SD) B-cell count in the STI group was 1.38 ± 0.94 × 10^8/L, a count approximately one half that of the mean values for the control group (2.67 ± 1.34 × 10^8/L) and the IFN-α group (2.61 ± 1.67 × 10^8/L) ($P = .0201$).

Although the absolute T-cell counts were significantly-lower in the 2 patient groups than in the control group but were comparable with each other, the CD4/CD8 ratios of the 3 groups were significantly different (2.08 ± 0.8 in the control group, 1.5 ± 0.90 in the IFN-α group, and 2.4 ± 1.2 in the STI group; IFN-α versus STI, $P = .0359$) (Figure 1E), clearly indicating a relatively CD8-dominant pattern in the IFN-α group. We also assessed the proportion of $CD26^{high}$ cells among $CD4^+$ cells. CD26 is known to be a dipeptidylpeptidase IV, which is mainly expressed on T-cells among blood cells and is up-regulated upon activation. $CD26^{high}CD4^+$ T-cells are considered to represent effector memory T-cells of a typical type 1 helper T-cell phenotype [15]. Recently, the allergen dose–dependent recruitment of $CD4^+CD26^+CD25^+$ T-cells was noted in a study of a rat model of asthma [16]. Intriguingly, the IFN-α group showed a higher proportion of $CD26^{high}$ cells among $CD4^+$ T-cells than the STI group (12.1% ± 5.0% in the IFN-α group and 7.9% ± 3.6% in the STI group) (Figure 1F), whereas the absolute counts were reduced in the 2 patient groups. Regulatory T-cells (Treg cells), which are characterized by their $Foxp3^+CD25^{high}CD4^+$ phenotype, are implicated in the maintenance of immunotolerance. Treg cells have an attenuated cytokine response to T-cell receptor stimulation and can suppress the proliferation and effector function of neighboring T-cells [17]. The expression of Foxp3, an essential transcription factor required for Treg cell development, is detectable by flow cytometry with an intracellular staining method. We studied whether absolute Treg cell counts or their relative proportions are influenced by the treatment option used. The absolute numbers and relative proportions of Treg cells were not different among the 3 groups (Figure 1G).

To assess the consequences of reduced peripheral blood B-cell counts in the STI group, we compared immunoglobulin A (IgA), IgG, and IgM levels in the 2 patient groups (Figures 1H-1J). Although all values were within the normal ranges, serum IgG and IgA titers were significantly decreased in the STI group compared with the IFN-α group. On the contrary, IgM values were not significantly different. Mean IgG and IgA values were 1563 ± 395 mg/dL and 370 ± 106 mg/dL, respectively, in the IFN-α group and 1032 ± 210 mg/dL and 232 ± 71 mg/dL in the STI group ($P < .0001$, and $P < .0013$, respectively). Therefore, these data suggest that STI therapy relatively impaired B-cell effector function, as has previously been reported [18].

Our data indicate that the 2 CML therapeutic agents induce a distinct immunologic status in CML patients and raise the possibility that immunologic surveillance of residual Ph^+ clones may be defective in STI-treated patients. Finally, periodic monitoring of immunologic parameters is recommended for CML patients undergoing prolonged STI therapy.

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