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図1. 胸部X線写真

下肢に浮腫は認めませんでした．その他は特に異常所見ありませんでした．

　北　普通に過ごしてきた50代男性にしては，るいそうが著明で，ツルゴールの低下があり，脱水が著しい．その上酸素飽和度も非常に落ちているということですね．fine crackleは間質性肺炎では下肺野で聴くことが多いですが，この方は広く全肺野で聴こえたということですね．

　発言者2　痩せているのにもかかわらず腹部膨隆とありますが，どんな感じの膨隆なのでしょうか．

　鳴河　そんなに著明ではなかったように記憶しています．

　北　腹水が貯まっている所見はありますか．

　鳴河　腹水を疑うものではありませんでした．

　北　この時点でプロブレムリストを整理してみます．まずは進行性の労作時呼吸困難．そして非常に著明なるいそう，全胸部で広く聴取するfine crackle，低酸素血症，さらには著しい下痢ですね．このような情報を得た時点で，皆さんでしたらどのような疾患を考えられますか．あるいはどのような検査を進められますか．

　発言者3　労作時呼吸困難は心不全なのかあるいは呼吸不全なのか．進行性の労作時呼吸困難でfine crackleも聴かれ浮腫がないということから，例えば間質性肺炎が急性に増悪してきたとも思われますし，下痢は何かというと，例えば間質性肺炎の原因はたくさんあるのでsystemicな疾患で腸管病変をきたしているのかも知れません．るいそうは急速に進んでいるのですか．

　鳴河　そうです．

　発言者3　そうするといわゆるcardiac dyspneaとかそういった長期間の原因によるものではなく，例えば下痢によるものか，あるいは消化器などの悪性腫瘍があるのかも知れません．

　林　龍二（富山大学第一内科）　呼吸器で考えるとこういう症状は日常的に遭遇するものではないと思います．亜急性ということですが，通常息が苦しいとなれば数日で患者さんは受診されます．もう少し慢性，例えば肺気腫とか間質性肺炎の場合はここまで急速には進みません．それからやはり感染症を考えなくてはいけません．微熱ということですが，どのくらいの発熱だったのか．本当に高熱がなかったか．また動けないということですがALS（筋萎縮性側索硬化症）のような神経疾患では呼吸困難とるいそうがみられます．神経学的に本当に異常がなかったのか，気になりました．

　鳴河　高熱は確認できていません．歩行困難でしたが，麻痺や完全な脱力はなく，るいそうに伴う筋力低下と考えられました．

　北　この方の場合，III音や頸静脈の怒張，心尖拍動の外側偏位などの心不全を疑う身体所見はなく，全胸部に広く聴取されるfine crackleということからフロアのご指摘通り呼吸不全を上位に挙げたいと思います．その中で感染症や神経疾患なども念頭に検査を進めるわけですが，実際の診療をシミュレーションする形でまず胸部X線写真から入らせていただきます．

　和倉　健朗（富山大学初期研修医）　軟部陰影，胸膜や横隔膜，縦隔の異常はなく，心肥大も認めません．両側の肺野にびまん性小粒状影あるいはややむらのあるすりガラス状陰影を認めます（図1）．

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表 1. 検査所見

【血算】		【生化学】			
WBC	22,670 /μl	TP	9.5 g/dl	BUN	50 mg/dl
Seg	78.5 %	Alb	4.0 g/dl	Cr	0.7 mg/dl
Band	4.0 %	Amy	62 IU/l	UA	6.3 mg/dl
Eos	0.0 %	AST	93 IU/l	TG	175 mg/dl
Baso	0.5 %	ALT	22 IU/l	T-Cho	207 mg/dl
Lymph	12.5 %	γ-GTP	45 IU/l	Na	129 mEq/l
Mono	4.0 %	ALP	288 IU/l	K	5.3 mEq/l
Myelo	0.5 %	LDH	1,162 IU/l	Cl	90 mEq/l
RBC	$423×10^4$ /μl	CPK	67 U/l	Ca	9.0 mg/dl
Hb	12.6 g/dl	T-Bil	0.4 mg/dl	CRP	17.6 mg/dl
Ht	38.0 %				
Plt	$65.9×10^4$ /μl				

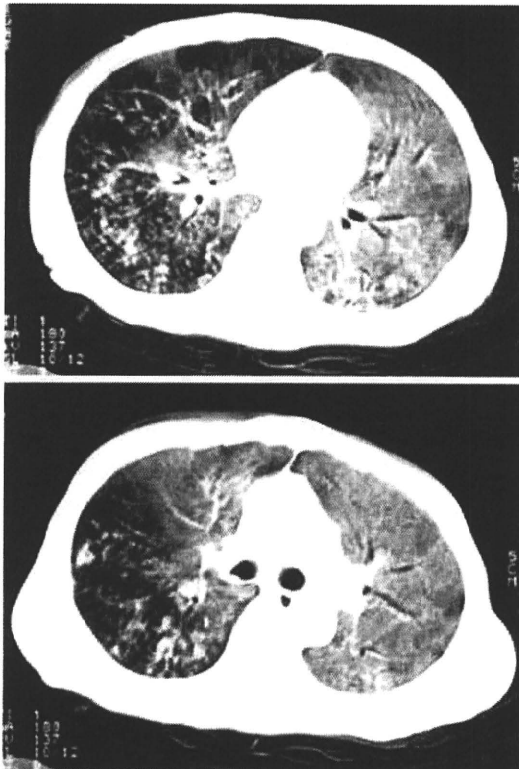| 【感染症他】		【動脈血ガス】	
HBs Ag	(−)	pH	7.511
anti-HCV	(−)	PaO_2	40.5 Torr
STS	(+)	$PaCO_2$	22.9 Torr
TP	(+)	HCO_3	18.2 mEq/L
β-D-glucan	52.6 pg/ml	BE	− 2.6
		A-aDO_2	82.02 Torr

図 2. 胸部単純 CT

北　血液検査結果をお願いします.

鳴河　白血球 22,670/μl, CRP 17.6 mg/dlと高度の炎症反応を認めます. その他AST 93 IU/l, LDH 1,162 IU/lと高値でした. Naは 129 mEq/l と低値でした（表 1）.

北　白血球数は非常に多いですが, 分画としては幼若なものは出ていないということですね. CRPも高く, 好中球が多く, LDHが著高している. ASTも高いですが肝疾患に伴うものとしてはその他の肝胆道系酵素の上昇はみられません.

鳴河　動脈血ガス（room air）ではpH 7.511, PaO_2 40.5 Torr, PCO_2 22.9 Torrと著明な低下を認めております. Aa-DO_2 は 82.02 Torrと開大を認めています.

北　アニオンギャップが 22 と開大しており, 代謝性アシドーシスも合併していると思われます.

鳴河　感染症スクリーニングの結果, 梅毒反応（STS）が陽性でした. 再検ではTPHA高値で, 活動性の梅毒と考えられました.

北　次にCTを呈示いたします.

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表2．プロブレムリスト

- ＃1　進行性の労作時呼吸困難（1カ月）
- ＃2　るいそう
- ＃3　全肺野で広く聴取するfine crackles
- ＃4　強い炎症所見
- ＃5　下痢
- ＃6　低酸素血症
- ＃7　胸部X線写真・CT異常像
- ＃8　高LDH血症
- ＃9　低Na血症
- ＃10　トレポネーマ陽性

表3．鑑別疾患

1. 間質性肺炎
2. サイトメガロウイルス肺炎
3. ニューモシスチス肺炎
4. 過敏性肺臓炎
5. 粟粒結核
6. 癌性リンパ管症

図3．肺胞洗浄液（Diff-Quik染色1,000倍）

　林　肺野の濃度上昇があります．呼吸不全とあわせて急性間質性肺炎（AIP）と読んでいいと思います．ただしAIPにしては肺野濃度の上昇がびまん性にみられる点が異なります．広範なすりガラス陰影とも取れますが，一部健常部が残っています（図2）．1カ月の微熱と呼吸困難があるので，結核も鑑別に挙がります．

　北　ここまでのプロブレムをまとめてみました（表2）．鑑別診断を挙げる際には，なるべく一つの疾患で説明できないかと考えていく方法と，複数の疾患が併存していると考える方法がありますが，まずは一つの原因で説明できないかなと考えていくのがセオリーだと思います．

　野上　和也（富山大学和漢診療部）　性的な傾向というのも大事な情報だと思います．感染症検査にHIVを追加しておくべきです．

　発言者4　HIVに伴う肺病変の可能性もあると思います．LDHの高値とCRPの上昇，低ナトリウム症等も画像を合わせれば間質性肺炎でも説明可能と思います．ただし下痢という症状が合いませんので，それはHIVの急性感染によるものかも知れません．また，膠原病肺の場合も肺病変が急速に進み得るので，一元的に間質性肺炎と消化管を結びつけるとなりますと，例えばRAの間質性肺炎に消化管アミロイドーシスが合併した場合も考えられます．抗CCP抗体とか各種自己抗体等の免疫系の検査も追加すべきと思います．
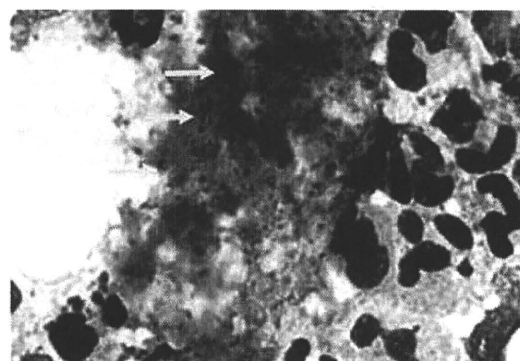
　北　フロアから挙げていただいた他に過敏性肺臓炎や粟粒結核，サイトメガロウイルスのようなウイルス性肺炎，癌性リンパ管症なども鑑別疾患として挙げてみました（表3）．その後の経過をお願いします．

　鳴河　この患者さんには気管支鏡検査を行いました．肺胞洗浄液をDiff-Quik染色で観察すると，目玉が2つ付いているようなニューモシスチスの虫体（嚢子）が多数固まってみえました（図3）．その後，HIV抗体陽性，RNA定量で17万コピーという結果が得られました．入院時のCD4細胞数は591/μlでしたが，脱水を補正した後の再検では166/μlとかなり低値を示していました．

　北　フロアからもご指摘頂きましたように症例はニューモシスチス肺炎（pneumocystis pneumonia；PCP），その背景としてHIV感染症という診断に至りました．

　鳴河　今回は診断過程を重視して，意図的に性的傾向については触れませんでしたが，この患者さんはバイセクシャルで，男性も女性も不特定のパートナーがいたということでした．
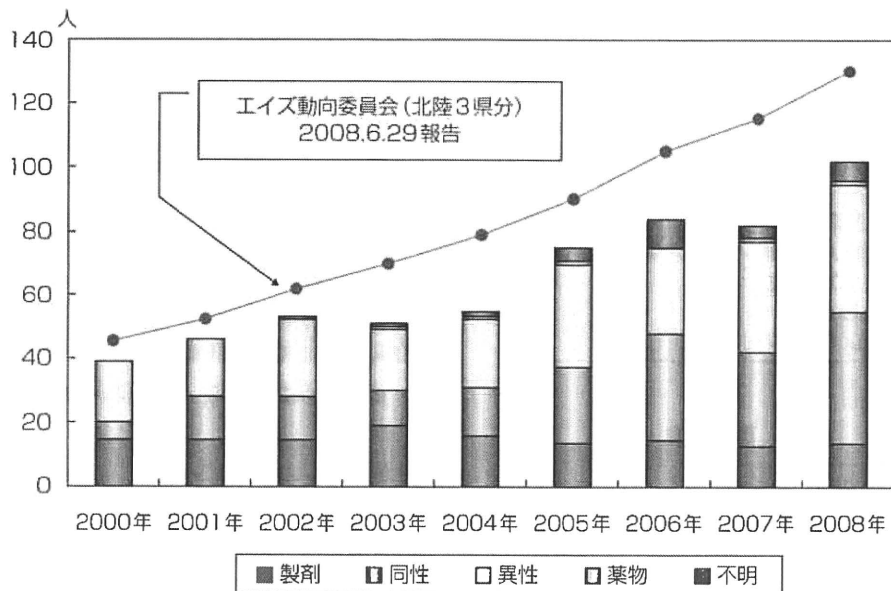
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図4. 北陸ブロックのHIV/AIDS患者数年次別推移
エイズ予防情報ネット　エイズ動向委員会報告（2008年6月29日）を改変

北　鍵となる情報の有無で，診断が容易にも難しくもなるのですね.

鳴河　臨床経過です. ST合剤とステロイド・パルス療法を併用しました. 経過中にST合剤に対する薬剤熱が出現したためにペンタミジン静注に変更しました. 41病日に両側気胸を併発しました. 両側気胸を来たしたケースは一般に予後不良ですが，何とか乗り切り，AIDSに対する治療を開始しました.

北　PCPやHIV感染症は日常的に出会う疾患ではないと思いますが，我々は診療科を問わず，こういう患者さんと出会う，出会ってしまう可能性が今後少なからずあると思われます. そこで，一般外来における診療のポイントについて解説していただきます.

鳴河　健康そうで今まで何もない若年者が急にこういう肺炎になった場合，まずニューモシスチス肺炎を疑うべきです. あくまで私見ですが，年配で独身の男性，海外，特に東南アジアに長期滞在していたという生活歴は,HIV感染症を疑うポイントの一つだと思います. また最初

は教えてくれなくても,HIV感染が判明した後で再度伺うとMSM（Men who has Sex with Men）であると答えてくれる場合があります. 診察所見では聴診所見のわりに高度な低酸素血症を来たすことが特徴です. PCPの場合,白血球増多や炎症反応は一般に軽度と言われています. このケースの場合，著明な脱水や重複感染症による修飾があったと思われます. さらにLDH上昇，低酸素血症，両側びまん性のすりガラス陰影，これらをみたらβ-D-グルカンとHIV検査は必ずしていただきたい. PCPは適切な初期治療によって，よくなるケースが多いので，できれば早期に気管支鏡検査を行い，確定診断をつけた方がよいと考えています. 治療はST合剤（15 mg/kg），副作用などで使用困難な場合はペンタミジン（3 mg/kg）を用います. 3週間続けるのが標準です. さらに呼吸不全を来たした重症例では治療開始後48時間以内にステロイド・パルス療法を行うことが重要です. HIV感染症の場合,ニューモシスチス虫体の量が多いので十分量のステロイドを投与することが成功のポイントです. 診断に
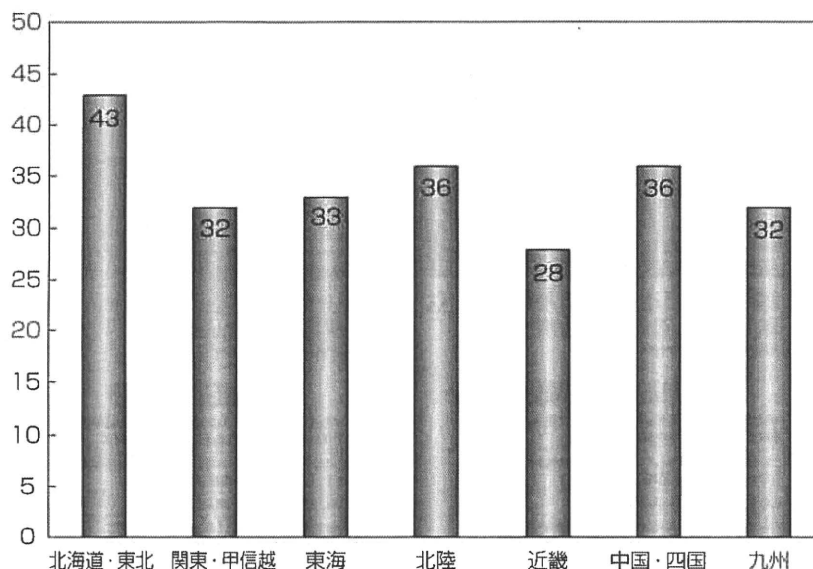
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図5. "いきなり AIDS" 患者比率（%）
2007 年エイズ動向委員会年報

迷う場合は，診断が確定するまでの間ST合剤を投与しておくとよいと思います．あくまで経験則ですが，ごく低用量でもST合剤を先行投与されていた症例の方が治療成績はよいようです．

　北　ST合剤の先行投与が診断の妨げになることはありませんか．

　鳴河　治療を開始しても2週ぐらいまではPCPは検出可能と言われています．

　北　今回の症例は背景にHIV感染症のあるニューモシスチス肺炎のケースでした．フロアからの貴重なご指摘・コメントありがとうございました．一般医としてはまずは疑うこと，そして重要な情報はこちらから聞き出すこと，さらには積極的にST合剤を使用して専門医にバトンタッチすることが重要であると考えます．これで本日の症例呈示を終わりにします．引き続き上田先生のミニレクチャーを頂きます．

　上田　司会者からHIV診療や検査における注意点を述べるよう，ご指示がありましたので，「北陸地方におけるHIV/AIDS診療の現状と課題」を中心に述べさせていただきます．

　北陸ブロック（3県）におけるAIDS治療拠点病院は，富山県に2病院，石川県に8病院，福井県に4病院が選定されています．HIV/AIDS患者さんの大半はこれらの病院で診療を受けていますが，一部の患者さんはこれ以外の病院で診療を受けています．富山県立中央病院，石川県立中央病院，福井大学医学部附属病院はそれぞれの自治体において中心的役割を担う中核拠点病院に指定されています．それぞれの中核拠点病院には十数名から数十名の患者さんが通っており，経験や知識を蓄積してきています．患者総数が多くない北陸においては，当面の間は中核拠点病院で重点的に診療を行い，院内体制の整備や経験の蓄積を行うことが望ましいと思います．

　図4の折れ線グラフは，AIDS動向委員会に報告されたHIV/AIDS患者の累積人数（北陸3県分）を，棒グラフは，3県の病院で定期的に診療を受けている患者総数を感染経路別に示しています．2008年現在，動向委員会報告での累積人数は130人で，病院へのアンケート調査でお答えいただいた実際の定期的通院患者数は102人です．その差は，他地域への転出や通院，あるいは死亡

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図6. 見落とし防止の HIV 検査支援ツール
厚生労働科学研究エイズ対策事業で作成した診療支援ツール（オリジナル）

などが考えられます．患者数が漸増してきているのは全国的な傾向で，近年は同性あるいは異性間の性行為による感染例が増加しています．図には示してはいませんが，HIVと他の性感染症の合併例をしばしば経験します．性感染症を診断された場合には，是非HIV感染のチェックも同時に施行していただきたいと思います．AIDS発症前の診断は，その患者さんの生命を救うことにつながりますし，社会にとっても望ましいことです．

図5は，"いきなりAIDS"患者比率（HIV感染が診断された時に，既にAIDSを発症していた人数の割合）を示します．これもAIDS動向委員会報告をもとに算出しましたが，北陸3県合計では36％（36％の人はエイズを発症して初めてHIV感染が診断された）と計算され，全国的にみても高い値です．このことは，北陸全体としてはHIV感染症診断の体制が脆弱であることを示すものと言えます．保健所では匿名・無料でHIV抗体検査を実施していますが，それにも種々の制約や限界があるので，われわれ医療者もHIV抗体検査を効率よく積極的に勧める必要があります．

繰り返しになりますが，AIDS発症前診断は，その人を救うことにつながるのです．

アンケートで得られた北陸3県における2004年から2008年までのHIV/AIDS関連疾患による死亡例8例の内訳は，リンパ腫2例，抗酸菌症，HBV/HCV感染（肝癌，肝不全），EBV感染，JCウイルス感染（進行性多巣性白質脳症），トキソプラズマ感染が各1例と免疫不全状態の日和見感染（日和見腫瘍）による死亡が多くを占めています．AIDSは治療法が進歩して慢性感染症になったと言われていますが，現在においても診断が遅れると死亡することも少なくない疾患です．このことを再度認識していただき，AIDS発症前診断に注意を払っていただきたく思います．HIV感染は，抗体検査陽性で感染の可能性があり，確認検査陽性で診断が確定します．いずれも採血検査になりますが，時々検査実施にあたり問題と思われる症例が見受けられます．検査前には説明や相談（カウンセリング）が必要ですが，説明がなく無断で検査が行われた例を時々経験します．また，抗体検査偽陽性例に「陽性」と伝えてしまう不幸な例を今でも稀に経験

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表4. HIV 検査の効果と重要性

1. 輸血関連の感染 ──→ 献血の HIV スクリーニングでほぼ排除した.
2. 母子感染 ──→ 妊婦へのルーチン検査と母子への予防投薬で, 児への感染は激減した.
3. 性行為による感染 ──→ スクリーニングが行われず, うまくいっていない.

します. これらのケースは, 事実を知った後で, いやな思いや時には医療者に対する怒りの感情を抱くことにつながります. ですから, 抗体検査前には必ず一言説明をして, 承諾を得るようにして下さい. 承諾は必ずしも文書でなくてよいと言われています.

図6は, 私たちが作成した「見落とし防止のためのHIV検査支援ツール」です. A4サイズで表と裏にカラー印刷された1枚の用紙（耐久性があり頻回使用可能）で, 外来診療で使えるように工夫しました. 片面には性感染症, もう片面にはAIDS指標疾患が記載され, その下にはHIV検査の必要性と承諾を求める文章が続いています. 最後には, プライバシーへの配慮や不安を軽減するために「陽性の場合の対応」などが記載されています. HIVとかAIDSという言葉を声に出しにくい診察環境もあるかと思います. そのような場合, 医師が指さした単語や文章を患者が読むことにより理解できるよう, 文字を大きくし内容を簡潔にしました. また, このような道具を用いることにより, 要領よく短時間での説明が可能ですし, 患者さんには説明を受けたという記憶が残ります. 在庫は多数ありますので, 必要部数をお教えいただければ提供いたします. ご活用いただければ幸いです.

表4は, HIV検査の効果と重要性をまとめたものです. 本邦においては, HIV感染はそれぞれの感染経路への個別対策により, 感染拡大防止効果を認めています. すなわち, 献血検体のHIVスクリーニングで輸血関連の感染はほぼ排除されました. また, 妊婦へのルーチン検査と母子への予防投薬により, 母子感染も防止できています. 問題は性行為による感染（薬物の注射回し打ちも含まれますが）で, 効果的なスクリーニング検査ができず, 予防ができていません. その結果として, はじめにも申し上げましたが, 本邦においても北陸においても感染者数は増加し続けています. われわれ医療者, とりわけ内科医には早期発見や感染予防につながる行動が求められていると思います.

最後に, HIV検査にかかわる診療報酬について紹介します. 現在, HIV抗体検査の診療報酬を請求できるのは, 次に述べる3つの場合においてです. ①HIV感染症を疑わせる自他覚症状がある場合はもちろんですが, ②手術前医学管理料として月1回のみですが請求できます. これは, 尿検査, 血液検査, 心電図, X線の包括検査です. その他, ③輸血料として輸血前後の感染免疫検査が勧められていることは皆さんご存知と思います.

以上, 北陸におけるHIV/AIDS診療の状況を紹介しつつ, HIV診療や検査における注意点を述べさせていただきました.

最終診断

HIV感染症のあるニューモシスチス肺炎

研究分担者　渡辺　大

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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吉野宗宏、矢倉裕輝、桑原健、坂東裕基、小川吉彦、矢嶋敬史郎、谷口智宏、大谷成人、富成伸次郎、渡邊大、西田恭治、上平朝子、白阪琢磨。	ロピナビル・リトナビル配合剤 (LPV/r) の1日2回から1日1回投与へのスイッチ臨床試験結果。	日本エイズ学会誌	11(3)	250-4	2009

Sustained High Levels of Serum Interferon-g During HIV-1 Infection: A Specific Trend Different from Other Cytokines

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Abstract

The expression levels of various cytokines increase with the progression of HIV-1 infection. However, the effects of antiretroviral therapy (ART) on serum cytokine levels have not been fully determined. In this study we measured serum cytokine levels of 35 HIV-1-infected Japanese adults. We first performed a cross-sectional study and observed that TNF-a, IL-6, IL-10, IL-18, and IL-7 levels all showed significant increases in those with advanced disease, and that this had a significant negative correlation with the CD4 cell count. However, IFN-g levels did not show this relationship. A longitudinal study in 18 HIV-1-infected patients with a CD4 cell count <350/mL revealed that the introduction of ART reduced cytokine levels. Significant reductions of IL-7, IL-10, IFN-g, and IL-18 levels were observed on days 30, 60, 90, and 90 after the initiation of ART, respectively. These results indicate a discrepancy between cross-sectional and longitudinal studies of serum levels of IFN-g. To clarify this, we investigated serum IFN-g levels in each patient. In 5 of the 15 patients IFN-g levels did not decrease, even after ART initiation, and remained at 5 pg/mL or higher on day 120 after ART initiation. Higher IFN-g levels (>5 pg/mL) were also observed in 2 of 7 asymptomatic patients, and 2 of 11 patients who underwent ART for 1 year or longer. These data demonstrate that IFN-g levels in some patients increased and remained high even after the initiation of ART, which was a specific observation different from those of the other cytokines.

Introduction

CYTOKINES ARE INTERCELLULAR SIGNALING MOLECULES that regulate the differentiation, proliferation, and activation of immune cells (11,12). They are primarily secreted by immune cells, and they exert their biological effects by binding to specific receptors on these (autocrine) or other target cells. Cytokines serve as the immune response molecules against infective microorganisms, and also have various physiological functions in inflammation, allergy, development, and hematopoiesis. The cytokines include interferon (IFN), tumor necrosis factor (TNF), and colony-forming factor, as well as the interleukins (ILs), which all show different functions *in vivo*. Common characteristics shared by these cytokines include effectiveness, even in trace amounts, transient secretion, and a short half-life. Thus serum cytokine levels are generally low and are often undetectable in healthy individuals. Another common feature of cytokines is the presence of complicated networks or cascades, which regulate their mutual effects additively, synergistically, or antagonis-

tically. The Th-1/Th-2 balance represents one of these networks (6). This balance determines the differentiation of naive T cells into Th-1 cells for cellular immunity, or Th-2 cells for humoral immunity.

Human immunodeficiency virus (HIV)-1 infects CD4$^+$ cells to destroy the immune system, leading to the development of acquired immunodeficiency syndrome (AIDS). After HIV-1 infection, 5–10 y will pass without symptoms in most patients. This period is called the asymptomatic carrier (AC) period. Although apparently asymptomatic in this period, HIV-1 gradually destroys the immune system and decreases the number of CD4$^+$ T lymphocytes (the CD4 cell count). When the CD4 count drops below 200/mL, various opportunistic infections, including AIDS indicator diseases, will develop. Antiretroviral therapy (ART) with multiple agents was developed in the second half of the 1990s, markedly improving the prognosis of HIV-1-infected patients. This therapy is called highly-active antiretroviral therapy (HAART), which continuously suppresses viral replication and restores the function of the immune system in HIV-1-infected patients.

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Many studies have been conducted on the importance of cytokines in the pathogenesis of HIV-1 infection because it affects the immune system (11). In the pre-HAART era, the expression levels of various cytokines reportedly increased, along with the progression of immunodeficiency (1,3). Since in previous studies researchers have reported that a Th-1/Th-2 imbalance is strongly involved in HIV pathogenesis (5,7), the cytokines responsible for the Th-1/Th-2 balance, such as IL-2 (18), IL-6 (4), IL-10 (2), and IFN-g, have been studied in detail (1). On the other hand, in the post-HAART era, the increased cytokine expression was decreased by ART (14,16,22,23,25). However, only a few cytokines examined in the pre-HAART era that exhibit abnormal expression have been studied. It has not yet been determined whether this abnormal cytokine expression is caused by biological responses to viral proliferation, or whether it is induced secondary to other infections caused by the immunodeficiency. In fact, the abnormal cytokine expression may be caused by latent opportunistic infections due to a decreased CD4 cell count. In this study we investigated the cytokines with expression levels that differed from those of the other cytokines, by measuring their serum levels primarily in patients who underwent ART, in order to identify the cytokines directly involved in HIV-1 infection.

Materials and Methods

Patients

We first obtained written informed consent from 35 HIV-1-infected adults who regularly visited our hospital, and collected sera from these patients. The median age of the patients (34 male and 1 female) was 39 y (range 28–73 y). All of the patients were Japanese. The putative infection routes
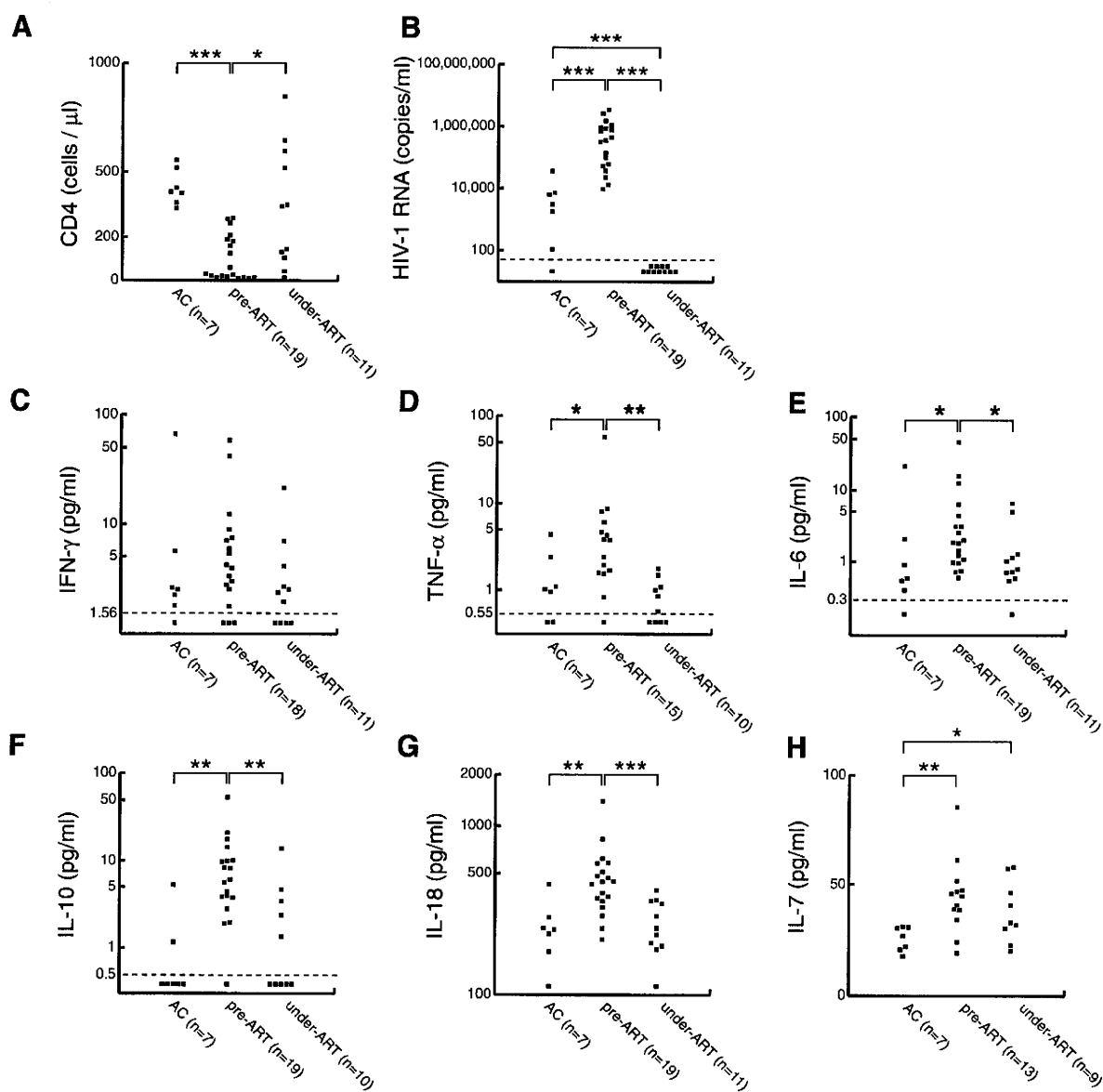

FIG. 1.   Serum cytokine levels in asymptomatic carrier (AC), pre-antiretroviral therapy (pre-ART), and under-ART groups. (A and B) Shown are the CD4 cell counts and HIV-1 RNA levels of the HIV-1-infected subjects in the three study groups. (C–H) These plots show the values of the indicated cytokines in the three study groups. Statistical comparisons were made using the Kruskal-Wallis test ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

of these patients included homosexual contact (29 patients), heterosexual contact (5 patients), and transfusion for hemophilia (1 patient). We then measured the CD4 cell counts by flow cytometry using the whole-blood lysis method. Plasma HIV-1 RNA levels were measured using reverse-transcriptase PCR (Amplicor HIV-1 monitor test; Roche Molecular Diagnostics Corp., Indianapolis, IN). This study was reviewed and approved by the Institutional Review Board, National Hospital Organization, Osaka National Hospital (approval number 0542).

Cross-sectional and longitudinal studies

The patients were divided into the following three groups: AC, pre-ART, and under-ART. They were then subjected to a cross-sectional study to compare serum cytokine levels. The AC group included asymptomatic patients with CD4 cell counts of 350/mL or higher. The pre-ART group included untreated patients (ART naive) with CD4 cell counts less than 350/mL, from whom samples were collected after treatment for opportunistic infections. The under-ART group included patients who underwent ART for 1 year or longer, and whose plasma HIV-1 RNA levels were below the detection limit. Patients in the pre-ART group were subjected to a longitudinal study, and samples were collected from them periodically after ART introduction for comparison with the baseline.

Measurement of serum cytokines

Serum cytokine levels were measured with a sandwich enzyme-linked immunosorbent assay (ELISA). The following reagents were used: IFN-g (human IFN-g ELISA; Bender MedSystems, Vienna, Austria), IL-6 (Quanti Glo human IL-6 chemiluminescent immunoassay; R&D Systems, Inc., Minneapolis, MN), IL-10 (human IL-10 ultra-sensitive ELISA kit; Invitrogen, Carlsbad, CA), IL-18 (human IL-18 ELISA; Medical & Biological Laboratories Co., Ltd., Nagoya, Japan), IL-1 (Quantikine HS human IL-1 immunoassay; R&D Systems), IL-2 (Quantikine human IL-2 immunoassay; R&D Systems), IL-4 (human IL-4 ultra-sensitive immunoassay; R&D Systems), IL-12 (Quantikine HS human IL-12 immunoassay; R&D Systems), IL-7 (Quantikine HS human IL-7 immunoassay; R&D Systems), TNF-a (Quanti Glo human TNF-a chemiluminescent immunoassay; R&D Systems), IL-17 (Quantikine human IL-17 immunoassay; R&D Systems), and IL-23 (Quantikine human IL-23 immunoassay; R&D Systems). The measurements were carried out according to the manufacturers' instructions.

Statistical analysis

Multiple comparisons were carried out using the Kruskal-Wallis non-parametric analysis of variance (ANOVA) test. The Spearman rank test was used for correlation, and the Wilcoxon signed-rank test was used for paired data. The significance level was set at $p < 0.05$.

Results

We measured the serum cytokine levels in 35 HIV-1-infected patients. IL-1b, IL-2, IL-4, IL-12, IL-17, and IL-23 were not considered in the analysis because they were below the detection limits in the majority of the samples (60% or above) (data not shown). To examine the relationship between the

serum cytokine levels and HIV-1 infection, the patients were classified into three groups on the basis of their CD4 counts and the introduction of ART: AC, pre-ART, and under-ART groups, and their serum cytokine levels were compared (cross-sectional study). The CD4 counts and plasma HIV-1 RNA levels of the groups are shown in Fig. 1A and B. As presented in Fig. 1 D–G, serum TNF-a, IL-6, IL-10, and IL-18 levels in the pre-ART group showed significant increases compared with the corresponding values in the AC and under-ART groups. The IL-7 levels were significantly higher in the pre-ART group than in the AC group, but comparable
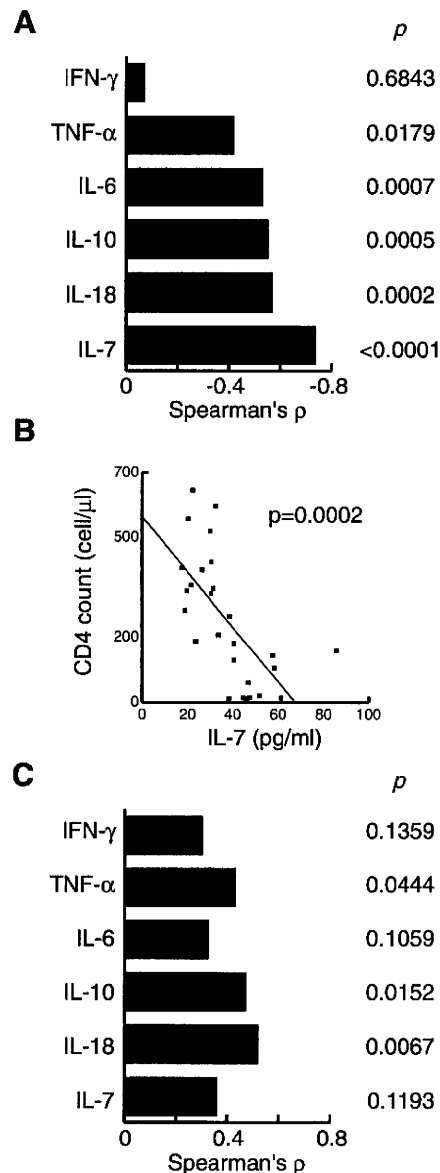

FIG. 2. Association of serum cytokine levels in HIV-1-infected patients with CD4 cell counts and plasma HIV-1 RNA levels. (A) The bars indicate Spearman's rank correlation coefficients (r). (B) Linear regression analysis was used to investigate the relationship of IL-7 levels with CD4 cell counts (slope = −8.3, $r^2 = 0.42$). (C) Correlation with plasma HIV-1 RNA levels. The bars indicate Spearman's rank correlation coefficients (r).

with those in the under-ART group (Fig. 1H). On the other hand, IFN-g levels did not differ significantly among the three groups (Fig. 1C). Thus, except for IFN-g, all of the other cytokines were found to be increased in the immunocompromised patients.

Subsequently, the correlation between the serum cytokine levels and CD4 counts was analyzed using Spearman's rank test. Except for IFN-g, all of the other cytokines exhibited a significant negative correlation with the CD4 cell counts (Fig. 2A). Among the cytokines we examined, IL-7 levels showed the strongest negative correlation (Spearman's r = −0.74); a similarly negative correlation was noted in the regression analysis (Fig. 2B). Serum cytokine and plasma HIV-1 RNA levels were also examined. Since the plasma HIV-1 RNA levels were below the detection limit in all of the patients in

the under-ART group, and this might have led to bias-based errors, the under-ART group was excluded from this assay. A significant but weak correlation was noted between TNF-a/ IL-18 and plasma HIV-1 RNA levels (Fig. 2C). Thus except for IFN-g, the levels of the cytokines analyzed in this study increased with disease progression, and correlated with clinical indicators such as decreased CD4 cell counts and increased plasma HIV-1 RNA levels.

Finally, a longitudinal study was conducted on the pre-ART group to examine the effects of ART on serum cytokine levels. ART was introduced for all 19 patients in the pre-ART group. One patient in this group did not show an optimal virological response 24 wk after the introduction of ART, so this patient was excluded from the analysis. Fig. 3 shows the serum cytokine levels before the introduction of ART, and on
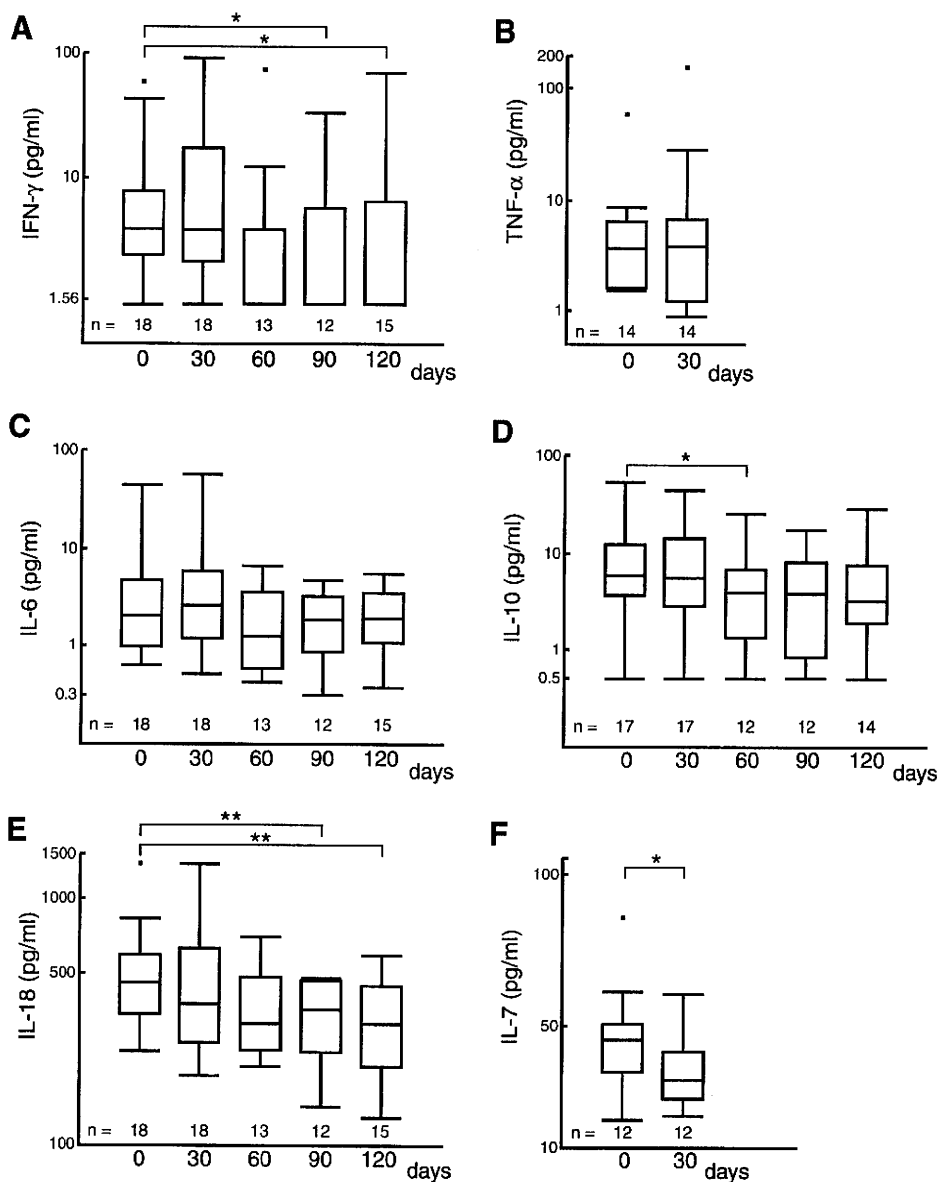

FIG. 3. Serum cytokine levels in HIV-1-infected patients before initiation of and during ART (A–F). The values shown of the indicated cytokines before, and at 30, 60, 90, and 120 d from the start of ART, are shown using box-and-whisker plots, representing the minimum, 25th percentile, median, 75th percentile, maximum, and outlying values. Statistical analyses versus baseline levels were carried out using Wilcoxon's matched-pair signed-rank test (*$p < 0.05$, **$p < 0.01$).

days 30, 60, 90, and 120 after its introduction. On day 30, only IL-7 levels showed significant decreases compared with baseline levels. Some cytokines (IL-10 and IL-18) showed no change, while others (IFN-g, IL-6, and TNF-a) showed upward trends. Four cytokines (IFN-g, IL-6, IL-10, and IL-18), when measured over time, revealed downward trends on day 60 and beyond; IL-10 showed a significant decrease on day 60, and IFN-g and IL-18 showed significant decreases on days 90 and 120. Thus, the IL-7 level rapidly declined after the initiation of ART, while the expression of the other cytokines decreased slightly later.

There was no correlation between the pre-ART and under-ART groups for IFN-g (Fig. 1C). However, our longitudinal observations demonstrated a significant decrease in IFN-g levels upon initiation of ART (Fig. 3A). To resolve this discrepancy, we examined the patients in the pre-ART group in more detail. In most of the patients, the IFN-g levels were gradually suppressed by ART. However, in 5 of the 15 patients, IFN-g levels did not decrease, even after the initiation of ART, and remained at 5 pg/mL or higher at day 120 after ART initiation, regardless of the ART-induced virological response. The data of two representative patients are shown in Fig. 4. In the patients shown in Fig. 4A, whose plasma HIV-1 RNA levels were maintained below the detection limit, the IFN-g levels were above 30 pg/mL, even at 3 y after the initiation of ART. In addition, 2 of 7 patients in the AC group, and 2 of 11 patients in the under-ART group, had higher IFN-g levels (>5 pg/mL). Thus, during the AC period or later, the IFN-g levels in some patients increased, and remained high even after the initiation of ART.

Discussion

Here we demonstrated changes in the serum cytokine levels in HIV-1-infected patients. The serum levels of many cytokines increased with disease progression, and were decreased by the initiation of ART. The abnormal cytokine expression patterns may be explained by two possible mechanisms: the direct effect of immune destruction by HIV-1, and the effects of opportunistic infection. In this study, 14 (74%) patients in the pre-ART group developed AIDS. However, since the samples were collected from all of the patients after the treatment of opportunistic infections, the effects of these opportunistic infections on abnormal cytokine expression patterns may be limited. On the other hand, it is important to note that there were changes in the cytokine levels after the initiation of ART. On day 30 after ART introduction in the pre-ART group, all cytokines except for IL-7 remained unchanged or increased. At this point, the HIV-1 RNA levels in the blood were decreased in all 18 patients, and the CD4 cell counts were increased in 16 (89%) patients. This indicates that the cytokine levels increased despite virological suppression and immune restoration. Immune reconstitution inflammatory syndrome (IRIS), a seemingly paradoxical pathological condition, has been extensively described (20). This is a condition in which the existing opportunistic infection is exacerbated, and/or a new opportunistic infection develops after the introduction of ART, presumably due to the restoration of immune responses against a pathogen that existed prior to ART. Of the 18 patients in the pre-ART group, only 2 developed clinically apparent IRIS. One patient experienced a relapse of an existing CMV infection, and the other patient newly developed an atypical mycobacterial infection, an AIDS-indicator disease, after the introduction of ART. In addition to these 2 patients, several other patients developed IRIS, but required no specific treatment, at 2–4 weeks after initiation of ART. In these patients, the increased cytokine levels observed on day 30 after ART introduction were associated with immune restoration, suggesting that these immune responses may have been mounted against a potential
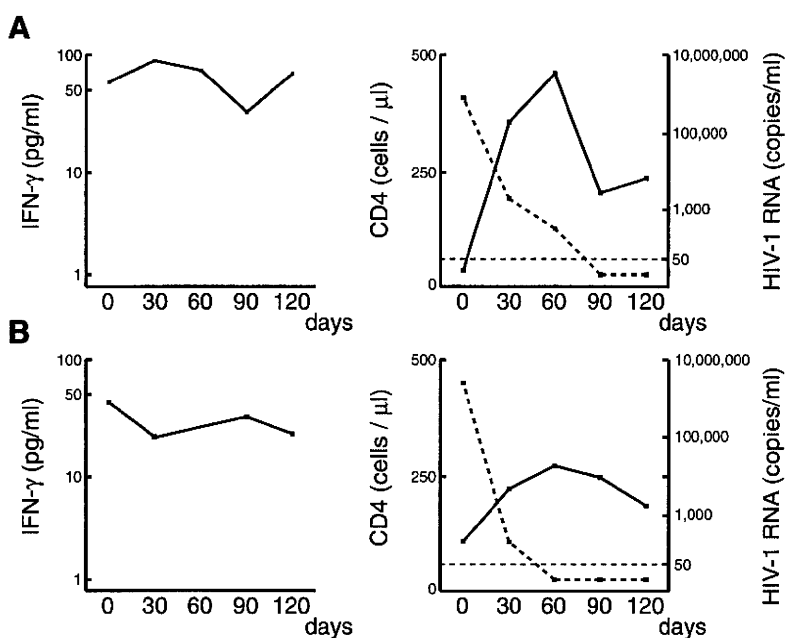

FIG. 4. Sustained elevation of serum IFN-g levels during ART in HIV-1-infected individuals (**A** and **B**). Shown are values of serum IFN-g (left), CD4 cell counts (right, solid lines), and levels of plasma HIV-1 RNA (right, dashed lines), of two typical patients at the indicated time points after the initiation of ART.

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infectious agent. The direct effects of HIV-1 infection cannot be completely overlooked. However, the abnormal cytokine levels seen in immunocompromised patients may be at least partially associated with opportunistic infections.

After ART initiation, many cytokines showed no change or showed upward trends; on the other hand, IL-7 rapidly decreased. This appeared to be associated with the physiological actions of IL-7. Other cytokines are associated with immune responses and inflammatory reactions against microorganisms; however, the main function of IL-7 is for hematopoiesis (24). IL-7 mainly acts on hematopoietic stem cells, and induces their differentiation into T lymphocytes. The IL-7 levels were higher in the under-ART group, including those patients with poor recovery of CD4 cell counts, compared to the AC group (Fig. 1H). In addition, the IL-7 levels and CD4 counts show a marked negative correlation (Fig. 2B). These results are consistent with findings previously reported (14,15), suggesting that IL-7 is physiologically induced by decreased CD4 cell counts. Currently, IL-7 is receiving attention as a cytokine that increases the CD4 cell count (17), and is the focus of many clinical studies investigating whether IL-7 administration may induce $CD4^+$ lymphocyte expansion in HIV-1-infected patients (13,19).

In this study we demonstrated characteristic increases in IFN-g levels due to HIV-1 infection. ART has been reported to decrease the serum levels of many of the cytokines that are elevated in patients with HIV-1 infection (16,22,23,25), and increases the levels of IL-21, which are reduced in patients with HIV-1 infection (9,10). To the best of our knowledge, ours is the first study to report that cytokine levels remain unchanged by ART, despite their abnormally high levels. IFN-g levels were high in some patients not only during the AC period, but also in patients with sustained suppression of viral replication and immune restoration by ART. This suggests the potential induction of IFN-g expression by HIV-1. This may be the first report on the above-mentioned phenomenon, probably because these high levels are not necessarily sustained in all cases, and thus changes occurring following the initiation of ART should be studied in more detail. Although the sustained high IFN-g levels observed in some patients is thought to be due to individual differences in immune responses against HIV-1, or the genetic characteristics of HIV-1 (8), or both, we could find no clinical data associated with increased serum IFN-g levels to support this. Unlike the total CD4 cell counts and viral loads presented here, the total CD8 cell counts and their kinetics after the initiation of ART were not associated with changes in IFN-g levels (data not shown). However, the IFN-g levels were increased in 9 of 33 patients (27%), and these patients account for a significant proportion, thus yielding important findings. IFN-g is a cytokine used as an immunocompetence indicator in HIV-1 vaccine studies (21). It has been reported that Th-2 cell numbers tend to increase with the progression of HIV-1 infection, and that IFN-g is one of the key cytokines for differentiation into Th-1 cells (6). Thus IFN-g may play an essential role, different from those of other cytokines, in the pathogenesis of HIV-1 infection. One possible mechanism behind the sustained high serum IFN-g levels seen despite ART's introduction may be that IFN-g production by HIV-1-specific $CD8^+$ T lymphocytes is driven by HIV-1 viremia, and could even be induced by ongoing viral replication during ART. Only a small population of $CD8^+$ T lympho-

cytes may be involved in IFN-g production, because there was no association between total CD8 cell counts and serum IFN-g levels. In future studies, we intend to investigate the role of this cytokine with a focus on acute HIV-1 infection, in which HIV-1-specific $CD8^+$ T lymphocytes are preferentially expanded to control viral replication.

Acknowledgments

Author Disclosure Statement

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CASE REPORT

Immune reconstitution to parvovirus B19 and resolution of anemia in a patient treated with highly active antiretroviral therapy

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Abstract Immune reconstitution inflammatory syndrome (IRIS) is an unsolved problem in the treatment of human immunodeficiency virus (HIV)-1 infection. Despite the high seroprevalence of parvovirus B19 (PVB19) among HIV-1-positive patients, reports on PVB19-induced anemia, especially that associated with PVB19-related IRIS, in these patients are limited. We present the case of a man with acquired immunodeficiency syndrome who developed severe transfusion-dependent anemia and was seropositive and borderline positive for immunoglobulin-M and IgG antibodies against PVB19, respectively. PVB19-DNA was also detected in his serum. The patient was diagnosed with pure red cell anemia (PRCA) caused by a primary PVB19 infection and was treated with periodical blood transfusions. However, he subsequently tested negative for IgG antibodies and developed chronic severe anemia with high levels of PVB19 viremia. This indicated a transition from primary to persistent infection. After initiation of highly active antiretroviral therapy, the patient showed an inflammatory reaction with rapid deterioration of anemia and seroconversion of the IgG antibody to PVB19. Subsequently, PRCA was completely resolved, but the patient's serum still contained low levels of PVB19-DNA. Thus, this was a case of IRIS associated with PVB19 infection. Our report highlights the significance of seroconversion to PVB19 in the diagnosis of IRIS and re-emphasizes the finding that persistently high levels of PVB19 viremia after primary infection are probably because of the lack of protective antibodies.

Keywords HIV-1 infection · Parvovirus B19 · Pure red cell anemia · Immune reconstitution inflammatory syndrome

Introduction

Human immunodeficiency virus (HIV)-1 is known to infect $CD4^+$ T lymphocytes and cause acquired immunodeficiency syndrome (AIDS) by decreasing the number of $CD4^+$ cells. In the mid-1990s, a new and specific treatment, namely, highly active anti-retroviral therapy (HAART), was developed to treat HIV-1 infection; HAART is a combination therapy comprising administration of two or three classes of antiretroviral drugs. This therapy induces long-term suppression of viral proliferation and immunological reconstitution in HIV-1-infected patients and thus increases their survival rate. Although HAART cures opportunistic infections by restoring the immune system, it can also induce an inflammatory reaction that is characterized by the aggravation of a preexisting opportunistic infection and the emergence of other infectious diseases that were not observed before the initiation of HAART. This phenomenon, termed as immune reconstitution inflammatory syndrome (IRIS), is thought to be caused by an immunological reaction to a pathogen that was present in the host before the antiviral therapy [1]. This paradoxical syndrome poses a major problem in the patients who undergo HAART.

Human parvovirus B19 (PVB19) belongs to the genus *Erythrovirus*. PVB19 is the predominant pathogenic erythrovirus in humans and is the prototype strain for

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genotype 1 [2]. PVB19 has been shown to cause erythema infectiosum in children as well as acute red cell aplasia in patients who have conditions causing hematopoietic stress, such as hemolytic anemia; this virus has also been implicated in the pathogenesis of rheumatic arthritis, myocarditis, nephritis, fulminant liver disease, and many other conditions [3]. In HIV-1-positive patients, PVB19 may persistently infect erythroid precursor cells, evade elimination by the immune system, and cause transfusion-dependent pure red cell anemia (PRCA) [4]. PVB19-related anemia can be resolved by treatment with intravenous immunoglobulin (Ig) [5]. However, this treatment often has a transient beneficial effect, and AIDS patients might experience a relapse of anemia. Therefore, AIDS patients may require periodic administration of intravenous Ig or blood transfusions. In recent years, some reports have shown that complete remission of PVB19-associated PRCA can be achieved by treating patients with HAART [6–8]. Although patients with HIV-1 infection show high seroprevalence of PVB19 [9], few reports have been published on primary or persistent PVB19 infection, particularly PVB19-related IRIS, in HIV-1-infected patients. In this report, we describe the case of a man with AIDS who presented with chronic PVB19-induced PRCA and IRIS after undergoing HAART. We focus on the relationship between the clinical presentation and immunological status in this condition.

Case report

A 54-year-old HIV-1-positive man visited our hospital in May 2006. He had been diagnosed with *Pneumocystis jiroveci* pneumonia and treated with sulfamethoxazole/trimethoprim in February 2006. His initial CD4 cell count was 35 cells/μl, and the plasma HIV-1 RNA level was 250,000 copies/ml. The results of other laboratory analyses were normal, except for the presence of slight anemia (hemoglobin level 11.5 g/dl). He reported that he had traveled abroad to Southeast Asia for personal reasons.

In November 2006, he re-visited our hospital, and his hemoglobin level had decreased to 7.7 g/dl. He did not show any other symptoms, such as fever, rash, or arthralgia, or any signs of cardiac, renal, or hepatic disorders. He did not report any direct contact with patients having erythema infectiosum. Two weeks later, he experienced dyspnea and was hospitalized immediately. Severe anemia was detected (hemoglobin 5.3 g/dl), and blood transfusions were performed (Fig. 1; Table 1). Gastrointestinal bleeding and hemolytic anemia were ruled out. PVB19 infection was suspected, and an immunoassay [Parvo B19 IgM-enzyme immunoassay (EIA); "SEIKEN," Denka Seiken, Tokyo, Japan] revealed anti-PVB19 IgM antibodies in the serum.
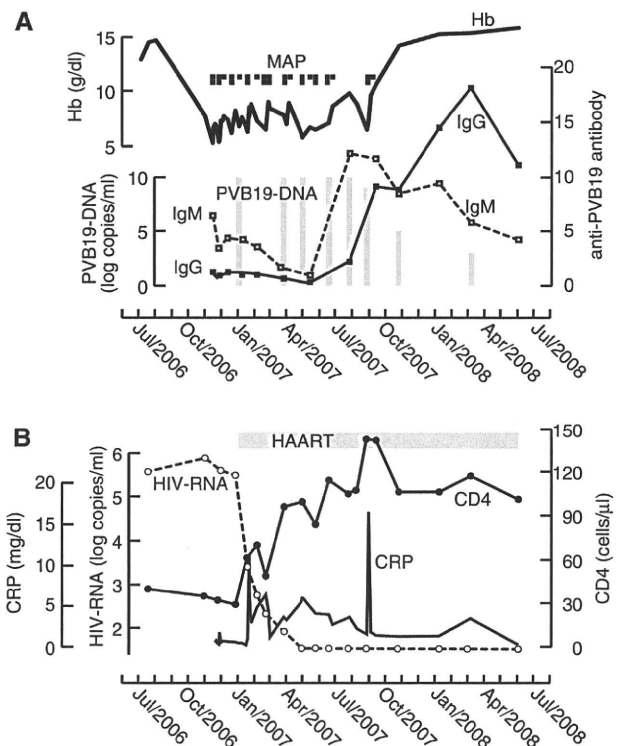

Fig. 1 The patient's clinical course with the changes in the hemoglobin levels and immunological status at the primary and persistent PVB19 infection and at the resolution of PRCA. **a** The *upper solid line* shows the time course of changes in the hemoglobin (Hb) level. The *closed boxes* indicate transfusion of 2 U of packed red blood cells. The *lower solid line* and the *broken line* represent the EIA indices of anti-PVB19 IgG and IgM antibodies, respectively. The *gray bars* show the concentration of serum PVB19-DNA. **b** Time course of changes in the CD4 cell count, plasma HIV-1-RNA levels, and CRP concentrations as well as the duration of HAART are shown

A qualitative polymerase chain reaction (PCR) analysis performed at BML Inc. (Tokyo, Japan) revealed the presence of erythrovirus DNA in the serum. The commercial assays for PVB19-DNA can detect erythrovirus DNA, including the DNA of the newly described erythrovirus variants (genotype 2 and 3) [2]. Quantitative assessment, i.e., real-time PCR analysis, was not performed at this point. The anti-PVB19 IgG antibody index assessed using Parvo B19 IgG-EIA (Seiken, Denak Seiken) was borderline positive (0.92). Examination of a bone marrow aspirate revealed an aplastic marrow (myeloid/erythroid ratio 63:1). Neither parasites nor hemophagocytic cells were found in the aspirate. Although typical giant proerythroblasts were not observed, acute PRCA caused by primary PVB19 infection was diagnosed. The patient was transfused with 6–8 U of blood per month. The anti-PVB19 IgG antibody index gradually reduced and changed from borderline-positive to negative, and the anemia did not improve; these findings indicated a transition from primary PVB19 infection to chronic and persistent infection. Intravenous Ig

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Table 1 Summarizing conditions of PVB19 infection, anemia, and immunological findings

Date (year/month)	PVB19 infection	Anemia	Decision of EIA		PVB19-DNA (log copies/ml)	CD4 cell count	HAART
			PVB19-IgG	PVB19-IgM			
2006/11	Primary infection	Acute anemia	+−	+	ND	35	−
2007/1	Persistent infection with high level viremia	Chronic anemia	−	+	10	29	−
2007/9	IRIS	Deterioration	+	+	9	142	+
2007/11	Low level viremia	Remission of PRCA	+	+	3–5	106	+

ND not determined

therapy was not administered because it is an expensive procedure.

From January 2007, HAART with tenofovir, emtricitabine, and efavirenz were initiated. The patient's CD4 cell count gradually increased, and his HIV-1 viral load became undetectable after May 2007. At the beginning of July 2007, the CD4 cell count had increased to 105 cells/μl, and seroconversion of IgG antibody was observed. Although the serum PVB19-DNA level was unchanged, the hemoglobin level increased to 9.8 g/dl, and the periodical blood transfusions were discontinued.

Two months after the last transfusion, the patient experienced episodes of dizziness and visited our hospital. His hemoglobin level rapidly deteriorated to 6.5 g/dl, and blood transfusion was repeated. The serum PVB19 load had reduced tenfold (from 10^{10} to 10^9 copies/ml). After 3 days, he developed fever and neutropenia (1,100 cells/μl), and circulating atypical lymphocytes were detected. Serum biochemical assessments showed elevated concentrations of lactate dehydrogenase (LDH) (395 IU/l) and C-reactive protein (CRP) (16.47 mg/dl). No other symptoms such as rash or arthralgia and no signs of cardiac, renal, or hepatic disorders were observed. The patient's symptoms disappeared, the abnormal test results reverted to normal within a few days, and the anemia rapidly improved. No further red blood cell transfusions were required. In October 2007, the patient's hemoglobin level was within the normal range, and the PVB19-DNA load decreased to 10^5 copies/ml. Although reexamination of bone marrow aspirate was not performed, PVB19-induced PRCA was completely resolved, and PVB19 IgG antibody was persistently detected; PVB19-DNA (10^3 copies/ml), however, was still detected.

Discussion

IRIS is a serious condition that can occur after the initiation of HAART. This syndrome is usually self-limited, but it may worsen and necessitate intervention. In our case, the patient presented with transient inflammatory responses, such as fever, shortly before the remission of PRCA. The laboratory results revealed leucopenia, atypical lymphocytes, and elevation of the serum LDH level; these findings were similar to those of a nonspecific response to viral infections. Additionally, his anemia rapidly worsened despite showing some improvement shortly before this episode. His immunological state was improving: the CD4 cell count rose, seroconversion to anti-PVB19 IgG antibody was observed, and the serum PVB19-DNA level showed a slight but significant decrease. On the basis of these paradoxical findings, we thought that this was an episode of IRIS. The recent literature contains only two reports of severe IRIS. In one of the cases, the patient presented with acute encephalitis [10]. In that case, the patient had persistent PVB19 infection, and the complication of chronic PRCA was treated with intravenous Ig therapy. Four weeks after initiating HAART, anemia developed rapidly with acute onset of ataxia and aphasia. Such a progression was unexpected because encephalitis is a rare complication in PVB19 infection. In the other case, acute and transient anemia developed after the initiation of HAART, although no anemia and PVB19 infection were detected before HAART [11]. Serum antibodies to PVB19 had not been fully confirmed in either of these cases. In all three cases, rapid deterioration of anemia was observed after HAART; this finding seems to be a typical presentation in IRIS associated with HAART for PVB19 infection. With the exception of anemia, the symptoms and pathogenic conditions observed in our case are different from those observed in the two above-mentioned cases. Our case seems to be the most typical presentation of IRIS because (1) the patient was proven to have a chronic PVB19 infection before HAART, (2) the immunological parameters, such as the CD4 cell count and IgG antibody production, showed an improvement during the course of IRIS, and (3) the patient developed symptoms resembling those of acute viral infection. The diagnosis of PVB19-associated IRIS with atypical features may be difficult because of the lack of diagnostic criteria. However, the

findings in our case suggested that seroconversion to antibody against PVB19 and the presence of anemia are helpful in diagnosing PVB19-related IRIS.

The production of neutralizing antibodies plays a pivotal role in the immune control of PVB19 infection [3]. Specific IgM and IgG antibodies are produced 2 and 3 weeks, respectively, after primary PVB19 infection, and these antibodies are responsible for the elimination of PVB19. The EIA kits used in this case could be used only for qualitative assessments. However, the EIA indices of anti-PVB19 IgG and IgM antibodies can indicate the titer of antibodies because these EIA kits include strong positive controls with EIA indices that are at least higher than 1.5, and the EIA indices of the clinical samples were up to 15 and showed good reproducibility. When evaluated on the basis of EIA indices, the anti-PVB19 IgM antibody level showed moderate elevation during primary PVB19 infection. In contrast, the samples were weakly negative for the anti-PVB19 IgG antibodies. These observations suggested that the class-switch recombination of B lymphocytes was markedly disturbed during the infection, and this was probably because of the dysfunction of CD4^{+} T lymphocytes. Lack of these protective antibodies may lead to the transition from primary to persistent infection and permit high-level PVB19 viremia [12].

In the pre-HAART era, some cases of chronic PRCA with persistent PVB19 infection were treated with intravenous Ig therapy [5], in which the patients were administered neutralizing antibodies to PVB19. This treatment results in a rapid decrease in the copy number of blood PVB19-DNA from 10^{10} to 10^{6} copies/ml and improvement of anemia [13]. However, this treatment has a transient effect, and many patients show recurrence of PRCA after the treatment. It should be noted that this therapy cannot clear the PVB19-DNA in the blood, and the DNA persists at levels of about 10^{6} copies/ml. These observations suggest that administration of neutralizing antibody alone is insufficient for eliminating PVB19. In our case, the patient developed chronic PRCA, and the serum PVB19-DNA level was 10^{10} copies/ml. Even after seroconversion to anti-PVB19-DNA IgG antibody and the resolution of PRCA, the viremia persisted, and the patient had viral loads of 10^{3}–10^{5} copies/ml. We cannot exclude the possibility that this might be caused by the production of an incomplete neutralizing antibody [7]. PVB19 was, however, not eliminated in our patient, as was also the case in previous reports of treatment with intravenous Ig. This indicates the importance of immune mechanisms other than humoral immune responses [14], such as those involving cytotoxic T lymphocytes.

In our case, the relationship among clinical observations, immunoserological findings and the serum viral load could be evaluated because intravenous Ig was not administered and immune recovery was prolonged. Physicians must note that PVB19 can cause severe anemia in HIV-1-infected patients [14], and detection of PVB19-DNA must be performed in immunosuppressed patients because of the lack of specific antibodies [12]. In addition, detailed investigations on immune reactions to PVB19 will facilitate a better understanding of the mechanism underlying immune reconstitution by HAART.

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review from the journal's Editor-in-Chief.

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