特集
日本の精神科医は
米国DSM-5を
どう読むか

〈総　論〉

社会精神医学におけるDSMシステム

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KEY WORDS
DSM，ICD，診断システム（diagnostic system），社会精神医学（social psychiatry）

抄録：精神医学診断システムは，社会精神医学あるいは疫学的精神医学的手法によってなされる精神障害を対象とした研究や，司法精神医学において大いに活用されており，精神医学診断と社会精神医学の関わりは深いといえる。DSM-5改訂により精神障害の呼称が変更され，今後スティグマの克服に向けた活動や多軸評定システムの不採用に伴う影響は検証されていくべきであろう。また診断システム改訂に伴って，社会精神医学分野の研究への影響も無視できない。今後，精神医学診断と社会に関わる相互の影響性を検証する調査研究が必要と考える。

1 はじめに

2013年に米国でDSM-5が発表されて，1年遅れて日本語版[3]が出版された。最近のインターネットの活用によって，その改訂作業については多くの精神科医が興味を持って，動向を注視していた。

精神医学は，臨床心理・精神病理学的側面，生物学的側面，および社会学的側面からなっており，その前提として精神医学診断の影響が少なくないことから精神医学診断の確かさは，ますますその重要性を増している。日本社会精神医学会のホームページにおいて，「社会精神医学」は，疫学的手法や社会科学的手法を用いて，社会的文脈からこころの健康問題の予防，疾患の診断・治療・リハビリテーション，社会保障制度のあり方などの研究を学際的に行う精神医学の一分野であり，精神医学関連領域に加え，看護学，心理学，社会学，教育学，公衆衛生学など多くの関連学問分野と関心を共有すると説明されている[13]。これらから推察するに，社会精神医学は，各精神疾患の疫学的データなどをもとに，その発症や経過および転帰に関わる社会的要因の役割を明らかにするとともに，その発症防止策や経過・転帰改善への糸口を探るというのが作業の中核であろう。

本稿では，社会精神医学における精神医学診断システムの役割や影響を中心に概説したい。

Meaning of the DSM system in social psychiatry
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2 DSMの果たした役割とその必要性

第二次世界大戦以後に，米国精神医学会(American Psychiatric Association：APA) は，新たな診断システムとしてWHOが作成したICD-6をベースに106の診断カテゴリから構成されたDSM (Diagnostic and Statistical Manual of Mental Disorders)-Ⅰ(1952年)を出版した。その後，DSM-Ⅱ(1965年)が出版され，その中心軸を徐々に生物学的精神医学に移しつつある過渡的なものであった。

DSMシステムの改訂過程の中で，アメリカにおける統合失調症概念がイギリスのそれよりも広く，イギリスで躁うつ病や神経症と診断された症例もアメリカでは統合失調症とみなされていたことがUS-UK project(1971年)[5]で明らかにされた。このため，精神医学的疫学研究や精神科薬物療法の効果判定の比較などの観点から，診断の一致率を高める必要性が認識され，操作的診断基準を持った精神障害分類体系構築が要請されるようになった。

このような動向に応えるかたちで登場したDSM-Ⅲ(1980年)[1]では，1. 精神科的問題に対する医学モデルの回復(データ交流)，2. 限定的な包括基準と除外基準の採用，3.「障害」概念の採用，4. 記述的診断を中心(神経症の排除)，および5. 多軸診断の採用といった特徴を有していた。日本語版も本国アメリカに遅れることわずか3年で導入され，日本でも急速に浸透していった[8,9]。DSM-Ⅲの改訂版としてDSM-Ⅲ-R(1987年)が出版され，DSM-Ⅳ(1994年)[4]へと引き継がれた。DSM-Ⅳでは，記述内容や操作的診断基準の根拠の有無からさらに実証性を重視した改訂となった2002年には，DSM-Ⅳ-TR精神疾患の診断・統計マニュアル[2]が発行されている。このDSM-Ⅲ以降の大きな変化は，精神医学の普遍化・標準化を助ける一方で，科学的試みであったはずのDSMが，一方では精神科臨床との乖離，診断システムの教条化・硬直化をもたらすという批判もあった。

3 社会からの影響　精神科診断と性

セクシャルマイノリティーに関しては，当事者の活動によりその精神科診断の扱いが大きく変化したことが知られている[7]。DSM-Ⅰにおいて同性愛は「病的性欲をともなった精神病質人格」と規定されていた。1973年にDSM-Ⅱの第7刷以降「同性愛」という診断名は削除され，代わって「性的指向障害」という診断名になったものの，同性愛を精神病理的異常とみなすか正常とみなすかに二分されていた。1980年のDSM-Ⅲでは同性愛という一般的ではない性指向を持つことがすなわち精神疾患というのではなく，そのことに苦悩を抱いている時に初めて疾患とみなされ，「自我異質性同性愛」という診断名が登場した。しかし，社会的偏見を助長するという批判から「同性愛」という疾患概念は，1987年のDSM-Ⅲ-Rでは削除されて以降，DSM-Ⅳ，DSM-Ⅳ-TR，そしてDSM-5でも精神疾患として扱われなくなっている。

またこれに呼応するようにWHOの国際疾病分類第9版ICD-9 (1975年)では「性的逸脱及び障害」の項の1つに「同性愛」という分類名があげられていたが，1990年採択のICD-10[20,21]では「同性愛」の分類名は廃止され，F66. 性の発達と方向づけに関連した心理及び行動の障害で同性愛的という分類名が用いられたが，同性愛自体は障害とされなくなった。日本の厚生省は1994年にWHOの見解を踏襲し，日本精神神経学会も1995年にWHOなどの見解を尊重すると表明し，「同性愛はいかなる意味でも治療の対象とはならない」と宣言している。

今回のDSM-5の改訂において，性同一性障害もその扱いが議論された。草稿の作成時において，WPATH (World Professional Association for Transgender Health)からの声明[22]など多くの関心を集めた。結局，DSM-5で，Gender Identity DisorderからGender Dysphoria (性別違和)に呼称が変更された。この変更には，精神科医だけでなく，関連領域の医師をはじめと

する医療専門職のほか，当事者，家族などの支援者の意見が大きく反映されている。性科学の進展で人間の多様な性行動が明らかになってきたことや，性的権利思想の出現に伴い価値規範が変化してきたことなどにより，性的問題への考えも変わりつつあり社会からの影響の1つの表れといえよう。

4 社会に与える影響

1. 診断名の重要性

先に述べたように，Gender Dysphoriaをはじめとして DSM-5改訂作業に際し精神医学診断の呼称については変更が加えられた。日本に導入する際にわが国で用いる精神科診断の呼称をはじめとする専門用語についても，同様に非常に重要な課題といえる。わが国の保険診療システムについてはICDシステムが用いられており，保険病名の管理やさまざまな医療保険福祉サービスにも広く活用されている。

しかしその一方で，精神障害や精神医療に対するスティグマが少なからず存在している[6]。マスメディア，インターネットを通じた精神医学診断をはじめとする精神医学の関連情報は，当事者，家族あるいは一般住民へ大きく影響を与える。最近では，いわゆる「新型うつ病」をめぐる社会への影響は看過できないものである。否定的な内容が広く流布することで，「うつ病」の正しい理解を妨げることが危惧される。また発達障害についても同様の傾向があり，精神科診断が周知される際には，診断名のみではなく付随する情報についても十分な理解を促すことが必要であろう。

さらに日常臨床の場面においても，治療を進めるうえで病名の告知や情報の提供がなされる場合も同様である。以前の精神科治療においては，明らかな病名告知がなされない状況下で診療・治療が行われていたものが，昨今の精神医療を取り巻く環境の変化から，適切な医療情報をその都度伝え，治療を進めていくかたちへと変わりつつある。特に2002年8月に行われた「精神分裂病」から「統合失調症」への呼称変更[17]に伴い，患者・家族に精神障害の情報を伝える機会が増したと感じられる。西村は，日本精神神経学会精神科医全員を対象に，2002年(変更直後)，2003年(1年後)，2004年(2年後)の3年間にわたる調査[15]を行った。この結果，本人に告知しないとした群は，変更直後は44％であったものが，1年後に21％，2年後に15％に減少し，告知する群は3年間で37％，65％，70％と漸増した。統合失調症は精神科医の間でほぼ9割普及し，精神分裂病やそのほかの病名を併用することなく単独で使用できる病名となった。呼称の変更という表面的な変化だけではなく，スティグマの軽減という効果などからも，その精神障害のコンセプトや疾患に対する枠組みが変わることが示されたわが国の取り組みが国際的に評価され，この試みは海外においても呼称変更がなされた。わが国においては，精神分裂病が「統合失調症」に変更される以前にも，これまでに，操作的診断基準においては，躁うつ病が「感情障害」に，また自閉症が「広汎性発達障害」にと呼称が変更されている。これ以外にも，認知症，知的障害，社交不安障害など，多くの精神科診断に関わる病名について変更がなされてきている。このような動きを受けて，日本精神神経学会の精神科病名検討連絡会によってDSM-5病名・用語翻訳ガイドライン[14]が出版された。DSM-5において，日本語診断あるいは症状について多くの検討・変更がなされており，医療福祉サービスの利用や精神科薬物療法などを含めた社会的影響について今後評価していく必要があるだろう。

2. 多軸評定システムの有用性

先にも述べたように多軸評定システムを日常診療の現場で生かそうというのが，DSM-Ⅲ以降の大きな特徴の1つでもあった。Axis Ⅰと Axis Ⅱは精神科臨床症状評価で，Axis Ⅲは身体疾患・身体状態に関する評価，Axis Ⅳは心理・社会的ストレスの重症度，Axis Ⅴは機能に

関する全体的評定である。米国や諸外国でいかに利用されていたか明らかではないが，少なくともわが国ではほとんど生かされなかったのが実情のようである。各事例について総合的な評定を行うことは理想的方策であると考えられたはずであるにもかかわらず，現実的な利用に至らなかったのは残念である。ICD-10Fにおいても，児童・思春期領域疾患についてはDSM以上の多軸が提案・出版され，成人事例についても検討された経緯はあるが，汎化することはなかった。特に社会精神医学分野では，精神障害者のリカバリーや社会参加を考えるうえで，もっと活用の余地があったのではなかろうか。DSM-5に至り多軸評定システムは採用されず，GAFをWHO-DAS2.0[18]に置き換えることが推奨された。このことからDSM-5が包括的な評価から精神医学臨床診断に重心を移したようにも見える。多軸評定については，確かに日常臨床的には煩雑なきらいはあるが，精神医学がbio-psycho-socialな視点に立とうとするなら，今一度考えなおすべきであろう。

5 精神障害に関するコンセプトの変化あるいは診断システム改訂に伴う疫学研究への影響

社会精神医学あるいは疫学的精神医学的研究においては，精神障害(者)を対象とした発病率，有病率調査や転帰研究を施行することが少なくない。たとえば，WHOは，統合失調症やうつ病の症例発見研究と長期転帰研究，プライマリ・ケアにおける精神科医療の実態に関する調査，最近ではcommon mental disordersに関する世界精神保健調査WMH（World Mental Health Surveys）(日本を含め世界10数カ国参加)を実施してきている。そこでは，一定の基準をもとに採用症例を抽出し，所定の方法に則って精神症状を面接評定し，基準に合わせて診断された事例について，有り様，経過や転帰について多国間比較するものである。こうした国際比較の前提として，診断基準・指針は絶対に欠かせず，これらなしでは有意の比較は成立しない。多施設間比較という場合，国内における共同研究であってもほとんど同じ次元である[12]。

長期にわたる研究の場合，途中で診断システムが改訂されることは，しばしば経験される。そのような場合に実際どのようなことが生じるか詳細に検討が必要であろう。長崎大学で行われた「重度精神障害の転帰決定因に関する国際共同研究(International Collaborative Study on Determinants of the Outcome of. Severe Mental Disorders; DOSMeD Study)」において統合失調症の亜型分類をICD-9とICD-10で比較検討した。DOSMeD研究において，スタートした当時はICD-9であったが，1992年にはWHO総会にてICD-10に改訂され，それに従って経過観察中に対象症例を再分類することになった。ICD-9は，分類体系としてほぼ確立されていたものの，詳細な診断指針は提言されておらず慣用的な診断が許容されており，各地域の従来診断に則っていた。そこで，われわれの長崎センターで採用された症例をICD-10Fで再分類した(表1)。全症例107例のうち，ICD-9で破瓜型とみなされた53例中，15例(28％)だけがICD-10Fでも同じ亜型に分類されたのに対し，妄想型についてはICD-9で31例のうち18例(58％)がICD-10Fでも同じ診断であった。この結果，日本では諸外国と違って永いこと，破瓜型が統合失調症における中核的亜型とされていたが，ICD-10Fになると諸外国で受け入れられていたと同様の傾向を確認できるようになった。DSM-5では，統合失調症の亜型分類が採用されなかった。特定用語やClinician-Rated Dimensions of Psychosis Symptom Severityを用いることで特徴的な臨床像の記述を行うようになっているが，その妥当性の検証が今後必要であろう。

また，WHOが主催した国際的精神障害大規模疫学調査WMH[19]に，長崎大学も参加して，common mental disorderの有病率や受診行動を

表1　WHO/DOSMeD-Japan 採用症例の ICD-9 診断および ICD-10F 再診断分類

ICD-9 ICD-10F	295.1 破瓜型	295.3 妄想型	その他	合計（%）
F20.0 妄想型	14	18（58%）	1	33（30.8）
F20.1 破瓜型	15（28%）	0	1	16（15.0）
F20.3 鑑別不能型	9	4	3	16（15.0）
F23 急性一過性精神病性障害	7	6	7	20（18.7）
F25 統合失調感情障害	0	0	1	1（0.9）
その他	8	3	10	21（19.6）
合計	53	31	23	107（100）

調べた。この調査では，CIDIを用いてDSMシステムおよびICDシステムで精神医学診断を行い，それぞれの有病率を求めることができる。少数であるが長崎市の対象者(男性83人，女性125人)をCIDIの結果から両システムでの有病率を調べてみた。この結果，長崎市における精神障害の生涯有病率(表2)を見ると，同じデータを基にDSMシステムとICDシステムでは同じ精神障害であれば，多くは類似する傾向にある。しかし，Dysthymia，Generalized Anxiety Disorder，Hypomania，Posttraumatic Stress Disorderなどでは異なった有病率を示していた。なかでも顕著なのは，Major Depressive Disorder(DSM：12.2，ICD：5.2)，Social Phobia(DSM：0.7，ICD：3.3)である。

DSM-5の改訂の際には，当然疫学研究のレビューも行われ，診断分類の妥当性についても検討されている。しかし，このように精神医学診断システムの違いによっても，調査研究の結果にも違いが現れる。今後，精神医学の関連する評価尺度のアップデートも行われていくと思われるが，DSM-5への改訂後の調査研究の結果を読み解く際に考慮する必要があると考える。

6 おわりに 社会的に期待される精神医学診断とは

米国においては，DSM-5が出版され1年を経過することから，改めて診断システムの評価に関する報告がなされている。特に社会的影響については無視できないことからPickersgill[16]は，DSM-5が医療対象化プロセスについて多くの意見が得られたことから，社会学的視点の必要性を報告している[16]。これは，精神保健サービスや政策決定に役立ち，精神保健・医学に対する偏見・差別の克服にも重要な意味を持つものと考える。

精神医学診断システムの改訂は，精神医学や精神科臨床への多大な影響を引き起こす。われわれはこれまで私家本として「精神障害の分類システム，ICD-10，ICD-9とDSM-Ⅳ-TRの対照表」(第一版[10]，第二版[11])を出版してきた。今後も，それらの適正な運用のためにDSM-5，DSM-Ⅳ-TR，ICD-10などさまざまな精神医学診断システムとの互換性を担保することが重要であろう。

今回DSM-5は，Living-documentの性質を持ったものという位置づけであることから，さらなるアップデートが考えられる。DSMを用いて動的な精神医学診断システムを考えることが，精神医学の発展にもつながると思われる。現在，ICD-11の改訂には多くの時間を要しているが，ICD診断システムは初期には10年ごとの改訂が予定されていた。頻繁な改訂は混乱を招くおそれもあり，改訂作業には多くの時間を要することから，一定サイクルでの実現は難しいのかもしれない。このため，ICD-10が出版された際には10年サイクルにはこだわらないとされていた。しかし本来，精神医学診断システムは，精神医学の発展や社会的状況も考慮して変化するべきものなのかもしれない。本稿でみてきたよ

表2 長崎市における精神障害のDSMおよびICDによる重み付け推定生涯有病率

	男性（n=83）		女性（n=125）		合計（n=208）	
	生涯有病率		生涯有病率		生涯有病率	
	DSM-Ⅳ	ICD-10	DSM-Ⅳ	ICD-10	DSM-Ⅳ	ICD-10
Agoraphobia	2.4	2.4	3.3	4.0	2.9	3.3
Alcohol Abuse	2.5	2.5	1.1	1.1	1.7	1.7
Alcohol Dependence	0.6	0.0	0.0	0.0	0.3	0.0
Dysthymia	1.9	3.4	2.2	3.9	2.0	3.7
Generalized Anxiety Disorder	2.5	2.5	9.6	7.1	6.4	5.0
Hypomania	0.0	1.4	1.7	2.8	0.9	2.2
Mania	0.0	0.0	0.5	0.5	0.3	0.3
Major Depressive Disorder	7.5	5.1	16.0	5.3	12.2	5.2
Panic Disorder	0.0	0.6	0.0	2.2	0.0	1.4
Posttraumatic Stress Disorder	4.4	6.4	5.6	6.2	5.0	6.3
Social Phobia	1.0	5.3	0.5	1.7	0.7	3.3
Specific Phobia	3.1	4.1	8.5	7.7	6.1	6.1

うに，精神医学診断システムは，単なる診断，統計データの収集といったものからその役割を変えてきており，精神科医だけのものでなくなってきている。今後，精神医学診断と社会との相互の影響を検証する調査研究が必要と考える。

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Selection of TI8-8V Mutant Associated with Long-Term Control of HIV-1 by Cross-Reactive HLA-B*51:01–Restricted Cytotoxic T Cells

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Elite controllers of HIV-1–infected HLA-B*51:01⁺ hemophiliacs, who remain disease free and have a very low plasma viral load for >30 y, had the 8V mutation at an immunodominant Pol283-8 (TI8) epitope, whereas the 8T mutant was predominantly selected in other HIV-1–infected HLA-B*51:01⁺ hemophiliacs, suggesting an important role of the 8V mutant selection in long-term control of HIV-1. However, the mechanism of this selection and the long-term control in these elite controllers remains unknown. In this study, we investigated the mechanism of the 8V mutant selection in these controllers. TI8-specific CTLs from these individuals evenly recognized both TI8 peptide–pulsed and TI8-8V peptide–pulsed cells and effectively suppressed replication of wild-type (WT) and the 8V viruses. However, the results of a competitive viral suppression assay demonstrated that CTLs from the individual who had WT virus could discriminate WT virus from the 8V virus, whereas those from the individuals who had the 8V virus evenly recognized both viruses. The former CTLs carried TCRs with weaker affinity for the HLA-B*51:01-TI8-8V molecule than for the HLA-B*51:01-TI-8 one, whereas the latter ones carried TCRs with similar affinity for both molecules. The reconstruction of the TCRs from these CTLs in TCR-deficient cells confirmed the different recognition of the TCRs for these epitopes. The present study showed that the 8V mutant virus could be selected by cross-reactive CTLs carrying TCR that could discriminate a small difference between the two molecules. The selection of the 8V mutant and elicitation of these two cross-reactive CTLs may contribute to the long-term control of HIV-1. *The Journal of Immunology*, **2014, 193: 4814–4822.**

Cytotoxic T lymphocytes play an important role in the control of HIV-1 (1–9). However, HIV-1 can escape from CTL-mediated immune pressure by various mechanisms such as Nef-mediated HLA class I downregulation and mutation to allow escape from HIV-1–specific CTLs (10, 11). The acquisition of amino acid mutations within CTL epitopes and/or its flanking regions leads to reduced ability for peptide binding to HLA class I molecules, impaired TCR recognition, and defective epitope generation (12, 13), resulting in lack of CTL activities to suppress replication of HIV-1 mutant virus as well as in the selection and accumulation of escape mutant viruses (10, 14–19).

A minority of HIV-1–infected individuals, who are known as elite controller or long-term nonprogressors, remain disease free and have a very low viral load (VL), even in the absence of antiretroviral therapy (20–22). A majority of these elite controllers carry the HLA-B*57/58:01, HLA-B*27, or HLA-B*51 allele associated with slow progression to AIDS (23, 24), suggesting that HIV-1–specific CTLs restricted by these HLA alleles control HIV-1 in elite controllers. The mechanism of the control by these CTLs has been well studied in elite controllers and slow progressors carrying HLA-B*57/58:01, HLA-B*27 or HLA-B*13. These studies showed strong Gag-specific CD8⁺ T cell responses in elite controllers or slow progressors carrying these alleles, suggesting that they may control HIV-1 (15, 16, 25–27). HLA-B*57–mediated immune pressure selects the escape mutation T242N in the Gag TW10 epitope. This mutation impairs viral replication, resulting in control of HIV-1 in these HLA-B*57⁺ individuals (28, 29). In the case of HLA-B*27⁺ individuals, the presence of Gag KK10–specific CD8⁺ T cell is associated with the control of HIV-1 (4, 30–32). The immunodominant KK10 epitope is almost invariably targeted by CD8⁺ T cells, and the KK10-specific CD8⁺ T cells display potent effector functions (4, 30, 31, 33). The conservation of this response is thought to account for the control of HIV-1 in these individuals.

A previous study showed that the HLA-B*51:01 allele was associated with long-term control of HIV-1 in HIV-1–infected Japanese hemophiliacs, and the frequency of HLA-B*51:01–restricted Pol283-290 (TI8: TAFTIPSI)–specific CD8⁺ T cells was inversely associated with plasma VL in HIV-1–infected ones (34), suggesting an important role of TI8-specific CD8⁺ T cells in the long-term control of HIV-1 infections. Four mutations (8T, 8L, 8R, and 8V) at position 8 of the TI8 epitope were significantly detectable in HLA-B*51⁺ individuals more than in HLA-B*51⁻ individuals, suggesting that these mutations were selected by TI8-specific CTLs (35). The 8T mutation is predominantly found in HIV-1–infected HLA-B*51:01⁺ donors. TI8-specific CTLs have a strong ability to suppress the replication of wild-type (WT) and the 8V mutant viruses in vitro but fail to suppress that of the 8T, 8L, and 8R mutant viruses (35, 36). A study using a Japanese hemophiliac cohort showed that the 8V mutation is found in only

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Abbreviations used in this article: 7-AAD, 7-aminoactinomycin D; RT, reverse transcriptase; VL, viral load; WT, wild-type.

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HLA-B*B51:01^{+} elite controllers (34), suggesting that the selection of the 8V mutant virus is a critical factor for long-term control of HIV-1 in Japanese hemophiliacs. However, the mechanism underlying selection of the 8V virus in HLA-B*51:01^{+} elite controllers remains unclear.

In the present study, to clarify the mechanisms of the 8V mutant selection, we investigated how TI8-specific CTLs from elite controllers select the 8V mutant. We established TI8-specific CTL clones from three elite controllers and then analyzed their abilities to suppress the replication of the 8V virus and to select this mutant in vitro. In addition, we assessed the TCR affinity of the CTLs for HLA-B*51:01 with the 8V peptide or WT peptide and evaluated the function of TCRs isolated from the CTL clones by reconstructing them in TCR-deficient cells. In the present study, we clarified the mechanism of the 8V selection and suggested its role in the long-term control of HIV-1 in Japanese hemophiliacs.

Materials and Methods

Patients

Three HIV-1–infected, antiretroviral-naive Japanese hemophiliacs were recruited for the current study, which was approved by the ethics committees of Kumamoto University (RINRI number 540, GENOME number 210) and the National Center for Global Health and Medicine (ID-NCGM-A-000172-00). Written informed consent was obtained from all subjects according to the Declaration of Helsinki.

Cells

C1R cells were purchased from American Type Culture Collection. C1R cells expressing HLA-B*51:01(C1R-B*51:01) were previously generated by transfecting C1R cells with *HLA-A*51:01* genes (37, 38). They were maintained in RPMI 1640 medium containing 5% FBS (R5) and 0.15 mg/ml hygromycin B. TCR-deficient mouse T cell hybridoma cell line TG40 cells were provided by T. Saito (RIKEN Institute, Saitama, Japan). TG40 cells expressing human CD8α (TG40/CD8) were previously established by transfecting TG40 cells with CD8α genes (39). TG40/CD8 and T1 cells, purchased from American Type Culture Collection, were maintained in R5.

HIV-1 clones

A previously reported infectious proviral clone of HIV-1, pNL-432, was used (40). Pol283-8V mutant viruses were previously generated on the basis of pNL-432 (34, 35).

Generation of TI8-specific CTL clones

HLA-B*51:01–restricted TI8-specific CTL clones were generated from HIV-1–specific bulk-cultured T cells by limiting dilution in U-bottom 96-well microtiter plates (Nunc). Each well contained 200 μl cloning mixture (~1×10^6 irradiated allogeneic PBMCs from healthy donors and 1×10^5 irradiated C1R-B*51:01 cells prepulsed with the corresponding peptide at 1 μM in RPMI 1640 medium containing 10% FBS [R10], 200 U/ml human rIL-2, and 2.5% PHA soup). These CTL clones were cultured in RPMI 1640 medium containing 10% FBS, 200 U/ml human rIL-2, and 2.5% PHA soup. The CTL clones were stimulated biweekly with irradiated target cells pulsed with the peptide.

Cytotoxic assay of CTL clones

The cytotoxic activity of TI8-specific CTL clones was determined by the standard ^{51}Cr release assay described previously (36). Briefly, C1R-B*51:01 cells were incubated with 100 μCi $Na_2{}^{51}CrO_4$ in saline for 60 min and then washed three times with R5. Labeled target cells (2×10^3/well) were added to each well of a U-bottom 96-well microtiter plate (Nunc) with the appropriate amount of the corresponding peptide. After a 1-h incubation, effector cells were added at an E:T ratio of 2:1, and then, the cultures were incubated for 4 h at 37°C. The supernatants were collected and analyzed with a gamma counter. Spontaneous ^{51}Cr release was determined by measuring the number of counts per minute in supernatants from wells containing only target cells (cpm spn). Maximum ^{51}Cr release was determined by measuring the cpm in supernatants from wells containing target cells in the presence of 2.5% Triton X-100 (cpm max). Specific lysis was defined as (cpm exp − cpm spn)/(cpm max − cpm spn) × 100, where cpm exp is the number of cpm in the supernatant in the wells containing both target and effector cells.

HIV-1 replication suppression assay

The ability of TI8-specific CTL clones to suppress HIV-1 replication was examined as described previously (41). CD4^{+} T cells were isolated from PBMCs of HLA-B*51:01^{+} healthy donors and incubated with the desired HIV-1 clones for 4 h at 37°C. After three washes with R5, the cells were cocultured with TI8-specific CTL clones. From days 3 to 7 post infection, culture supernatants were collected, and the concentration of p24 Ag in them was measured by use of an ELISA kit (HIV-1 p24 Ag ELISA kit; ZeptoMetrix).

In vitro competitive viral suppression assay

T1 cells (HLA-B*51:01^{+}) were coinfected with NL-432 and NL432-Pol283-8V mutant viruses at a ratio of 9:1. The infected cells were incubated with TI8-specific CTL clones at an E:T ratio of 0.05:1. From days 4 to 7 postinfection, culture supernatants were collected; and the concentration of p24 Ag in these supernatants was measured by using the HIV-1 p24 Ag ELISA kit (ZeptoMetrix). Viral RNA was extracted from the culture supernatant by using a QIAamp Viral RNA Mini kit (Qiagen) and subjected to RT-PCR by use of a SuperScript III One-Step RT-PCR System (Invitrogen). Nested PCR was subsequently performed for direct sequencing. The ratio of WT to mutant virus was determined by the relative peak height on the sequencing electrogram.

Tetramer binding assay

HLA class I–peptide tetrameric complexes (tetramers) were generated as described previously (34). CTL clones were stained with PE-conjugated tetramers at 37°C for 30 min. The cells were then washed twice with R5, followed by staining with FITC-conjugated anti-CD8 mAb and 7-aminoactinomycin D (7-AAD) at 4°C for 30 min. Finally, they were washed twice with R5. For the analysis of tetramer association, CTL clones were stained with PE-conjugated tetramer at a concentration of 1 μM at 37°C for various times (0, 2, 5, 10, 15, 30, and 60 min) and then washed as described above. For the analysis of tetramer dissociation, CTL clones were stained with PE-conjugated tetramer at a concentration of 1 μM at 37°C for 30 min. A portion of the cells was then removed periodically (0, 5, 10, 15, 30, 60, 80, and 110 min), and the cells were washed as described above. The cells stained with tetramer were then analyzed by flow cytometry (FACSCanto II).

Sequencing of plasma RNA

Viral RNA was extracted from the plasma of chronically HIV-1–infected individuals by using a QIAamp Mini Elute Virus spin kit (Qiagen). cDNA was synthesized from the RNA with Superscript III and random primer (Invitrogen). We amplified HIV reverse transcriptase (RT) sequences by nested PCR with RT-specific primers 5′-ACACCTGTCAACATAATTGG-3′ and 5′-TGTATGTCATTGACAGTCCA-3′ for the first-round PCR and 5′-GGGCCTGAAAATCCATACAA-3′ and 5′-GGTGATCCTTTCCATCCC-TG-3′ for the second-round PCR. PCR products were sequenced directly or cloned with a TOPO TA cloning kit (Invitrogen) and then sequenced. Sequencing was done with a BigDye Terminator version 1.1. cycle sequencing kit (Applied Biosystems) and analyzed by use of an ABI PRISM 310 or 3100 Genetic Analyzer.

TCR clonotype analysis

CTL clones were stained with PE-conjugated tetramers, anti-CD8 mAb, and 7-AAD, and then, tetramer^{+}CD8^{+}7-AAD^{-} cells were sorted by using a FACSAria. Unbiased identification of TCR-αβ-chain usage was assessed as described previously (42). TCR gene designations were based on the ImMunoGeneTics database.

Reconstruction of TCRs on TCR-deficient T cells

The cDNAs encoding full-length TCRα and TCRβ of TI8-specific CTL clones were obtained by a previously described method (42). cDNA was then amplified by nested PCR with TCR-α–specific primers 5′-GGAATTCGCCGCCACCATGCTCCTGCTGCTCGTCCCAG-3′ and 5′-ATTTGCGGCCGCAGATCTCAGCTGGACCACAGCCGCAG-3′ or 5′-GGAATTCGCCGCCACCATGGAAACTCTCCTGGGAGTGT-3′ and with TCR-β–specific 5′-GGAATTCGCCGCCACCATGGCCTCCCTGCTCTT-CTTCT-3′ and 5′-ATTTGCGGCCGCCTAGCCTCTGGAATCCTTTCTC-TTGA-3′ or 5′-GGAATTCGCCGCCACCATGGGCACCAGGCTCCTCT-GCT-3′. The amplified genes were separately cloned into a retrovirus vector pMX and used to transfect TG40/CD8, as described previously (39). Briefly, the genes of TCR were subcloned into the retroviral vector pMX. First, the ecotropic virus packaging cell line Platinum-E was transfected with the constructs. Two days later, the culture supernatant containing recombinant

virus was collected and then incubated with TG40/CD8 cells in the presence of 10 μg/ml polybrene for 6 h. The cells were cultured for an additional 2 d for analysis of TCR gene expression. Finally, the cells showing bright staining with PE-conjugated anti-mouse $CD3^+$ mAb (2C11; BD Pharmingen) were sorted by using the FACSAria.

IL-2 secretion assays

TG40/CD8 cells transfected with TCRs (4×10^4/well) were cultured with C1R-B*51:01 (4×10^4/well) in 200 μl R5 in the presence of various concentrations of peptides in U-bottom 96-well microtiter plates (Nunc) for 48 h at 37°C. The culture supernatants were then collected, and the concentration of IL-2 in them was measured by use of an IL-2 ELISA kit (eBioscience).

Results

*Slow selection of Pol283-8V mutant in HLA-B*51:01$^+$ Japanese elite controllers*

We previously revealed that only 3 (patients KI-021, KI-051, and KI-124) of 108 Japanese hemophiliacs who had survived without antiretroviral therapy for ~15 y, from 1983 to 1998, exhibited a strong inhibition of HIV-1 for an additional 10 y (34). All three of these elite controllers recruited from 1997 to 1999 had HLA-B*51:01 and the 8V mutation at position 8 in the TI8 epitope. To identify when the 8V emerged, we performed longitudinal sequence analysis of the TI8 epitope in these three patients. KI-021 and KI-124 already had the 8V mutant in September 1997 and January 1998, respectively. KI-051 had only the WT sequence in October 1999, the 8V mutant in 63% of the clones analyzed in July 2002, and then only the 8V mutant in October 2006 (Table I). Since KI-051 had been infected with HIV-1 before 1985, these results indicate that the WT virus predominantly existed for >17 y after HIV-1 infection. Thus, this mutant was slowly selected and had accumulated in this patient.

Recognition of the 8V mutant by TI8-specific CTLs

A previous study showed that the 8V mutation weakly reduced the recognition of TI8-specific CTL clones established from KI-051 in July 2002 (34, 35). We first reconfirmed this finding by using three TI8-specific CTL clones (2B5, 2C6, and 2D1), which were established from KI-051 in July 2002 when the WT virus was still detectable. The results for a representative CTL clone (2C6 clone) are shown in Fig. 1A. This clone effectively suppressed both WT and 8V mutant viruses but revealed slightly weaker ability to suppress the replication of the 8V mutant than the WT virus at higher E:T ratios. All three CTL clones revealed significantly weaker ability to suppress the replication of the 8V mutant than the WT virus at an E:T cell ratio of 1:1 (Fig. 1B, 1C). We further established 11 TI8-specific CTL clones from three elite controllers (KI-051 in June 2009, KI-021 in September 1997 and January 2005, and KI-124 in August 2001) and investigated the ability of these TI8-specific CTL clones to recognize the WT and the mutant epitopes. We first measured the killing activity toward target cells prepulsed with the WT or the 8V mutant peptide. All 11 CTL clones as well as 2B5, 2C6, and 2D1 clones showed the same killing activity toward target cells prepulsed with the 8V peptide as that of those prepulsed with the WT peptide (Supplemental Fig. 1). We next analyzed the ability of these CTL clones to suppress the replication of the 8V virus. CTL clones 2B, 7B, and 7F, which were established from KI-051 in June 2009 when only the 8V virus was detectable, exhibited a similar ability to suppress both the 8V and the WT virus at the same level (Fig. 1B, 1C). The CTL clones from KI-021 and KI-124 also showed characteristics similar to those of these clones from KI-051 (Fig. 1B, 1C).

Table I. Sequence of Pol283-8 epitope in three HLA-B*51:01$^+$ elite controllers of HIV-1–infected Japanese hemophiliacs

Patient	HLA Allele						Sample Date (month/date/year)	Pol283-8 Epitope			VL (copies/ml)	CD4 (cells/ml)	Name of CTL Clone
	A Allele		B Allele		C Allele			Sequence	Frequency				
								TAFTIPSI[a]	Direct (%)[b]	Cloning[c]			
KI-021	2402	2602	5101	6701	0702	1402	9/12/1997	- - - - - - - - V	100	12/12	<400	727	3B, 4C, 3D
							3/27/1998	- - - - - - - - V	100	12/12	<400	808	
							1/27/2005	- - - - - - - - V	100	12/12	<50	646	10, 20, 52
KI-051	0206	3101	4002	5101	1402	1502	10/26/1999	- - - - - - - - -	100	NT[d]	<400	629	
							7/25/2002	- - - - - - - - -	37	6/12	63	911	2B5, 2C6, 2D1
								- - - - - - - - V	63	6/12			
							10/24/2006	- - - - - - - - V	75	NT[d]	<50	966	
								- - - - V - - V	25	NT[d]			
							6/30/2009	- - - - - - - - V	90	9/12	<50	1040	2B, 7B, 7F
								- - - - V - - V	10	3/12			
KI-124	1101	0206	5101	1501	0401	1402	1/13/1998	- - - - - - - - V	100	15/15	<400	745	
							8/16/2001	- - - - - - - - V	100	18/18	600	511	12E, 12H

[a]The sequences for KI-021 in March 1998 and January 2005 were obtained from proviral DNA, whereas those for all other patients and KI-021 in September 1997 were from plasma RNA.
[b]Direct, direct sequence.
[c]Number of clones carrying the indicated sequence/number of clones tested.
[d]NT, not tested.

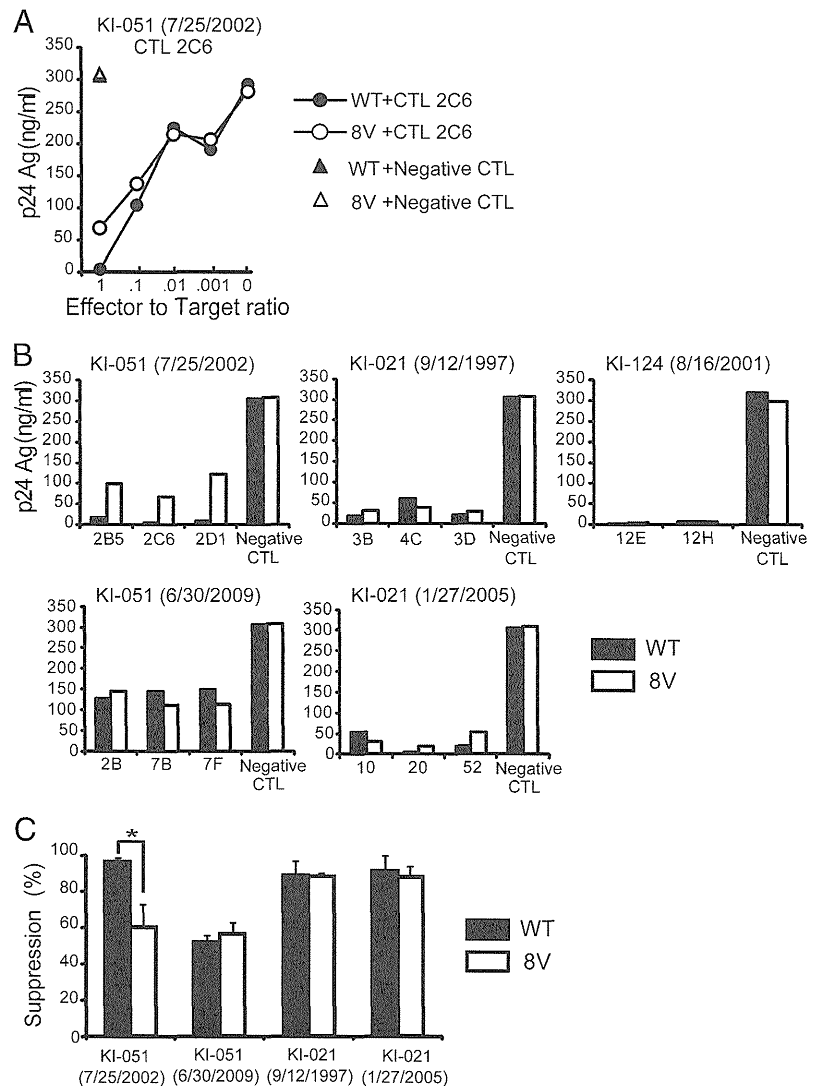

FIGURE 1. Abilities of TI8-specific CTL clones to suppress the replication of the 8Vmutant virus. (**A**) Ability of TI8-specific CTL clone 2C6, which was established from KI-051 in July 2002 when WT virus was still detectable, to suppress the replication of NL432 virus and the 8V virus. $CD4^+$ T cells from an HLA-B*51:01$^+$donor were infected with NL432 or NL432-Pol283-8V virus and then cocultured with clone 2C6 at different E:T ratios. An HLA-mismatched (HLA-A*11:01–restricted Pol675 specific) CTL clone was used as negative control at an E:T cell ratio of 1:1. HIV-1 p24 Ag levels in the supernatant were measured on day 6 postinfection by performing an enzyme immunoassay. (**B**) Summary of the ability of other TI8-specific CTL clones established from elite controllers (KI-051, KI-021, or KI-124). The results at the E:T ratio of 1:1 are shown. (**C**) Percent inhibition of three clones. Statistical analysis was performed by using the paired *t* test. $*p < 0.05$.

In vitro selection of the 8V mutant by TI8-specific CTLs

The results described above strongly suggest that the 8V was an escape mutant selected by TI8-specific CTLs having the ability to discriminate this mutant from WT. The 8V virus had the same replication capacity as the WT virus in a competitive proliferation assay for 7 d (Supplemental Fig. 2). Therefore, to confirm this selection by the TI8-specific CTLs, we investigated whether TI8-specific CTL clones could select this mutant virus in vitro by competitive viral suppression assay for 7 d. The TI8-specific CTL clones were cultured with HLA-B*51:01–positive $CD4^+$ T cells infected with NL-432 and the 8V mutant virus together at a ratio of 9 to 1. The ratio of the 8V mutant virus to the WT one increases if the TI8-specific CTLs have ability to suppress WT virus more than the mutant virus. Indeed, CTL clones 2B5, 2C6, and 2D1 from KI-051, which exhibited weaker ability to suppress replication of the 8V virus compared with that to suppress that of the WT (Fig. 1), selected the 8V mutant virus in this competitive viral suppression assay (Fig. 2A). In contrast, other CTL clones, which exhibited similar ability to recognize the 8V as the WT (Fig. 1), did not select the 8V mutant virus (Fig. 2A). These results indicate that the 8V mutant was selected as an escape mutant by CTLs that could discriminate the 8V virus from the WT virus but not by other CTLs, which could not do so. HIV-1 p24 Ag levels in the culture supernatant in the presence of the CTL clones were very low compared with those in the absence of these clones (Fig. 2B), confirming that all CTL clones used in this experiment strongly suppressed the replication of both viruses.

TCR affinity of TI8-specific CTL clones

We found two types of TI8-specific CTL clone for the 8V recognition. To further characterize the 8V recognition by these CTL clones, we investigated the TCR affinity of these clones by using tetramers of HLA-B*51:01 with TI8 peptide (WT tetramer) and with the 8V mutant peptide (8V tetramer). Clone 2B5 exhibited significantly weaker affinity for the 8V tetramer than for the WT one (EC_{50}: 60.7 ± 14.3 nM for WT and 332.5 ± 32.7 nM for 8V; $p < 0.00019$; Fig. 3A). In contrast, clone 2B from KI-051 after the emergence of the 8V mutant and clone 3B from KI-021 showed almost the same affinity for both tetramers (EC_{50}: clone 2B, 116.3 ± 52.3 nM for WT and 115.1 ± 39.2 nM for 8V; $p < 0.98$; clone 3B, 104.5 ± 16.5 nM for WT and 112.5 ± 56.1 nM for 8V, $p < 0.82$; Fig. 3A). The TCR affinity for the 8V tetramer of all CTL clones was compared in terms of EC_{50} ratio of WT to 8V tetramer. The EC_{50} ratio of the CTL clones from KI-051 in July 2002 was significantly lower than that of the CTL clones from KI-051 in June 2009 and from KI-021 in September 1997 and January 2005 (Fig. 3B). These results taken together indicate that 2B5 carried TCR's with weaker affinity for the 8V tetramer than for the WT one, whereas 2B and 3B carried TCR's with similar affinity for both tetramers.

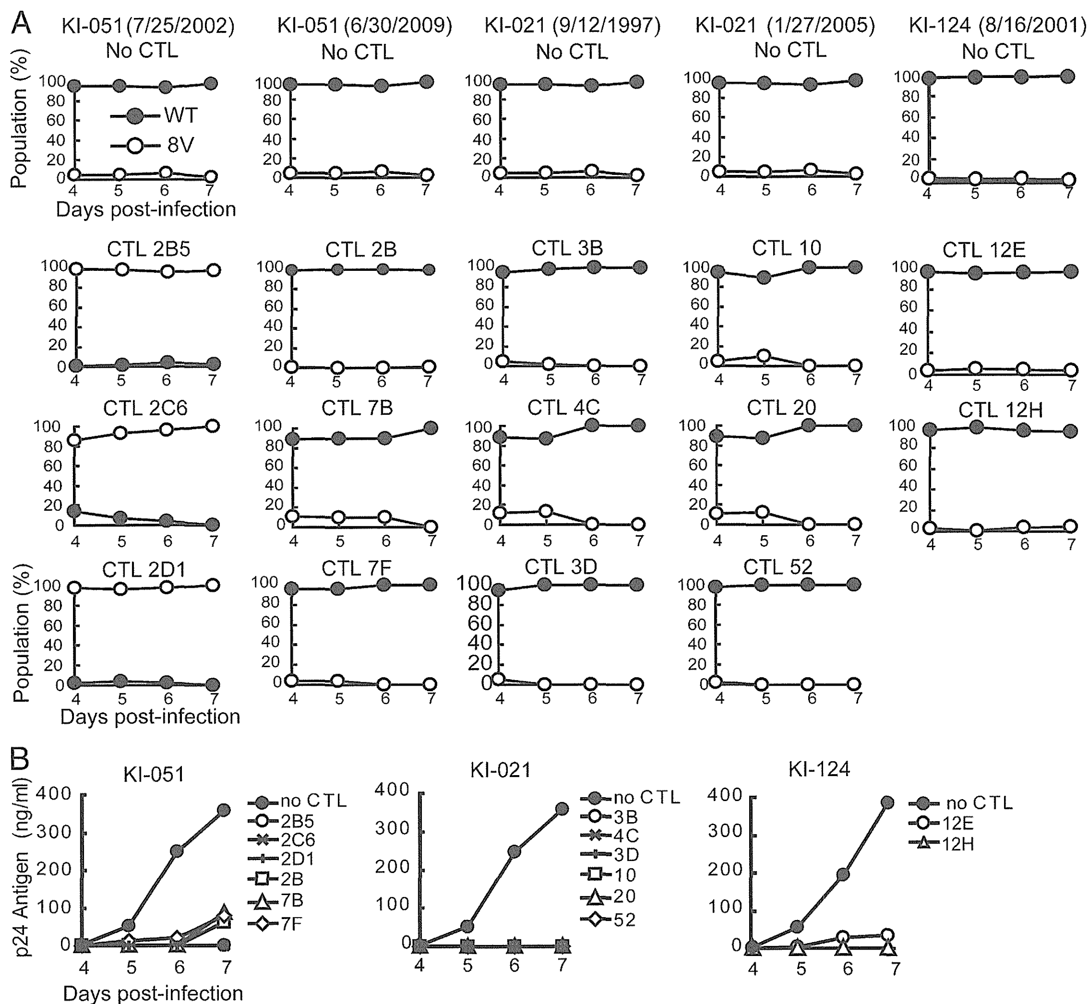

FIGURE 2. In vitro selection of the 8V mutant virus by TI8-specific CTL clones. (**A**) Ability of TI8-specific CTL clones to select the 8V mutant. T1 cells expressing HLA-B*51 and CD4 molecules were infected with NL-432 or NL432-Pol283-8V virus together at a ratio of 9:1. The infected cells were cocultured with TI8-specific CTL clones at an E:T ratio of 0.05:1. The culture supernatants were collected from days 4 to 7 postinfection. The population change in the viral mixture was determined by the relative peak height on the sequencing electrogram. (**B**) Ability of TI8-specific CTL clones to suppress the replication of HIV-1. HIV-1 p24 Ag levels in the supernatant were measured by use of an enzyme immunoassay (sensitivity: 7.8 pg/ml). The concentrations of HIV-1 p24 Ag in the collected supernatants were >0.1 ng/ml.

We next examined the kinetics of the TCR/HLA–peptide interaction. We first measured the time for half-maximal binding of these CTL clones by using the tetramers. The results showed that the time of this binding was nearly identical among these three clones (time of half-maximal binding: clone 2B5, 1.70 ± 0.48 min for WT and 1.71 ± 0.23 min for 8V; $p < 0.99$; clone 2B, 3.30 ± 0.66 min for WT and 3.06 ± 1.78 min for 8V; $p < 0.83$; clone 3B, 1.81 ± 0.36 min for WT and 1.61 ± 0.13 min for 8V; $p < 0.43$; Fig. 4A). Because the affinity of the TCR/HLA–peptide interaction is mainly controlled by its dissociation rate (43, 44), we additionally performed a tetramer dissociation assay to compare the stabilities of tetramer–TCR binding among these three clones. Clone 2B5 showed a faster dissociation rate of the 8V tetramer compared with that of the WT one (half-lives: 1530 ± 407 min for WT and 140 ± 53 min for 8V; $p = 0.027$; Fig. 4B), whereas clone 2B and 3B showed similar dissociation kinetics of both tetramers (half-lives: 2B, 1347 ± 75 min for WT and 2058 ± 382 min for 8V; $p = 0.50$; 3B, 300 ± 68 min for WT and 471 ± 189 min for 8V; $p = 0.50$; Fig. 4B). These results demonstrated that the binding stability between clone 2B5 TCR and HLA-B*51:01-TV8 peptide was weaker than that between the TCR and HLA-B*51:01-TI8, suggesting that the reduction in the 8V recognition and selection of the 8V virus resulted from this lower stability.

Reconstruction of the TCR function in TCR-deficient cells

To characterize TI8-specific CTLs at the molecular level, we analyzed the TCR-αβ genes of a TI8-specific CTL clone. Clone 2B5 expressed the TRAV8-2/TRBV24-1 clonotype, whereas clone 3B expressed the TRAV17/TRBV7-3 one (45). The TRAV17/TRBV7-3 clonotype is a public clonotype in TI8-specific CTLs and predominantly detected in KI-021 and KI-051 (45). This TCR clonotype was expressed on all CTL clones from KI-021, whereas the TRAV8-2/TRBV24-1 clonotype was on all three clones from KI-051 in July 2002 (data not shown). To confirm TCR function, we cloned their TCRs and then reconstructed them in TCR-deficient mouse T cell line TG40 transfected with human CD8α (TG40/CD8). TG40/CD8 cells transfected with 2B5-TCR or 3B-TCR genes (TG40/CD8-2B5 TCR or TG40/CD8-3B TCR) expressed CD3 (Fig. 5A), suggesting that these TCRs had been successfully reconstructed on the surface of the TG40/CD8 cells. In addition, TCR-transfected TG40/CD8 cells could produce IL-2 in response to stimulation with anti-CD3mAb (Fig. 5B), confirming functional TCR/CD3-mediated signaling in these cells. To

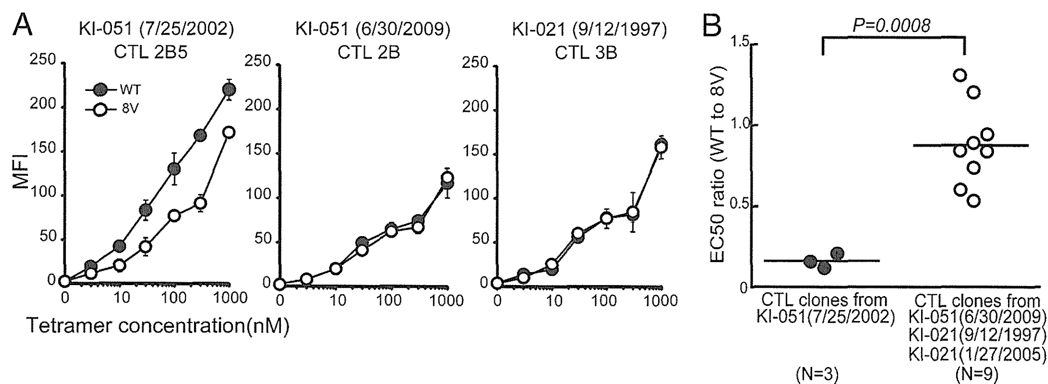

FIGURE 3. Difference in TCR affinity for HLA-B*51:01 with TV8 peptide complex among TI8-specific CTL clones. (**A**) The ability of the TCRs on TI8-specific CTL clones to bind WT or 8V tetramer was measured in terms of the mean fluorescence intensity (MFI) of each CTL clone stained with the tetramers at concentrations of 3–1000 nM. Data are shown as mean ± SD of n = 3 samples. An independent experiment gave similar results. (**B**) Comparison of TCR affinity for 8V tetramer of TI8-specific CTL clones in terms of EC_{50} ratio for WT to 8V tetramer (EC_{50} for WT tetramer/that for 8V tetramer). ● and ○, CTL clones from KI-051 in July 2002 and CTL clones from KI-051 in June 2009 and from KI-021, respectively. Statistical analysis was performed by using the t test. Bar indicates the average.

investigate whether 2B5 TCR and 3B TCR had Ag specificity, we coincubated TCR-transfected cells with C1R-B*51:01 prepulsed with various concentrations of the TI8 peptide. Both TG40/CD8-2B5 TCR and TG40/CD8-3B TCR cells showed IL-2 secretion in response to the peptide (Fig. 5C), indicating that TRAV8-2/TRBV24-1 and TRAV17/TRBV7-3 TCRs, respectively, were functionally expressed on these transfected cell lines. We further analyzed the recognition by these cells for the 8V mutant peptide. The cells expressing TRAV17/TRBV7-3 TCR exhibited a similar response to 8V as that to WT (EC_{50}: 1.38 ± 0.07 μM for WT and 1.90 ± 0.35 μM for 8V; p = 0.14), whereas the cells expressing TRAV8-2/TRBV24-1 exhibited a weaker response to 8V than that to WT (EC_{50}: 0.74 ± 0.23 μM for WT and 4.12 ± 0.39 μM for 8V; p = 0.0002) (Fig. 5C). These results confirmed that the recognition of the 2B5 TCR for 8V was weaker than that for WT and that 3B TCR evenly recognized both 8V and WT.

Discussion

We established TI8-specific CTL clones from three elite controllers at various time points and then analyzed the function of these clones. All CTL clones established from two of the elite controllers (KI-021 and KI-124) having only the 8V mutant virus and those from KI-051 after the emergence of the 8V mutant had almost the same ability to suppress the replication of the 8V as they did that of the WT one, whereas CTLs established from KI-051 having the WT virus had weaker ability to suppress the replication of the 8V virus than that of the WT one. These findings indicate that two types of CTLs for recognition of the 8V mutation were elicited in these elite controllers. Furthermore, the results of the in vitro competitive viral suppression assay revealed that the CTL clones established from KI-051 having the WT virus could select the 8V virus, whereas those from KI-051 having the 8V virus and other patients could not. Taken together, these results indicate that the 8V was an escape mutant selected by the former CTLs that could discriminate the 8V from WT and that HIV-1 was controlled by the latter ones after the emergence of the 8V virus.

The tetramer binding assay revealed that clone 2B5 carried TCR's with a little weaker affinity for the 8V tetramer than for the WT one, whereas clones 2B and 3B carried TCRs with similar affinity for both ligands. In addition, the tetramer dissociation assay demonstrated that the half-life of binding of the 2B5 TCR to the 8V tetramer was ~10-fold shorter than that to the WT one but that the 2B and 3B TCRs exhibited similar half-lives to both tetramers. Thus, the weaker recognition of clone 2B5 for the 8V mutant epitope resulted from a reduced binding stability between 2B5 TCR and HLA-B*51:01-TV8 peptide. To confirm the ability of these TCRs to recognize WT and mutant viruses, we reconstructed 2B5 TCR or 3B TCR on the surface of TG40/CD8 cells by transfecting the cells with these genes. The analysis using the cells expressing these TCRs also demonstrated the reduced ability of the 2B5 TCR to recognize the 8V peptide. These results confirmed that 2B5 TCR recognized the 8V peptide weaker than the WT one and that 3B TCR evenly recognized both peptides.

Our recent study demonstrated that 2B5 and 3B CTLs expressed TRAV8-2/TRBV24-1 and TRAV17/TRBV7-3 TCR clonotypes, respectively (45). The latter clonotype was predominantly detected in TI8-specific CTLs from KI-021 and KI-051 after emergence of the 8V virus, whereas the former was found in seven TI8-specific CTL clones established from KI-051 when WT virus was still detectable (45). The CTL clones expressing TRAV8-2/TRBV24-1 clonotype were predominantly established from PBMCs in July 2002 from KI-051 but not detected in ex vivo PBMCs (45), suggesting that the CTLs expressing this clonotype were in the minority but had a strong ability to proliferate by Ag stimulation. Unlike the TCR detected in HLA-B27–restricted KK10-specific CTLs, the 3B TCRs exhibited very weak affinity for HLA-B*51:01-TI8 as compared with pathogenic epitope-specific TCRs. However, the parental CTLs had strong ability to suppress the replication of HIV-1 in vitro. These findings indicate that even CTLs having weak TCR affinity could effectively control HIV-1.

The substitution from Val (GTA) to Leu (TTA or CTA) is easily produced by only a single nucleotide substitution because Val at position 8 is encoded by the "GTA" nucleotide codon, whereas more than two nucleotide substitutions are required for the change from Val to Thr (ACA) or to Arg (CGA or AGA). TI8-specific CTLs having the ability to discriminate the 8V mutant from WT highly cross-recognized the 8L mutant peptide but failed to suppress the replication of the 8L mutant virus (34, 35), suggesting that the 8L mutation has a deleterious effect on Ag processing of TI8 epitope. Therefore, it is likely that TI8-specific CTLs established from KI-051 at the different time points or from the other patients also failed to suppress the 8L virus. These facts suggest that the 8L mutant can be selected by a second immune response. Indeed, a previous study of a Chinese cohort infected with the 8V mutant virus as the founder virus showed that most of the HLA-B*51:01^{+} patients had the 8L mutation, not the 8T one (46). However, selection of the 8L from the 8V mutant was not driven

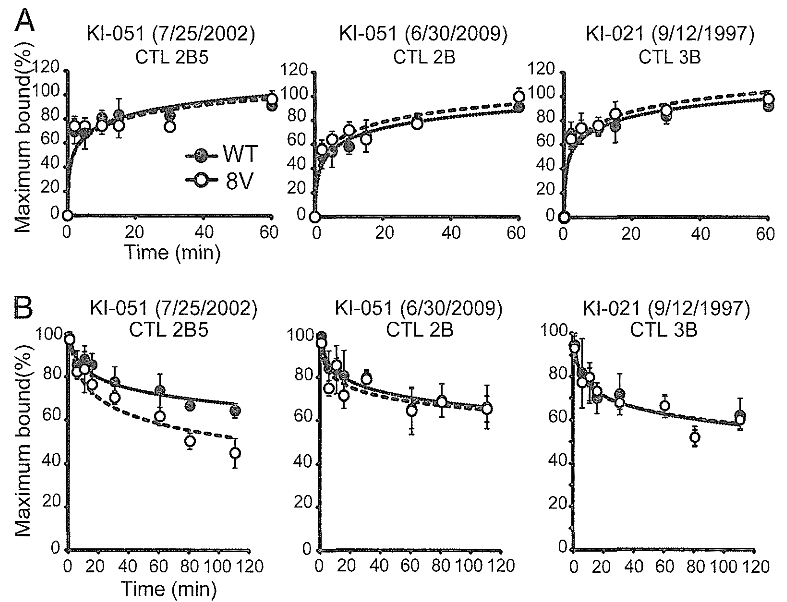

FIGURE 4. Kinetics of interaction between HLA-B*51:01–peptide complex and TI8-specific CTL clones. (**A**) Kinetics of tetramer association with TCR of TI8-specific CTL clones. The CTL clones were incubated with WT or 8V tetramer at a concentration of 1000 nM and taken periodically (2, 5, 10, 15, 30, and 60 min), fixed, and analyzed for the intensity of tetramer staining. Tetramer staining is expressed as a percentage of maximal staining intensity (maximum bound). Data are shown as the mean ± SD of $n = 3$ samples. (**B**) Kinetics of tetramer dissociation from TCRs of TI8-specific CTL clones. The CTL clones were stained with WT or 8V tetramer at a concentration of 1000 nM for 30 min, washed, and resuspended in R5. The cells were then taken periodically (5, 10, 15, 30, 60, 80, and 110 min), fixed, and analyzed for the intensity of tetramer staining. Tetramer staining is expressed as percentage of maximal staining intensity (time 0 min: 100%). Data are shown as the mean ± SD of $n = 3$ samples.

by the TI8-specific cross-reactive CTL response in our three elite controllers. We speculate that the CTLs elicited in these three elite controllers strongly inhibited HIV-1 replication in the acute phase of HIV-1 infection and maintained VL at a very low level for the long time so that selection of the mutant virus may have been very slow in these patients. The exact mechanism by which the 8L mutant was not selected in these elite controllers remains unclear. Therefore, further study is required to clarify it.

It is well known that escape mutants are selected by WT epitope-specific CTLs that cannot recognize the mutant virus (10, 16, 18, 47). In the present study, we demonstrated that the 8V virus was selected by TI8-specific CTLs that could discriminate 8V from WT, although the CTLs could effectively recognize the 8V mutant. A similar mutation is known in the KK10 epitope. The L268M mutation in the KK10 epitope restricted by the HLA-B*27 allele is selected by WT KK10-specific CTLs under the control of virus replication, and then, cross-reactive KK10-specific CTLs effectively recognizing WT and L268M are elicited after the emergence of this L268M mutation (48, 49). The cross-reactive TI8-specific CTLs evenly recognizing 8V and WT epitopes were elicited after the emergence of the 8V mutant, but they failed to select further escape mutants such as the 8L and 8T. In contrast to TI8-specific CTLs, cross-reactive CTLs together with WT KK10-specific CTLs finally select the R264K mutant with the compensatory S173A

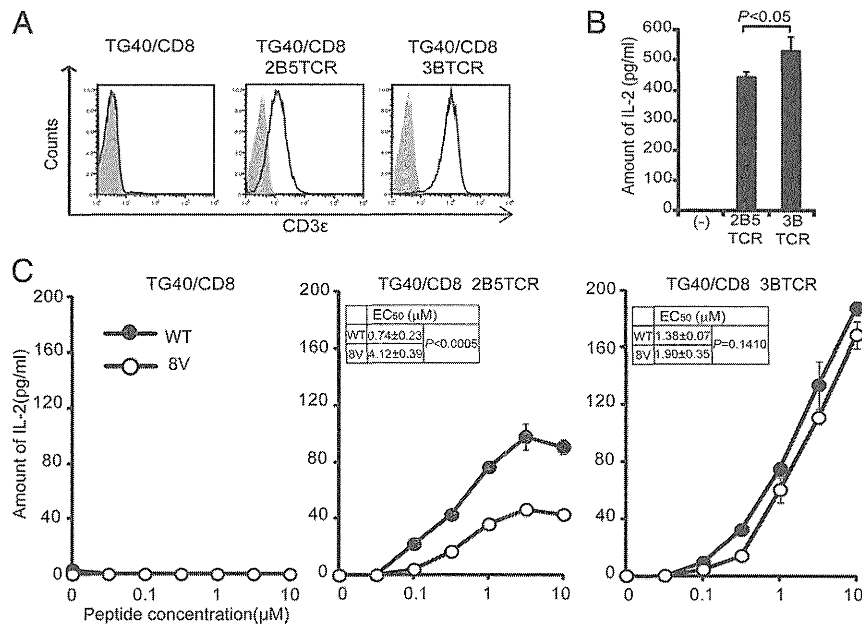

FIGURE 5. Reconstruction and recognition of TI8-specific TCRs on TCR-transfected TG40/CD8 cells. (**A**) CD3 expression in TG40/CD8 cells transfected with 2B5 TCR or 3B TCR. The TCR-transfected cells were stained with anti-CD3ε mAb (open histogram) or with an isotype control (shaded histogram), and then, CD3 expression on the cells was analyzed by flow cytometry. (**B**) IL-2 secretion by 2B5 TCR- or 3B TCR-TG40/CD8 cells stimulated with anti-CD3ε mAb. The cells were cultured in CD3ε mAb–coated wells for 48 h, and the amounts of IL-2 in supernatants were measured by use of an enzyme immunoassay. The data are shown as the means and SD of triplicates (2B5 TCR: 445 ± 16 pg/ml; 3B TCR: 529 ± 47 pg/ml). Another independent experiment gave similar results. (**C**) IL-2 secretion by 2B5 TCR- or 3B TCR-TG40/CD8 cells stimulated with TI8 (WT) or TV8 peptide. These cells were coincubated for 48 h with C1R-B*51:01 cells prepulsed with various concentrations of TI8 or TV8 peptide (0.03–10 μM). The amounts of IL-2 in supernatants were measured by the enzyme immunoassay. The data are shown as the means and SD of triplicates. Another independent experiment gave similar results.

mutation (49, 50). These mutations (S173A/R264K/L268M) critically reduce the binding to HLA-B*27 so that WT and cross-reactive KK10-specific CTLs fail to control the escape mutant virus (16, 17, 51). Thus, the events of the 8V selection and the control of HIV-1 by TI8-specific CTLs were quite different from those in the case of the KK10-specific CTLs.

A previous study showed that the 8T and the 8R mutation were selected within only 6 and 12 mo after the first test, respectively, in acutely infected HLA-B*51:01^{+} patients who had been infected with the WT virus (35). Longitudinal sequence analysis of the TI8 epitope in KI-051, who had been infected with HIV-1 around 1983, revealed that 37% of the HIV-1 isolates were the WT virus in July 2002, with the 8V mutant being predominantly detected at this time, indicating that the WT virus had existed for ~20 y in this patient. Thus, the 8V mutant was very slowly selected in KI-051, whereas the L268M mutation was mostly selected within 1–3 y after HIV-1 infection by highly effective KK10-specific CTLs recognizing the WT but not the L268M mutant (49, 52). We found that CTLs had a strong ability to inhibit the 8V virus in vitro and that elite controllers carrying the 8V mutant maintained a very low level of VL for a long time. However, because the highly effective WT-specific and L268M cross-reactive KK10-specific CTLs can select new escape mutants (49), other mechanisms may be involved in maintenance of the 8V in HLA-B*51:01^{+}elite controllers.

The HLA-B*51:01 allele was associated with slow progression to the disease in HIV-1–infected Japanese hemophiliacs, and the frequency of HLA-B*51:01–restricted TI8-specific CTLs is inversely correlated with the plasma VL in chronically HIV-1–infected Japanese hemophiliacs (34). These findings indicate that the TI8-specific CTLs control HIV-1 in these patients. In contrast, the escape mutations 8T, 8R, and 8L were found to accumulate in several populations (35). A recent analysis using Japanese cohorts also showed that the WT sequence 8I is present in only 13.5% of chronically HIV-1–infected Japanese individuals (53). These findings suggest that TI8-specific CTLs are hardly elicited in HLA-B*51:01^{+} individuals recently infected with HIV-1 because the escape mutations in the TI8 epitope accumulate in epidemic HIV-1. Thus, the HLA-B*51:01 allele is then no longer associated with slow progression to AIDS. Indeed, this allele is not associated with the slow progression in Japanese individuals recently infected with HIV-1 (54).

In conclusion, we found two different TI8-specific CTLs recognizing the 8V mutant in HLA-B*51:01^{+} elite controllers. We showed that 8V was an escape mutant selected by cross-reactive CTLs having weaker ability to recognize 8V virus–infected cells than WT virus–infected ones and that this 8V mutant could elicit CTLs evenly recognizing 8V virus–infected cells and WT virus–infected ones in the elite controllers. These two cross-reactive CTLs effectively suppressed HIV-1 for a long time. Our findings provide a novel mechanism concerning selection of the 8V mutant and long-term control of HIV-1 in HLA-B*51:01^{+} elite controllers. Further studies clarifying why these elite controllers carrying the 8V mutant do not select other escape mutants such as the 8L mutant may impact on the fields of pathogenesis and immunotherapy in AIDS research.

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Disclosures

The authors have no financial conflicts of interest.

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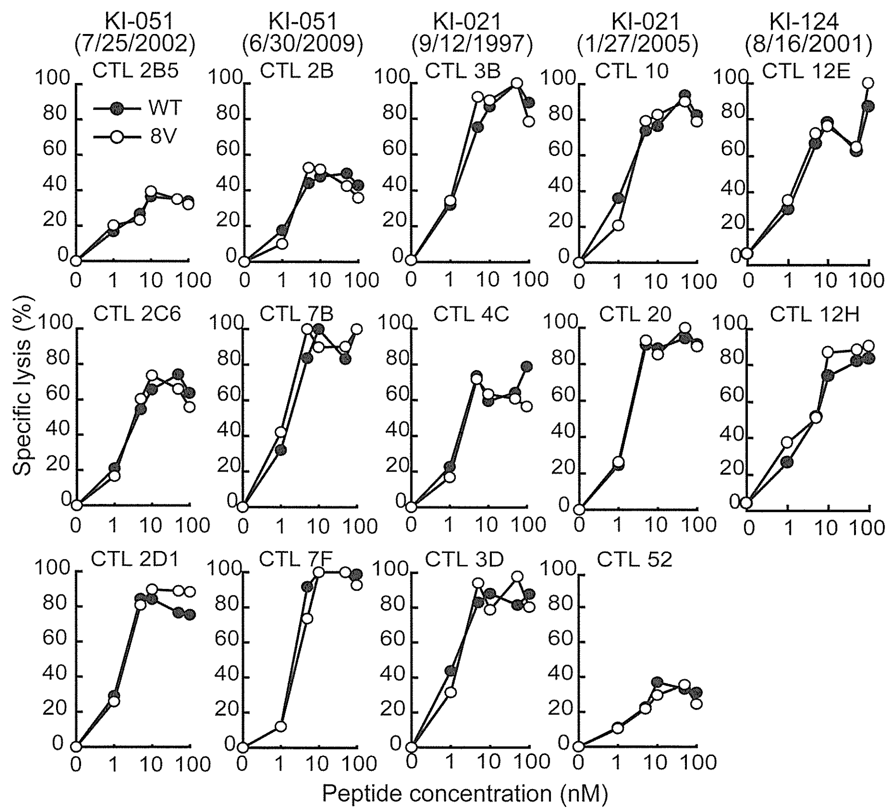
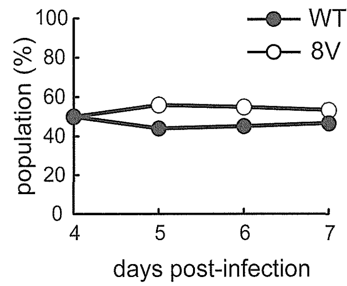


Supplementary Figure 1:

Recognition of TI8 (WT) and TV8 (8V) peptides by TI8-specific CTL clones.

Cytotoxic activity toward C1R-B*51:01 cells prepulsed with TI8 or TV8 peptide at concentrations of 1 to 100 nM was determined. The cytotoxic activity was measured at an E:T ratio of 2:1.



Supplementary Figure 2:

Replication capacity of WT and the 8V mutant viruses.

A competitive proliferation assay using WT and the 8V mutant viruses. T1 cells were infected with WT and the 8V mutant viruses at a ratio of 1:1. The culture supernatants were collected from day 4 to day 7 post infection. Viral RNA extracted from the supernatants was subjected to RT-PCR followed by nested PCR for direct sequencing. The population change in the viral mixture was determined by the relative peak height on the sequencing electrogram.

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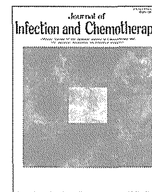

Original article

Low body weight and tenofovir use are risk factors for renal dysfunction in Vietnamese HIV-infected patients. A prospective 18-month observation study

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ABSTRACT

Background: The use of tenofovir has been rapidly increasing in Vietnam. Several studies identified low body weight as a risk factor for tenofovir-induced nephrotoxicity. However, little is known about the impact of tenofovir on renal function in HIV-infected Vietnamese with generally low weight.
Methods: An observational single-center cohort of adult HIV-infected patients on antiretroviral therapy at National Hospital of Tropical Diseases, Hanoi. Patients on tenofovir or with creatinine clearance $\leq$60 ml/min at baseline were excluded. The incidence of renal dysfunction was compared between patients who switched to tenofovir and those who did not. Renal dysfunction was defined as 25% decline of creatinine clearance from baseline. Time to renal dysfunction was analyzed by the Kaplan–Meier method between the two groups. The Cox hazard model was used to determine risk factors for renal dysfunction in uni- and multivariate analyses.
Results: Of 556 patients enrolled in this study, 403 were non-tenofovir group while 153 were the tenofovir-switched group. Renal dysfunction occurred at a higher rate in the tenofovir-switched group (92.5 per 1000 person-years) than the non-tenofovir group (47.8 per 1000 person-years)($p = 0.023$, Log-rank test). Multivariate analysis confirmed that tenofovir use, low body weight and glucosuria were significant risk factors for renal dysfunction (hazard ratio = 1.980; 95% confidential interval, 1.094–3.582, HR = 1.057; 95%CI, 1.016–1.098, HR = 5.202; 95%CI, 1.245–21.738, respectively).
Conclusions: Tenofovir use, low body weight and glucosuria were significant risk factors for renal dysfunction. We suggest close monitoring of renal function in patients with these risk factors even in resource-limited setting.
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Key points

Treatment with TDF and low body weight were significant risk factors for renal dysfunction in Vietnamese HIV-treated patients. Given that the average body weight of Vietnamese is small, close monitoring of renal function in HIV-1-infected patients is important during treatment with TDF.

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1. Introduction

Although renal dysfunction is an important cause of morbidity and mortality in HIV-infected patients [1–7], only limited information is available on renal function in Vietnamese HIV-infected patients. Along with the 2010 WHO guidelines which phased out stavudine and recommended tenofovir (TDF) (URL: httl://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf), the use of TDF had been increasing in Vietnam in recent years.

TDF-associated nephrotoxicity is well known adverse effect. However, a meta-analysis study that evaluated the safety of TDF concluded that TDF-associated nephrotoxicity can be considered negligible and thus there is no need to restrict TDF use even when regular observation of renal function is not feasible [8]. Other experimental and clinical studies, however; provide a different scenario: one study of rhesus macaques described a dose-dependent nephrotoxic effect for TDF [9] and several studies reported cases of TDF-associated nephrotoxicity in low-body-weight HIV-infected patients [10,11]. Our group also reported that low body weight and use of TDF were significantly associated with chronic kidney dysfunction in Vietnamese HIV-infected patients in a cross-sectional study [12]. Since Vietnamese have a considerably smaller body weight compared with Caucasians, and the use of TDF in Vietnam is increasing throughout the country, the potential risk for TDF-related nephrotoxicity is a concern in Vietnam. This is also true in all countries in the region since the Asian population is, in general, of low body weight. To examine this issue in more detail, we conducted a longitudinal study to evaluate the incidence of renal dysfunction in Vietnamese HIV-infected patients and the risk factors of such morbidity, including use of TDF and low body weight.

2. Patients and methods

2.1. Study design

We performed a prospective observational study of a single-center cohort of Vietnamese HIV-infected patients on antiretroviral therapy (ART) to evaluate the impact of TDF and low body weight on renal function. This cohort was established in 2007 at the National Hospital of Tropical Disease (NHTD) in Hanoi, one of the biggest outpatient clinics for HIV infected-patients in Vietnam. The population of the cohort consists of Vietnamese HIV-infected patients on ART aged more than 17 years referred to NHTD.

To evaluate renal function, serum creatinine had been measured since October 2011, which is the baseline of this study. Entry criteria were patients who were registered in this cohort on October 2011. Patients taking TDF or with serum creatinine clearance (Crcl) of $\leq$60 ml/min at baseline were excluded. Also excluded from the study were patients whose creatinine was not obtained twice at least. The follow-up period was 18 months (between October 2011 and April 2013). All patients of this cohort received ART at baseline. ART included Zidovudine (AZT)/Lamivudine (3 TC), Stavudine (d4T)/3 TC or TDF/3 TC as nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) in combination with efavirenz (EFV), Nevirapine (NVP) or ritonavir boosted lopinavir (LPV/r). To estimate the incidence of renal dysfunction in this population, patients were divided into those who switched to TDF and those who did not. Laboratory data, including serum creatinine, were measured twice a year (in April and October) in this cohort. The study was approved by the Human Research Ethics Committee of NHTD. Each patient included in this study provided a written informed consent for the clinical and laboratory data to be used for publication. The study was conducted according to the principles expressed in the Declaration of Helsinki.

2.2. Measurements

Clinical and laboratory data included demographic variables (age, sex and weight), serum creatinine (mg/dl, measured by Jaffe method), CD4 cell count (cell/mm^3, measured by flow cytometry), plasma HIV-RNA (copies/ml, measured by the Roche COBAS TaqMan HIV monitor assay), complete history of ART, use of cotrimoxazole, date of HIV diagnosis, and presence of other comorbidities such as hepatitis B and C virus, diabetes mellitus and AIDS defining diseases. Renal dysfunction was defined as 25% decline in Crcl estimated by the Cockcroft–Gault formula, relative to the baseline.

2.3. Statistical analysis

Baseline characteristics were compared between case patients and control patients by the Student's t-test for continuous variables and by either the χ^2 test or Fisher's exact test for categorical variables. The time from baseline to renal dysfunction was analyzed by the Kaplan–Meier method for patients who switched to TDF and those who did not, and the log-rank test was used to determine the statistical significance. Censored cases represented those who died, dropped out, or were referred to other facilities before the end of follow-up period. The Cox proportional hazards regression analysis was used to estimate the impact of TDF use on the incidence of renal dysfunction. The impact of basic demographics, baseline laboratory data, and other medical conditions was also estimated with univariate Cox proportional hazards regression. Variables significantly associated with renal dysfunction in univariate analysis ($p < 0.05$) were entered into multivariate analysis. Statistical significance was defined at two-sided p value < 0.05. We used the hazard ratio (HR) and 95% confidence interval (95%CI) to estimate the association of each variable with renal dysfunction. All analyses were performed in SPSS (version 22.0).

3. Results

At baseline, 793 Vietnamese HIV-infected patients on ART were registered in this study. However, 237 patients were excluded from the study due to existing renal dysfunction at baseline (Crcl < 60 ml/min, $n = 72$), had already been treated with TDF at baseline ($n = 143$), and lack of repeated measurements of Crcl ($n = 22$). Thus, 556 patients who received ART met the study criteria and were included in the study. Of these, 153 patients were switched to TDF during the study period, while 403 patients continued treatment with non-TDF-containing regimen. The criteria for switch to TDF were adverse event caused by ART or induction of treatment for chronic hepatitis B virus infection.

Table 1 compares the baseline demographics and clinical variables of patients of the TDF-switched group and the non-TDF group. The TDF-switched group was significantly more likely to be males, hepatitis B virus S antigen-positive and hepatitis C virus antibody-positive compared to the non-TDF group. The TDF-switched group had marginally significant trend to be older and have diabetes mellitus. Body weight, serum creatinine, CD4 count, HIV RNA viral load, duration of ART, frequency of proteinuria and glucosuria, use of ritonavir boosted lopinavir (LPV/r) and cotrimoxazole, and history of AIDS-defining disease were not significantly different between the two groups. The mean CD4 count was >300/mm^3 and the mean HIV RNA load was <100 copies/ml in both groups.

During the observation period, renal dysfunction, defined as 25% decline in Crcl, was observed in 19 (12.4%) of the TDF-switched group and 27 (6.7%) of the non-TDF group, with an estimated incidence of 92.5 and 47.8 per 1000 person-years, respectively. Fig. 1 depicts the time from the baseline to the development of