Table 2
Comparison of the effect of fresh BMC and DC prepared from male donor on sensitization of female recipients

Experimental animals	Source of cell used for sensitization	Source of cells	Graft survivals (days)[a]	Median of survival times (MST)
LEW ♂ → ♀ LEW	IV[b]	Fresh BMC	12, 12, 13, 15, 16, 17	15
	SC[b]		14, 14, 15, 17, 78	14
	IV	DC[c]	21, 27, 42, 58, > 100	42
	SC		10, 11, 12, 18, 23, 57	15
DA ♂ → ♀ DA	IV	Fresh BMC	68, 9× > 100	100
	SC		19, 21, 23, 28, 2× > 100	25.5
	IV	DC	23, 17× > 100	100
	SC		17, 18, 24, 33, 48, 3× > 100	40.5
PVG.RT1U ♂ → ♀ PVG.RT1U		Fresh BMC	ND[d]	
	IV	DC	4× > 100	100
	SC		8× > 100	100

[a] Following the immunization of female rats with male cells, one month later the recipient rats received male skin isografts as described in Section 2. The fate of the graft was followed.
[b] Fresh male BMC without culture were prepared as described in Section 2. Two doses of 20×10^6/0.1 ml of cells were injected either in the foot pads (subcutaneous, SC) or through the tail vien (IV).
[c] Male DC from the cultured BMC as described in Section 2 were prepared. Two doses of 0.1×10^6/0.1 ml of cells were injected either in the foot pads (SC) or through the tail vien (IV).
[d] ND: not determined.

specifically. Although in mouse DC, CD11c is widely used to define a subset of DC, equivalent rat CD11b/c (OX42) was expressed at an extremely low level or null on rat DC described here. Furthermore, classification of mouse *plasmacytoid* DC generated by Flt3 ligand is characterized by CD11c expression with B220 (CD45R), and proposed by others to distinguish the *plasmacytoid* or *lymphoid* DC from myeloid DC. The latter differentially expressed CD11b but not B220 [28]. With this respect, the rat DC generated in this study appears to belong to neither typical plasmacytoid nor lymphoid DC subsets.

As for the DC specific marker, CD103 (MRC-OX62) has been characterized by Brenan and Puklavec [16] and has been considered to be a specific marker for a subset of DC. Two subsets of rat DC have been reported; (i) $CD103^-$ phenotype consisting of DC in the epidermal Langerhans cells [16], and (ii) $CD103^+$ phenotype consisting of DC-like veiled cells in the thoracic duct lymph (TDL) [16,29,30]. Additionally, $CD161a^+$ (also defined as NKR-P1A [23,31]) was found to be positive for a subset of rat DC. It is tempting to define rat DC by two markers, CD103 and CD161a. By this criteria, DC driven by Flt3/Flk2 ligand and/or IL-6 appear to be defined $CD161a^+$ $CD103^-$. In this study, we confirmed and extended the study by Brissette-Storkus et al. [32]. These investigators also described a short-term rat BMC culture driven by *Flt3/Flk2 ligand* in FBS based culture medium resulting in $CD161a^+$ $CD103^-$ phenotype DC, although the expression level of CD161a appears to be far below that of our study. Nevertheless, the level of CD161a as well as class II expression on rat DC cultured in the present study gradually increased during a long-term culture. It appears that the level of CD161a is intimately related to the maturation processes of DC progeny. Whether CD161a expression of DC during the maturation processes is a general feature of the DC subset or only a unique characteristic of rat DC remains to be determined.

The view of that GM-CSF plays a primary role in growth and differentiation of DC precursor cells, was first suggested by Steinman and his colleagues in mouse systems [8,9,33]. However, based on our study, it is tempting to postulate that GM-CSF per se has a limited capacity to increase DC progenitors of BMC but instead acts as a differentiation factor in general. It should be noted that both the GM-CSF deficient mouse and the GM-CSF receptor deficient mouse contained a substantial number of DC, although the level of the cell number was significantly lower than both the normal mouse and the GM-CSF transgenic mouse [34]. In this regard, our preliminary and on-going studies (manuscript in preparation) demonstrate that our culture systems are likewise applied to generate *myeloid DC* from BMC, not only from the GM-CSF deficient mouse and GM-CSF receptor deficient mouse [35], but also in the human system. Thus, our culture systems demonstrate that Flt3/Flk2 ligand combined with IL-6 is able to replace the effect of GM-CSF on the outgrowth of DC precursors from the BMC culture. These results strongly suggest a common pathway, which is *GM-CSF independent myeloid DC* development [15,36,37]. Different subsets of DC-precursors might require different cytokines, depending on the cell-differentiation stages. Nevertheless, at least in the rat system, the absolute requirement of exogenous GM-CSF for BMC-derived *myeloid DC induction* appears to be less likely.

As for the function of *GM-CSF independent myeloid DC*, the APC function of CD161a$^+$ DC generated by *GM-CSF independent* rat BMC culture driven by Flt3/Flk2 ligand and IL-6 was verified by in vitro syngeneic MLC as well as in vivo sensitization of minor histocompatibility antigen H-Y, which is considered to be a prototype of tumor immunology. Thus, the simple culture methods described here would facilitate the acquisition of a large number of highly uniform subsets of DC for cellular immunology research including transplantation biology as well as tumor immunology.

Heretofore, valuable information and knowledge have been accumulated on the prerequisite cytokines for DC development and classification of DC subsets. However, it remains to be determined whether and to what extent each cytokine, or cytokines in concert, play a crucial role in growth and/or differentiation of particular DC subsets from hematopoietic stem cells, and whether to what extent so-called DC specific markers are stably expressed during the their life span. Our simplified culture method described here may be applied to further investigation of the functional and developmental aspects of DC subset.

In summary, we have demonstrated that GM-CSF per se is not able to support a significant growth of DC progenitors in rat BMC, regardless of the sources (mouse, rat, human). Combined with Flt3/Flk2 ligand and IL-6, not with c-kit ligand and IL-6, undefined DC progenitors emerge, and these cytokines appear to be able to increase DC progeny from rat BMC. Thus, these expanded DC precursors are able to differentiate into fully mature DC in both phenotype and function as specialized APC. Functionally full-mature DC express at a high level of CD161a and hence the mature DC were highly purified by this single cell surface marker. With additional cytokines such as murine GM-CSF, TNF-α and IL-4, it appears that heterogeneity of phenotypic and functional DC [38–40] is generated. These additional cytokines in concert, not a single cytokine, might enable generating phenotypic and functional diversity of DC in vivo.

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Plasma exchange; a promising treatment for toxic epidermal necrolysis with AIDS

Toxic epidermal necrolysis (TEN) is a life-threatening, drug-induced mucocutaneous intolerance reaction. Patients with AIDS are reported to have a much higher risk of TEN than the general population [1]. Therapeutic strategies for TEN are still controversial. The administration of systemic corticosteroids or immunosuppressants for TEN has been an issue of debate. Furthermore, the use of immunosuppressants in AIDS patients remains controversial as there is a risk of further immune suppression from the viral disease. Here we report a case of TEN with AIDS that was successfully treated with plasma exchange.

A 41-year-old man from Zambia presented with fever, general fatigue and haematuria at the outpatients' clinic in July 2003. He was diagnosed as having urinary and miliary tuberculosis, which turned out to be a complication of AIDS. Isoniazid, rifabutin, ethambutol hydrochloride and pyrazinamide were administered to treat the tuberculosis. In addition, trimethoprim–sulphamethoxazole was also prescribed prophylactically for the treatment of *Pneumocystis carinii* pneumonia. After 2 months of these combined therapies, widespread erythema with fever subsequently appeared. Flaccid bullae developed shortly after over the erythema, which resulted in the formation of extensive erosions involving 90% of the body surface area (Fig. 1a). The Nikolsky sign was positive. Penile and oral mucosal erosions and bilateral conjunctivitis were also observed. A diagnosis of TEN was made, and he was admitted to our hospital on 8 October 2003. At the time, his HIV-RNA level was 121 600 copies/ml and his CD4 cell count was 15 cells/mm^3. All the treatments were discontinued and 1000 mg methyl-prednisolone pulse therapy was started on the admission day. As blister formation and skin detachment were not halted by systemic steroid therapy, plasma exchange was performed for three consecutive days from 9 October. In total, 10 l plasma were filtered. Three days after this plasma exchange, the patient's Nikolsky sign became negative. In 2 weeks, his skin condition also improved dramatically (Fig. 1b).

TEN is one of the most severe adverse drug reactions and the mortality rate has been reported to be approximately 30% [2]. This critical skin disorder is characterized by extensive apoptosis of keratinocytes, resulting in blistering and detachment of the epidermis. The incidence of TEN in AIDS patients has been reported to be much higher than in the general population [1]. The reason for this is speculated to be (i) that with the decrease in CD4 cell counts (especially less than 200 CD4 T cells/mm^3), the incidence of drug eruptions will increase significantly [1]; and (ii) AIDS patients are frequently exposed to trimethoprim–sulphamethoxazole, the prophylactic, and the treatment for *Pneumocystis carinii* infection [3], and antiretroviral drugs, which are reported to be the main causative drugs of TEN.

There is currently no evidence-based specific treatment for TEN. Some retrospective studies have claimed a benefit with the use of corticosteroids [4], whereas other reports showed no efficacy or even increased morbidity and mortality [5]. Meanwhile, intravenous immunoglobulin (IVIG) [6], plasma exchange [7] and immunosuppressants [8] have been reported to be effective in some individual cases. However, for AIDS patients with TEN, the administration of systemic corticosteroids or immunosuppressants has been an issue of debate because there is a risk of further immune suppression.

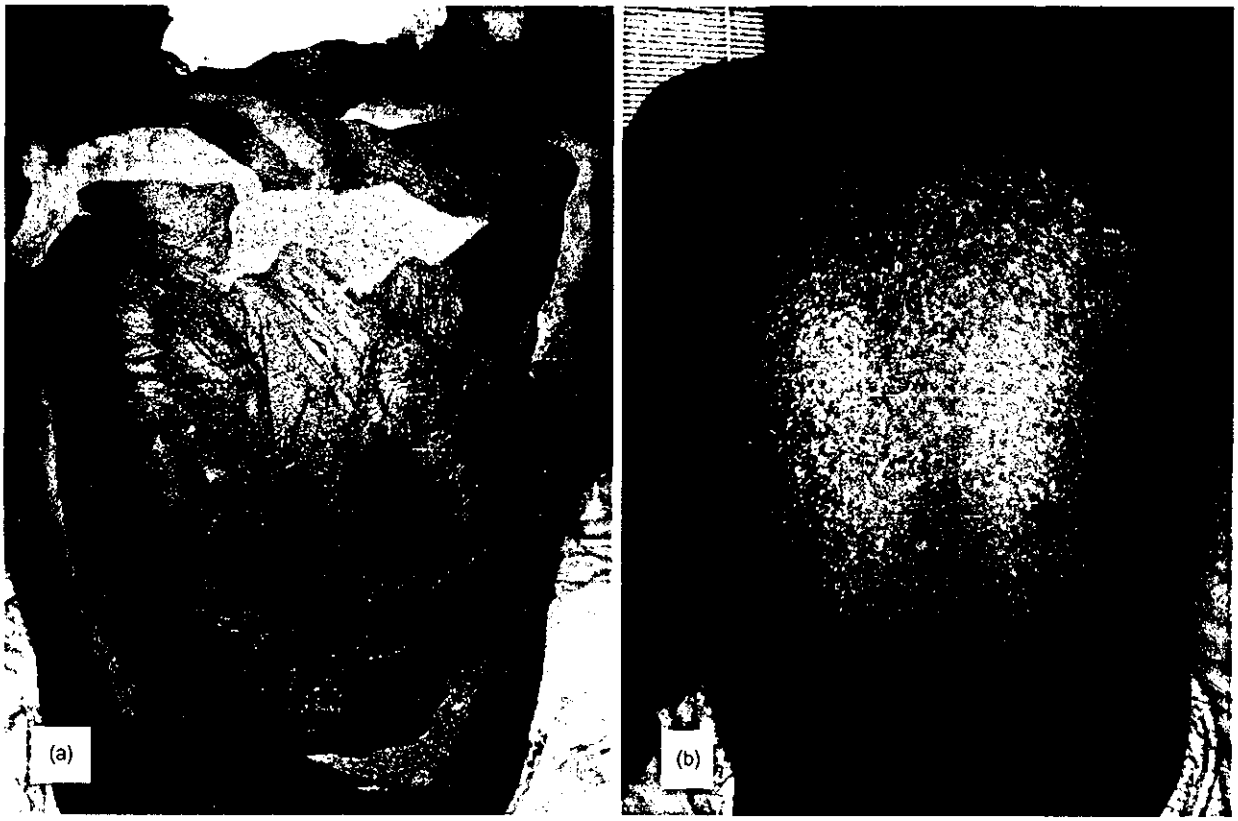

Fig. 1. Photographs of the patient's back. (a) Widespread erosions and bullae were seen on the back. The epidermis was easily detached (positive Nikolsky sign). Approximately 90% of the body surface area was involved. (b) Two weeks after plasma exchange. Significant re-epithelization and a drastic improvement were obtained.

Although the pathogenesis of TEN remains obscure, it is reported that the interaction of Fas, an apoptosis receptor, and soluble Fas ligand plays an important role in TEN by inducing the apoptosis of keratinocytes [9]. Plasma exchange and IVIG might inhibit Fas-mediated keratinocyte death by removing soluble Fas ligand and blocking Fas receptors [6], respectively. Some cases of TEN with AIDS have been reported to have been successfully treated with IVIG [10–12]. Plasma exchange might be considered a more appropriate treatment when patients have renal dysfunction, because acute renal failure might also be made more likely by the use of IVIG [13].

In the present case, systemic corticosteroids were used at first to retard the disease progression; however, no apparent efficacy was observed. Accordingly, we chose plasma exchange, which turned out to be very effective in a short time. This is the first case report of the effective use of plasma exchange for TEN with AIDS. As HIV infection is now spreading, TEN in AIDS patients is also expected to increase in numbers. We consider that plasma exchange might become a more promising treatment for TEN, especially when patients are in an immunologically suppressed condition such as AIDS.

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症例報告

AIDS治療中に免疫再構築症候群による肺非結核性抗酸菌（*Mycobacterium kansasii*）感染の寛解が得られた1例

A Case of AIDS treated with HAART (higly active antiretroviral therapy) which was in remission of nontuberculous mycobacterium (*Mycobacteriuum kansasii*) infection in the process of the immune reconstitution syndrome

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要　旨

症例は27歳男性．近医より呼吸器易感染性精査目的にて紹介入院．入院後CD4陽性リンパ球数5/μl，HIV-1RNA定量63万コピー/mlよりAIDSと診断．AZT＋3TC＋NFVの3剤によるHAART（highly active antiretroviral therapy）を開始した．投与開始7日目に高熱がありLevofloxacin（LVFX）を併用したが，投与開始14日目に胸部レントゲン写真上，左肺門周囲の肺炎像が出現．喀痰の抗酸菌培養同定にて*Mycobacterium kansasii*を認めた．HAARTとLVFXを継続投与し肺炎像は28病日をピークに改善した．以上から，AIDSに伴う免疫再構築症候群の経過中に*Mycobacterium kansasii*が検出されたものの，生体防御反応の正常化に伴い，感染症としての悪化，遷延化を免れたものと考えられた．

Key words：AIDS，免疫再構築症候群，HAART，非結核性抗酸菌

はじめに

免疫再構築症候群[1]は進行したAIDS患者において，すでに存在する病原性微生物に対し急激に回復した免疫能により一時的に感染症状の悪化をみることである．通常の感染のように病原性微生物の増殖と生体防御反応の伴った病態というより，生体防御反応の過剰反応による感染症状の悪化が主体であると考えられる．

今回，我々はHAART開始による免疫力の回復に伴い免疫再構築症候群をおこし，非結核性抗酸菌（*Mycobacterium kansasii*）が喀痰から培養同定され，肺炎像をきたしたが，HAARTと抗菌剤を継続併用することにより改善した1例を経験したので報告する．

症　例

患　者：27歳　男性．
既往歴・家族歴：特記すべきことなし．
現病歴：約2年前より口腔カンジダ症を反復．平成14年1月より発熱を繰り返していた．平成14年7月より呼吸困難，胸痛，発熱を主訴に近医入院．入院中に間質性肺炎と診断されメチルプレドニゾロンのパルス療法を施行された．呼吸器症状

は消失し画像上，間質性肺炎は軽快したものの呼吸器易感染性の精査目的にて当院転院となった．

転院当日，HIV-1陽性であることと，入院中1回のみ喀痰の抗酸菌鏡検にてGaffky1号であることが判明したとの報告を前医より電話で受けた．

入院時現症：身長162cm．体重60kg．体温37.0℃．脈拍66/分，整．血圧118/78mmHg．呼吸回数14/分，整．眼結膜に貧血，黄疸なし．胸部聴診上異常なし．心音は清，心雑音聴取せず．表在リンパ節触知せず．腹部平坦かつ軟，肝脾触知せず．神経学的異常所見なし．入院時呼吸器症状は全く認められなかった．

入院時検査所見（表1）：白血球数2800/μlと減少．CD4陽性リンパ球数5/μlと著明に減少．HIV-RNA量63万コピー/mlと高値．入院時の胸部レントゲン写真にて左下肺野にわずかに間質性陰影を認めた．

入院後経過：AIDSと診断し，AZT+3TC+NFVの3剤によるHAARTを開始した．治療開始7日目に39度を超える発熱と，左胸部痛，咳嗽，喀痰を認めた．このときは胸部レントゲン写真上，入院当初認めた間質影は消失しており，他にも異常なく，炎症所見も白血球4200/μl，CRP 0.1mg/dlと異常を認めなかった．免疫状態が極端に抑制されているAIDS患者であることを考慮し，細菌感染の合併も否定し得ないため，LVFX 300mgの投与を開始した．39度を超える発熱は3日続いた後，自然に解熱，呼吸器症状も消失した．

表1　入院時検査所見

・WBC	2800 /μl	T-Bil	0.3 mg/dl	HIV-1 EIA	
・Ly	12 %	LDH	237 IU/l	WBとも陽性	
・Hgb	12.8 g/dl	γGTP	53 IU/l	CD4陽性リンパ球数	
・PLT	24.6 × 10^4 /μl	GOT	55 IU/l	5 /μl	
・T.P	6.5 g/dl	GPT	63 IU/l	CD8陽性リンパ球数	
・Alb	3.8 g/dl	CK	24 IU/l	130 /μl	
・BUN	10 mg/dl	AMY	67 IU/l	HIV RNA定量	
・Cre	0.6 mg/dl	CRP	0.1 mg/dl	6.3 × 10^5 コピー/ml	
・Na	140 mEq/l				
・Cl	106 mEq/l	検尿 蛋白	(−)		
・K	4.0 mEq/l	糖	(−)		
・Ca	8.8 mg/dl	潜血	(−)		

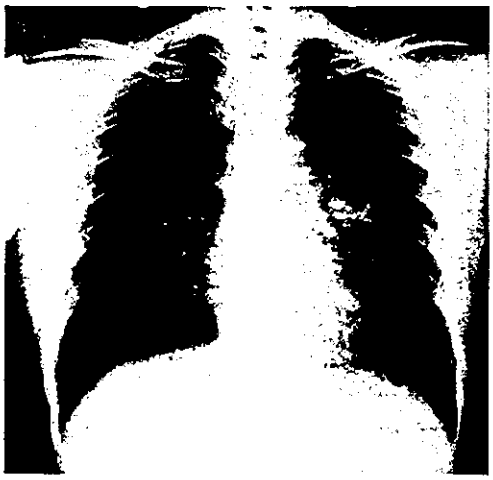

図1　胸部レントゲン写真・左肺門周囲に肺炎像を認める

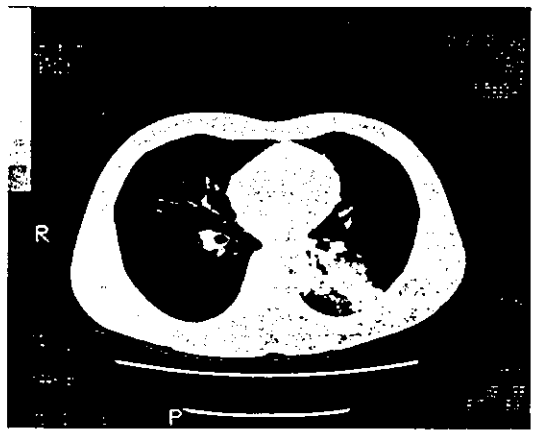

図2　胸部CT検査・左S6中心の肺炎像を認める

喀痰の一般培養を2回行ったが口腔内常在菌しか認められなかった．一方，喀痰塗抹検査でGaffky3号の抗酸菌が検出されたもののPCR法では結核菌，MACとも陰性であった．

HAART開始14日目に左肺門周囲に肺炎像の出現をみた（図1）．このときCD4陽性リンパ球数100/μl，HIV-RNA量7700コピー/mlと免疫能が改善しウイルス量は低下していた．炎症反応は白血球数12700/μl，CRP4.5mg/dlと上昇していたが発熱，呼吸器症状とも認められなかった．

HAART開始約1カ月後に胸部レントゲン写真上，左肺門周囲の肺炎像はピークに達しCT（図2）にてもS6中心の一部器質化した肺炎像を認めた．このとき白血球数7200/μl，CRPは7.0mg/dl

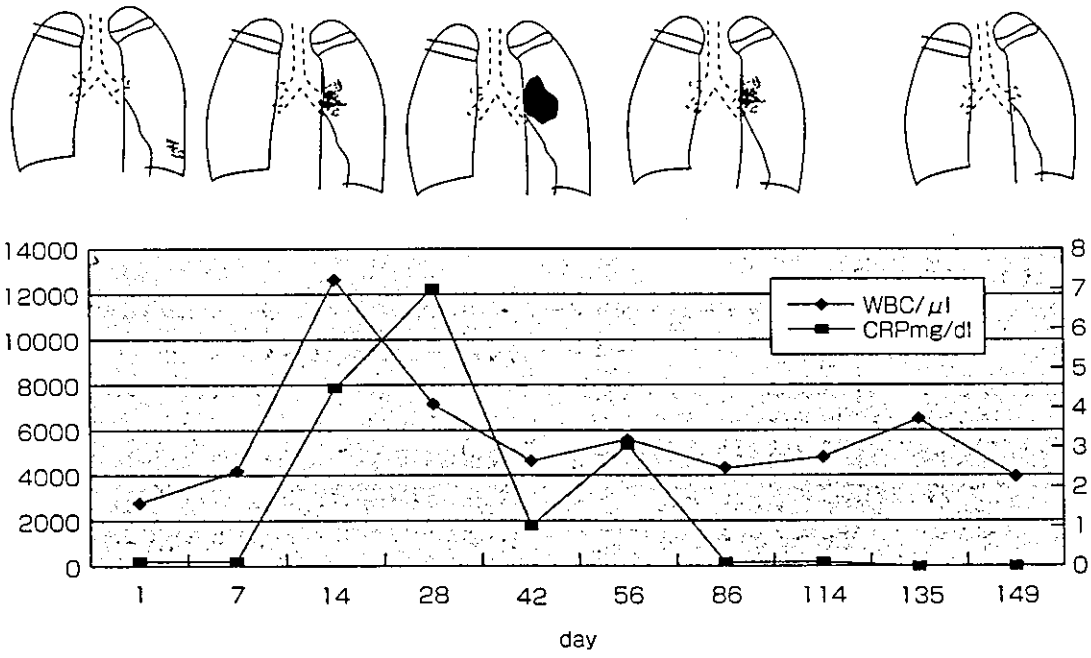

図3　臨床経過

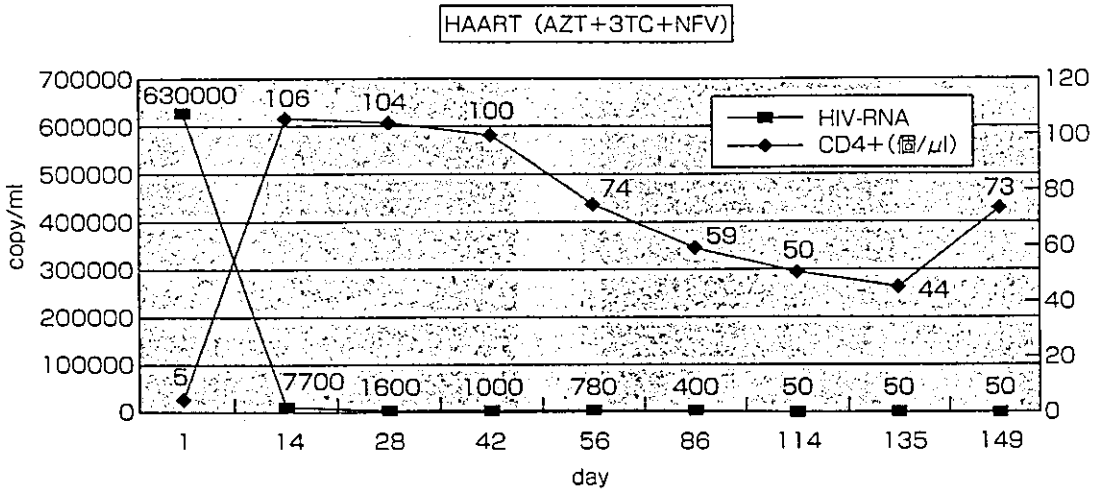

図4　臨床経過

と上昇していたが発熱，呼吸器症状を認めなかった．ほぼ同時期に喀痰の抗酸菌培養にて*Mycobacterium kansasii*が同定された．感染症状に遅れて白血球数が上昇し，さらに遅れて画像所見の悪化とCRPの上昇をみている．

画像所見は第28病日をピークに以降，次第に改善した．図3に白血球数，CRP定量の経過と胸部レントゲン所見のスケッチを記す．CD4リンパ球数は42病日に100個/μlを超え以降やや減少に転じたものの基本的には増加している．ウイルス量は激減し第114病日に測定感度以下となった．図4にCD4陽性リンパ球数，HIV-RNA量の変化を記す．

考　察

本例ではHAARTの効果により急激に免疫能が改善されたため，潜在していた病原性微生物に対して免疫再構築症候群による肺炎をきたしたと考えられたが感染症としての悪化，遷延化には至らなかった．すなわちHAARTを継続し抗菌薬を併用し，生体防御反応の正常化を待つことにより免疫再構築症候群を乗り切ることができたと考えられる．

AIDSの治療がプロテアーゼ阻害薬を含む強力なHAARTが中心になり，免疫再構築症候群は生体防御反応が正常化する過程で報告されるようになってきた[2]．非結核性抗酸菌感染についてはリンパ節炎の報告が多い[3,4]．また眼科領域ではCMV（サイトメガロウイルス）による網膜炎の報告がある[5]．CMV網膜炎は[1]進行したHIV感染症患者において，抗CMV治療を行わなくともプロテアーゼ阻害薬単独で軽快することが報告されている[6]．日和見感染で発症したAIDS患者ではHAART開始前に感染を制御することが重要である．本症例ではHAARTを開始する前に呼吸器症状，感染症状を認めなかった．しかしHAART開始後，免疫能の改善に伴い免疫再構築症候群を発症し，喀痰培養で肺炎の起因菌になりうる有意な一般細菌は同定されず塗抹検査で抗酸菌が検出された．これが培養により*Mycobacterium kansasii*と判明した頃にはすでに症状は消失している一方でWBC，CRP上昇と胸部陰影が明らかとなった．この時点で通常の感染というよりは免疫再構築症候群による感染の一時的増悪と判断し，非結核性抗酸菌感染に対して抗結核薬を使用せず，HAARTを中断することなく抗菌剤併用を継続した．その結果，生体防御反応の正常化とともに肺炎像は次第に改善した．HAARTによるAIDS治療においては，潜在している病原性微生物に対する注意が必要である．またHAART治療開始後に感染症状が出現した時には，あらたな感染と免疫再構築症候群に伴う感染との鑑別は困難である．しかし，免疫状態が改善されている時期に感染症状や炎症を示す検査所見，画像所見の時間的な乖離が著しいときは免疫再構築症候群による感染の悪化を疑い，慎重に経過を見極めるべきであると考えられた．なお本論文の要旨は第170回日本内科学会東北地方会（仙台，2003）にて発表した．

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わが国のHIV/AIDS患者に合併する寄生虫症

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Key Words：エイズ，HIV感染症，寄生虫症

はじめに

わが国では，エイズおよびHIV感染症に合併する寄生虫症は，その数が余り多くないために，患者の医療における重要性の認識が低く，適切な診断・治療の体制整備が遅れている。エイズ指標疾患に入る寄生虫症は，トキソプラズマ脳症，クリプトスポリジウム症，イソスポラ症の3疾患で，患者の免疫

Parasitic Diseases Complicated with HIV/AIDS Patients in Japan

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力が低下してきた際の日和見原虫感染症である。さらにエイズおよびHIV感染症に高率に合併する寄生虫症としては，赤痢アメーバ症，ランブル鞭毛虫症，糞線虫症などが知られている[1]。全国360余のエイズ拠点病院にアンケート用紙を配布し，エイズ／HIV感染症患者に合併した上記寄生虫症の調査を行うなど，わが国における同合併症の疫学特性を把握するための研究を展開したので報告する。

赤痢アメーバ症

全国のエイズ拠点病院（368施設）にアンケート用紙を配布し，平成15年度にHIV感染症患者に合併した赤痢アメーバ症の患者背景について調査を行った。

回収率は223施設（61％）で，175施設はHIV感染症患者の診療経験があり（175/223＝78％），赤痢アメーバ症の診療経験のある施設は28施設（28/223＝13％），症例数は38例であった。すべて男性症例で，平均年齢37歳，33例はMSM（Men who have sex with men）によるSTD（Sexually transmitted disease）感染と考えられた。CD4数の平均は306/μl，HIVウイルス量の平均は2.2×10^4コピー/mlであった。

赤痢アメーバ症の病型は，腸炎18例（18/33＝55％），肝膿瘍13例（39％），腸炎と肝膿瘍の合併3例，その他の部位の膿瘍2例であった。

すべての症例でメトロニダゾールが治療薬として用いられ，抗嚢子薬フロ酸ジロキサニド（フラミド®）が16例（42％）で使用されていた。転帰としては完治12例，改善24例，不変1例，死亡1例（アメーバ症によるものではなく原疾患である悪性リンパ腫によるもの）であった。

わが国のエイズ／HIV感染症に合併する赤痢アメーバ症は，CD4数の平均値から考えて患者の免疫状態は比較的良好であり[2]，また海外旅行歴が少ないのに対し，梅毒やB型肝炎などの合併が多かった。すなわち感染は，流行地における食事等を介した糞口感染としてではなく[3]，また日和見感染としてでもなく[4]，MSMにSTDとして感染していると考察できた。

トキソプラズマ症

全国のエイズ拠点病院362施設にアンケート調査を行った。回収率は235施設（65％）で，169施設はHIV診療経験があり（169/235＝72％），トキソプラズマ症の診療経験のある施設は29（29/235＝12％），症例数は55例であった。これは全5,147例のHIVの1.07％にあたる。男性48例，女性6例，不明1例。全症例の平均年齢は41.2歳（2例は年齢記載なし）。CD4数の平均値は44.3/μlであった。HIVウイルス量は測定限界以下から最高では8.8×10^5/mlまで幅広く分布した。すなわち，ウイルス量＜10^3/ml：6例，同＜10^4/ml：4例，同＜10^5/ml：13例，同＜10^6/ml：8例。感染経路の検討では，非加熱血液製剤によるHIV感染者は7例（12.7％），同性間性交渉による者は10例（18.2％），異性間性交渉による者は31例（56.4％），その他7例（12.7％）であった。

本調査結果は，対象施設をエイズ拠点病院と限定はしているものの，その症例数から信頼度は高いものと考えられた。また，調査期間を限定せず過去から現在までのすべての症例について回答を得たが，トキソプラズマ症合併例のHIV感染経路の大半は性感染によるもの（74.6％）であることや，58％の症例で抗HIV療法が施行されていることを考慮すると，集められた症例のほとんどは最近10年程度に経験されたものが多いものと思われた。合併率は1.07％であったが，一般には2％程度といわれていることから低い数値と思われるが，アンケート報告された5000例を超えるHIV症例が最近10年くらいの症例へのかたよりがあると過程すると，ST合剤を用いたカリニ肺炎の予防がトキソプラズマ症の発症の予防につながっている可能性があると考えられた。それと同時に，抗HIV療法は55症例中の半数強に施行されているのみであることから，感染予防をさらに充実させればトキソプラズマ症の合併頻度をさらに低下させることができる可能性があると考えられた。

ジアルジア症

愛知県内の大型10病院（500床以上）にアンケー

ト調査を行ったが，ジアルジア症の報告はなかった。ジアルジア症は，やはりMSMにSTDとして感染することが想定される。名古屋医療センターでは，従来一般患者（HIV陰性患者）からのジアルジア症の検出が続いていたが，最近報告がなくなっていることも考慮に値する。

国立国際医療センターでは，海外渡航歴の無い下痢を主訴とした患者に，HIV感染を認めた2例を最近経験したが，ジアルジア症の重症化がエイズで起こることが疑われた。

一般的にエイズでの合併の報告がわが国で少ないことは，検査法の非徹底ならびに技術の低下による見逃しが予想される。近年直接蛍光抗体法による検出キットが市販の状況にあるが，その導入も今後は検討する必要がある。しかしながら，従来のギムザ染色等の検査法でも，体制の整った検査室では診断が可能であるので，同検査技術の熟練の必要性を強く認める。

イソスポラ症・クリプトスポリジウム症

全国で発症したAIDS報告症例の集計（1985～2002）を行い，大阪医療センターの症例に関しては，過去約7年（1997.4.1.～2003.12.3）の実態を調査した。

全国集計の結果は，AIDS患者2,556名（日本国籍1,906名，外国籍650名）の内，イソスポラ症の合併報告はなく，クリプトスポリジウム症の合併は計9件（内外国籍8）であった。

大阪医療センターでは，HIV感染者524例のうち，イソスポラの合併は認められず，クリプトスポリジウム症の合併は3例であった。3例とも激しい下痢，体重の減少，CD4数の顕著な減少があり，AIDSの病態として重篤であった。すべての症例で，クリプトスポリジウムの特異的な治療は行わず，抗HIV療法による免疫力回復で治癒した。2例は，それぞれ赤痢アメーバおよびキャンピロバクターとの重複感染例であり，下痢の病態が特定しずらかった。感染経路については，他の下痢起因微生物の感染経路を鑑みて，性的接触によるクリプトスポリジウム症の感染の可能性が考えられた。

イソスポラ症およびクリプトスポリジウム症はAIDS指標疾患に入る寄生虫症であり，免疫力の低下による日和見感染が起こりうる[5]。しかしながら，わが国の合併症例はきわめて少なく，症例の蓄積が困難である。大阪医療センターにおけるクリプトスポリジウム症3例の報告はきわめて貴重であり，真に有効な治療薬が無いクリプトスポリジウム症に対して，補液による脱水と電解質バランスの補正や，適切な抗HIV療法による免疫力の回復を図る治療法の有効性が強く認識された。

糞線虫症

本症との合併に関するアンケート調査を，全国のエイズ拠点病院364施設に行った。回収率は201施設（55%）で，糞線虫症との合併例は6例［日本人2例，外国人3例（アフリカ人，タイ人，ブラジル人），国籍不明1例］（男5例，女1例）で，合併時の平均年齢は41歳であった。地域としては，沖縄が1例，その他は東京，大阪などの大都市であった。HIV感染経路は，同性間性交渉2例，異性間2例，両性間2例，不明1例であった。

糞線虫症合併時のCD4は3～793（平均206）/μlで，かなりの幅が見られた。本6例のうち，播種性糞線虫症が認められたのは1例のみであった。

糞線虫症は，熱帯・亜熱帯に広く分布する線虫類の寄生虫症で，本邦では，沖縄や南西諸島に未だに多くの感染者が認められる。特に消耗性疾患，栄養不良，悪性リンパ腫や成人T細胞性白血病，ステロイド投与など免疫力が低下すると感染が進行することが知られており，エイズ患者における糞線虫症の合併の危険性が考えられている。しかしながら今回の調査研究では，本邦における症例では重症化の傾向は見られなかった。HIV感染者における糞線虫症合併頻度はおよそ0.1%以下であろうと考えられ，なかなか特異的な症例の蓄積ができないのが現状である。

おわりに

わが国におけるエイズ・HIV感染症との寄生虫症の合併頻度やその重篤性は，諸外国の報告とはかなり異なり[6]，診断・治療法の選択も，諸外国の例をそのまま適用できないことが分かった。また，合併

例に対する適切な治療薬の配備もわが国には十分でない。たとえば，アメーバ赤痢におけるフロ酸ジロキサニドや，糞線虫症におけるイベルメクチンの使用は制限された状況にある。

それぞれの合併寄生虫症には，その疫学および臨床的特徴があることから，今後わが国における有効な治療法の確立のために，個別の症例研究をかさねてゆく必要があるものと考えられた。

追記：本報告は，「平成15年度エイズ医療共同研究（15公-6)」の成果による。

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Case Report

Rhabdomyolysis After Simvastatin Therapy in an HIV-Infected Patient with Chronic Renal Failure

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ABSTRACT

We encountered a 49-year-old HIV-infected man with chronic renal insufficiency who developed rhabdomyolysis after treatment with simvastatin. He recovered after initiating hemodialysis and discontinuing oral medications. Rhabdomyolysis most likely resulted from an excessive blood concentration of simvastatin caused by concomitant use of fluconazole in the presence of renal insufficiency.

INTRODUCTION

THE PROGNOSIS OF patients with HIV infection has been improved dramatically by highly active antiretroviral therapy (HAART), but adverse reactions associated with this long-term medication represent an increasingly important issue. HAART-associated hyperlipidemia, mainly an effect of HIV protease inhibitors (PIs), may increase long-term risk of cardiovascular disease[1]; therefore, drug therapy should be considered for patients with severe hypercholesterolemia. While □-hydroxy-□-methylglutaryl-coenzyme A (HMG-CoA)-reductase inhibitors (statins) are effective treatments for lowering cholesterol, these agents rarely cause rhabdomyolysis. In this literature, we report a case of HIV infected patient who developed rhabdomyolysis likely because of an excessive blood concentration of simvastatin.

CASE REPORT

A 49-year-old Japanese man with a medical history of HIV infection, hypercholesterolemia, and chronic renal failure presented to our hospital for evaluation of renal function on May 1, 2001. In August 1998 he had developed Pneumocystistis carinii pneumonia, and was found to have HIV infection. The CD4□ T-lymphocyte count at that time was 4 per microliter, and the HIV viral load was 69000 copies per milliliter. Beginning in November of the same year, he started HAART including sanilvudine, lamivudine, and nelfinavir. Chronic renal failure also first was noted in 1998. On October 1, blood urea nitrogen (BUN) was 42.5 mg/dL and serum creatinine (Cre), 1.7 mg/dL; subsequently, renal function continued to gradually decline. In February 2001 simvastatin (10 mg/d) was initiated to treat hypercholes-

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terolemia, a likely adverse effect from the antiretroviral agents. When initiation of maintenance hemodialysis was considered, the patient was referred to our hospital, located near his home town. Antiretroviral therapy had been stopped for 3 months prior to admission because of acute pancreatitis; medications at the time of admission included allopurinol (300 mg/d), amlodipine (2.5 mg/d), fluconazole (100 mg/d), mecobalamin (1.5 g/d), sucralfate (3 g/d), and simvastatin (10 mg/d). One day prior to admission he developed fever, malaise, and muscle weakness. He had no history of trauma, heavy exercise, or alcohol abuse.

Physical examination revealed a temperature of 38.5°C, blood pressure of 132/91 mm Hg, and heart rate of 100 beats per minute. Neurologic examination disclosed proximal muscular weakness in the lower limbs, accompanied by complaints of myalgia. The Achilles tendon reflexes were decreased. The remainder of the physical examination was unremarkable.

Laboratory data were notable for the following: BUN, 62 mg/dL; Cre, 4.5 mg/dL; aspartate aminotransferase (AST), 280 IU/L; and lactate dehydrogenase (LDH), 2210 IU/L. The serum creatine kinase (CK) concentration in serum was markedly elevated, at 6820 IU/L. The serum myoglobin also was elevated, at 61800 ng/mL. Test results for antinuclear antibody (ANA), rheumatoid factor (RF), and anti-JO 1 antibody all were negative. CD4□ T-lymphocytes declined to 150 per microliter, and the HIV viral load increased to 29000 copies per milliliter.

The urine was amber colored, and test results were positive for protein, occult blood, and myoglobin. The urinary sediment contained casts of various types, and urinary N-acetyl-□-D-glucosaminidase (NAG) and □$_2$-microglobulin also were elevated. A 24-hour urine collection contained 5.9 g of protein. Ultrasonography of the abdomen showed both kidneys to be hyperechoic and nearly normal in size.

The patient was diagnosed with rhabdomyolysis, and all oral medicines including simvastatin were discontinued 3 days after admission. To prevent further decline in renal function, he was given large quantities of intravenous fluid to establish urine flow. However, CK concentrations continued to rise steadily, peaking at 25,340 IU/L on May 7. The creatinine clearance rate (Ccr) was 6.7 mL/min, and worsening renal failure could no longer be controlled by conservative management. Hemodialysis was initiated on that day. Subsequently AST, LDH, and CK started to improve, and were normal by May 18. Muscle strength recovered, but renal dysfunction did not. The patient remained dialysis-dependent, and was discharged from the hospital early in July after placement of a native arteriovenous fistula.

DISCUSSION

Rhabdomyolysis is a syndrome characterized by elevated CK and myoglobinuria that frequently leads to renal insufficiency. Although the precise pathophysiology of statin-associated rhabdomyolysis is unknown, excessive serum concentrations of statins are believed to increase risk of rhabdomyolysis. Simvastatin, frequently used to treat hypercholesterolemia, is metabolized in the liver by the cytochrome P450

TABLE 1. CASE REPORTS OF STATIN-ASSOCIATED RHABDOMYOLYSIS THAT OCCURRED IN HIV-INFECTED PATIENTS

Reference	*Age (yr)*	*Gender*	*CK peak (U/L)*	*Statin drugs*	*CYP3A4-inhibiting agents*
[5]	70	M	78,000	Simvastatin	Nelfinavir
[6]	74	M	105,180	Simvastatin	Delavirdine
[7]	34	M	11,332	Atorvastatin	Clarithromycin, lopinavir, ritonavir
[8]	63	M	9,600	Atorvastatin	Delavirdine
[9]	57	M	76,000	Cerivastatin	Gemfibrozil, indinavir
[10]	51	F	23,968	Simvastatin	Indinavir, ritonavir
this case	49	M	25,340	Simvastatin	Fluconazole

CK, serum creatine kinase.

system (CYP3A4); concomitant administration with other drugs metabolized by this system, such as PIs, can increase serum concentrations of simvastatin. Most occurrences of rhabdomyolysis associated with simvastatin reported until now have involved combined use with drugs that inhibit CYP3A4, such as erythromycin, cimetidine, and itraconazole.

As our patient recovered with hemodialysis and withdrawal of oral medicines including simvastatin, simvastatin appear to have played a crucial etiologic role. Fluconazole was the only CYP3A4 inhibitor among drugs prescribed for him, so rhabdomyolysis most likely was caused by concomitant use of simvastatin and fluconazole. Within the azole class of antifungal agents, fluconazole inhibits CYP3A4 less strongly than itraconazole or ketoconazole. Rhabdomyolysis resulting from interactions between simvastatin and fluconazole accordingly is considered to be rare, and few cases have been reported.[2]

The serum concentration of simvastatin also can be elevated by advanced renal insufficiency, and a few consequent cases of rhabdomyolysis have been reported.[3] Our patient had already developed end-stage renal failure when rhabdomyolysis occurred, so serum concentrations of simvastatin and fluconazole may have been elevated, leading to development of rhabdomyolysis. Various renal lesions have been described in the course of HIV infection. Although renal biopsy was not performed considering the patient's end-stage renal disease, the clinical course, the presence of proteinuria, and hyperechoic kidneys, were consistent with HIV associated nephropathy (HIVAN), which histologically is characterized by sclerosing glomerulopathy and severe tubular changes.

A variety of musculoskeletal syndromes associated with HIV infection have been reported, ranging from nonspecific myalgia to rhabdomyolysis; causes include direct viral invasion and reactions to drugs. In our case, HIV virus load had rapidly increased at the time of occurrence of rhabdomyolysis 3 months following withdrawal of HAART because of pancreatitis. The patient's condition therefore resembled primary HIV infection. Rhabdomyolysis associated with primary HIV infection has been reported,[4] so suspending HAART may have contributed to development of rhabdomyolysis. However, HIV may not have been the main cause because recovery occurred without lowering of the HIV viral load or use of steroids.

In a search of the literature written in English, we found six previous cases of rhabdomyolysis associated with statins that occurred in HIV infected patients (Table 1)[5–10]; three of these also involved simvastatin. In all six cases, statins were used together with antiretroviral agents: PIs in four, and delavirdine, a non-nucleoside reverse transcriptase inhibitor, in two. Our case is unique in that the patient had coexisting renal failure and was not taking antiretroviral agents at the time of onset of rhabdomyolysis. This case suggests that multiple drugs should be prescribed cautiously in HIV-infected patients, and that particular care should be taken in prescribing simvastatin when these patients have renal insufficiency.

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□ ORIGINAL ARTICLE □

Interference of (1→3)-β-D-glucan Administration in the Measurement of Plasma (1→3)-β-D-glucan

Yasuo ISHIZUKA, Hiroki TSUKADA and Fumitake GEJYO

Abstract

Objective **Blood (1→3)-β-D-glucan (βG) measurement is widely used as an effective sero-diagnostic method for deep-seated mycosis. Antitumor βG (lentinan, schizophyllan) administration is known as one of the false-positive factors of blood βG measurement. To understand the influence of administered βG preparation to βG measurement in blood, we compared the interfering effect of βG administration in different βG measuring methods.**

Methods **βG concentration in plasma was measured by three different methods.**

Materials **βG concentration was measured in plasma of 18 samples of 7 cases with βG administration and 86 samples without βG administration. The period after last βG administration was three days to three years.**

Results **In the cases for which βG was administered, blood βG level drastically increased using the method which employs alkaline pretreatment. Even in the cases for which βG was administered three years previously; βG value measured by alkaline pretreatment was significantly high. Thus, interference of βG administration in blood βG measurement continued for years after the last administration.**

Conclusion **Disparity in βG values measured by different methods for βG administered cases is due to differences among sample pretreatment methods. Conformation of administered βG seemed to be transformed into a sensitive form to factor G by alkaline pretreatment. Especially in the case of the alkaline pretreatment method, βG administration disturbance was much stronger than for dilution-heating pretreatment. Therefore, in suspected cases, it is important to pay attention to βG administration during the previous few years.**
(Internal Medicine 43: 97–101, 2004)

Key words: (1→3)-β-D-glucan, deep-seated mycoses, false-positive reaction, lentinan, schizophyllan

Introduction

As an auxiliary sero-diagnostic method for deep-seated mycosis, the measurement of (1→3)-β-D-glucan (βG) concentration in blood is used widely in Japan (1–3). Since βG, which is the common cell wall component of fungi, is the target of measurement, it is impossible to specify a species of infectious fungi. However, βG measurement in blood is effective as the screening test for fungal infection, because the result can be derived in a short time period, and many species of pathogenic fungi have βG as a cell wall component. Furthermore, βG is measured quantitatively (rather than qualitatively), the βG value reflects the extent of illness and it is useful for the judgment of cure effect (2, 4). All the βG measurement methods utilize βG sensitive blood coagulation cascade reaction of horseshoe crab (5). However, methods differ in terms of sample pretreatment and/or principle of final enzyme activity measurement.

Antitumor βG preparation (lentinan and schizophyllan) is known to be a false-positive factor in βG measurement. This treatment has a direct influence on the βG concentration in blood, because the preparation is, in fact, βG itself, and administration is intra-muscular. To understand the influence of administered βG preparation on βG measurement in blood, βG concentration in the cases with βG administration was measured by three different methods. We found that administered βG preparation interfered with the βG measurement for several years, and the degree of interference differed among βG measuring methods.

Materials and Methods

Materials

We measured βG concentration in 18 plasma samples from 7 cases with prior βG administration (Table 1), and in 86 samples from 38 cases without prior βG administration. In all cases with βG administration, no signs of deep-seated mycoses and no signs of liver function failure were observed. In all 7 βG administered cases, hemodialysis with

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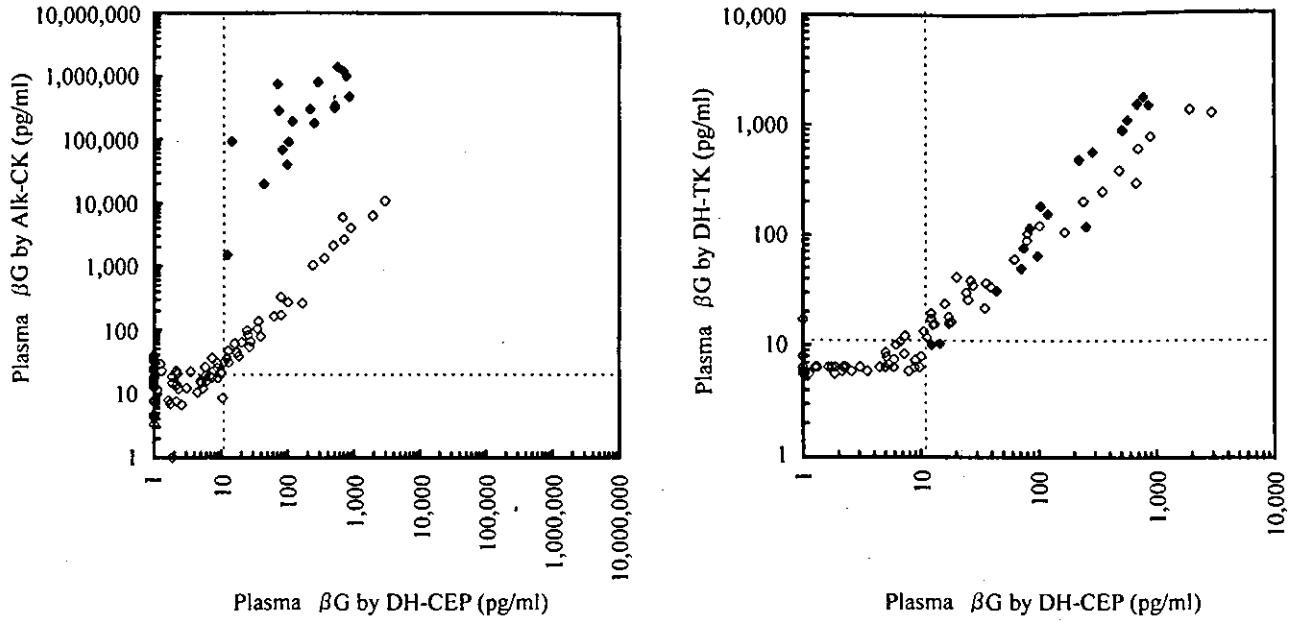

Figure 2. Correlation of plasma (1→3)-β-D-glucan (βG) concentration for three methods. ◆: βG administered cases, ◇: cases without βG administration, Dotted line shows cut-off value of each method. DH-CEP: Dilution and heating treatment—Chromogenic endpoint method, Alk-CK: Alkaline treatment—Chromogenic kinetic method, DH-TK: Dilution and heating treatment—turbidimetric kinetic method.

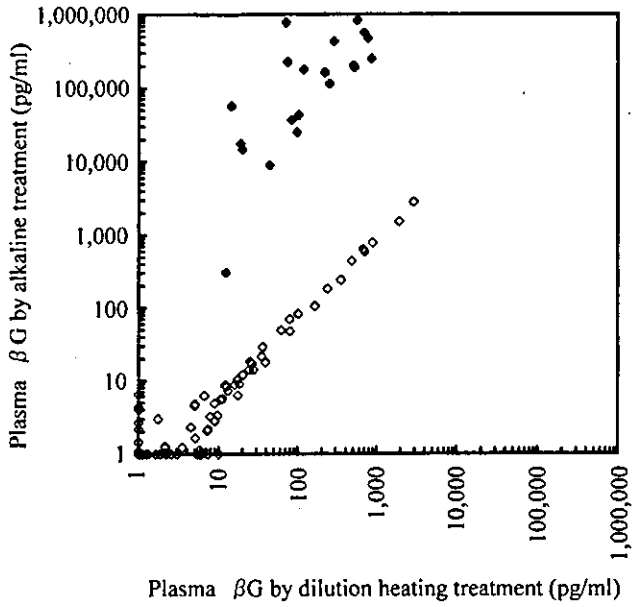

Figure 3. Correlation of plasma (1→3)-β-D-glucan (βG) concentration between two sample pretreatment methods. Plasma βG concentration was measured with chromogenic endpoint reaction reagent after alkaline or dilution heating pretreatment. ◆: βG administered cases, ◇: cases without βG administration.

sample pretreatment is necessary to eliminate the interference of blood component. Since the Alk-CK method, that employs alkaline treatment as sample pretreatment, showed high βG concentrations, we assumed that different sample pretreatment methods resulted in the disparities in measured βG concentrations for the different methods for βG administered cases. We compared βG concentrations measured using chromogenic endpoint reagent with alkaline or dilution-heating pretreatment. In the cases with prior βG administration, βG concentration measured by alkaline treatment showed significantly higher values than for the dilution and heating pretreatment. In the cases without βG administration, βG concentration was not changed by sample pretreatment method. We conclude that the high βG concentration by Alk-CK method in βG administered cases is due to the alkaline treatment.

βG concentration measured by the alkaline pretreatment method showed drastically higher values than those of the dilution-heating pretreatment in the cases with βG administration. If abnormally high βG concentrations judged from clinical symptoms were observed, we could estimate whether βG had been administered or not, by measuring βG using different sample pretreatment methods, even in the cases in which βG administration in the past was uncertain,

All the methods for βG measurement utilize βG sensitive factor G of horseshoe crab (5). Factor G activating activity of βG depends on conformation of βG, single-helical or random-coiled conformer exhibits stronger factor G activating activity than a triple-helical conformer (11, 12). Most βG in

aqueous solution of lentinan or schizophyllan preparation exhibits triple-helical conformer; it is converted to single-helical conformer under alkaline conditions (11, 12). If administered βG remained as triple-helical conformer in blood for long periods, converted to single-helical conformer and displayed stronger activity to factor G after alkaline treatment, the typical reaction pattern in βG administered cases should be appropriate.

In the cases without prior βG administration, a large difference in the βG concentration among the different sample pretreatment methods was not observed. Since βG concentration in deep-seated mycosis is of an order of ng/ml, even in the highest cases, it is very difficult to determine conformation of βG in blood. Alkaline pretreatment did not activate factor G activating activity of βG in the cases without βG administration. This suggests that most of the βG in the blood of deep-seated mycosis has single-helical conformer, which displays strong activity to factor G.

Blood concentration of ^{3}H labeled lentinan after intramuscular administration in mouse, rat and dog declines in a di-phasic manner, with a half-life below three hours and over fifty hours (13). Hase et al reported clearance of schizophyllan in humans (14). Blood concentration of ^{13}C labeled schizophyllan rises to a maximum concentration at 24 hours after intra-muscular single administration of 20 or 40 mg, and declines in a di-phasic manner. Blood concentration at 30 days after administration is tens of ng/ml. Thus, from these data it would not be expected that lentinan or schizophyllan administration affected βG measurement for years. Investigation of long period clearance of βG should clarify how long βG administration affects blood βG concentration.

βG concentration in deep-seated mycoses responds to cure effect and declines in a few months, if the cure effect is successful (2, 4). βG in blood as a result of prior βG administration might be different in terms of clearance rate from that originated in fungal infection. Since blood βG concentration in the cases with deep-seated mycoses is several ng/ml in the highest cases, the total amount of βG in circulating blood would be on the order of μg. A normal dose of lentinan is 2 mg per week and schizophyllan is 40 mg per week. Since they are administered continuously for long periods, the totals amount to hundreds of mg. Different clearance rates for βG originating from βG administration and fungal infection may be due to differences in the total amount of βG present in the whole body.

Clearance rates of different conformers of schizophyllan in mouse depend on its conformation; single-helical conformer clears more rapidly than triple-helical conformer (15). Long-term presence of administered lentinan or schizophyllan may be due to their triple-helical conformer.

Administered βG preparation is present for years in peripheral blood and interferes with blood βG measurement. βG preparation other than oral administration is only in the forms of schizophyllan and lentinan in Japan, and susceptible diseases are only stomach cancer and uterocervical cancer. If βG is measured in the cases with these diseases, it is important to confirm whether βG preparation had been administered or not over long periods.

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