発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
無治療の日本人HIV感染者におけるGag-Protease依存のウイルス増殖能と病態進行性の網羅的解析・口頭	阪井恵子、近田貴敬、長谷川真理、潟永博之、岡慎一、滝口雅文.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
血友病のHIV slow progressor 6例を対象としたdeep sequencingによるtropism解析・口頭	林田庸総、土屋亮人、潟永博之、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
HIV感染血友病患者の健康関連QOLの実態調査・口頭	大金美和、塩田ひとみ、小山美紀、柴山志穂美、久地井寿哉、岩野友里、柿沼章子、大平勝美、池田和子、潟永博之、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
HIV感染血友病患者の医療と福祉の連携へのアプローチ～療養支援アセスメントシートの検討・口頭	塩田ひとみ、大金美和、渡部恵子、坂本玲子、伊藤ひとみ、川口玲、石塚さゆり、山田三枝子、高山次代、羽柴知恵子、鍵浦文子、木下一枝、長與由紀子、城崎真弓、池田和子、潟永博之、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
成人と新生児におけるAZTリン酸化物細胞内濃度の比較・口頭	木内英、加藤真吾、細川真一、田中瑞恵、中西美紗緒、定月みゆき、田沼順子、潟永博之、矢野哲、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
ハノイの腎機能障害を有するHIV感染者におけるテノフォビル使用による腎機能予後・口頭	水島大輔、田沼順子、潟永博之、菊池嘉、Nguyen Kinh、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
プロテアーゼ阻害薬の骨密度低下メカニズムに関する研究・口頭	木内英、潟永博之、水島大輔、西島健、渡辺恒二、青木孝弘、矢崎博久、本田元人、田沼順子、源河いくみ、塚田訓久、照屋勝治、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
HIV感染者における新たな慢性炎症マーカーと動脈硬化症・口頭	本田元人、遠藤元誉、古川恵太郎、柴田怜、谷崎隆太郎、柳川泰昭、小林泰一郎、水島大輔、西島健、青木孝弘、木内英、渡辺恒二、矢崎博久、田沼順子、塚田訓久、潟永博之、照屋勝治、菊池嘉、尾池雄一、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内

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当院のHIV感染者における適応障害患者のHIV治療状況とカウンセリング介入についての検討･口頭	渡邊愛祈、仲里愛、小松賢亮、高橋卓巳、木内英、大金美和、池田和子、田沼順子、照屋勝治、塚田訓久、潟永博之、加藤温、関由賀子、今井公文、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
HIV感染者のターミナルケア　—HIV治療に消極的な感染者との心理面接—･口頭	小松賢亮、仲里愛、渡邊愛祈、塩田ひとみ、大金美和、西島健、矢崎博久、田沼順子、照屋勝治、塚田訓久、潟永博之、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
新規に開発されたイムノクロマトグラフィー法による第4世代HIV迅速診断試薬の臨床的有用性の検討･口頭	土屋亮人、潟永博之、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
当院における受診を中断したHIV感染症患者の傾向･口頭	中家奈緒美、小山美紀、木下真里、塩田ひとみ、伊藤紅、杉野祐子、大金美和、池田和子、塚田訓久、田沼順子、照屋勝治、潟永博之、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
（独）国立国際医療研究センターエイズ治療・研究開発センターにおける外国人患者対応—初診時のコミュニケーションについて—･口頭	木下真里、池田和子、中家奈緒美、塩田ひとみ、小山美紀、伊藤紅、杉野祐子、大金美和、塚田訓久、田沼順子、照屋勝治、潟永博之、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
HIV患者の梅毒治療におけるアモキシシリンの治療効果･口頭	谷崎隆太郎、青木孝弘、西島健、古川恵太郎、柴田怜、柳川泰昭、小林泰一郎、水島大輔、渡辺恒二、木内英、本田元人、田沼順子、塚田訓久、潟永博之、照屋勝治、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
HIV感染患者における赤痢アメーバ潜伏感染についての検討･口頭	渡辺恒二、永田尚義、柳川泰昭、小林泰一郎、水島大輔、西島健、青木孝弘、木内英、本田元人、田沼順子、塚田訓久、潟永博之、照屋勝治、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内

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HIV合併アメーバ性肝膿瘍の発症リスクとしてのHLA対立遺伝子の解析･口頭	小林泰一郎、渡辺恒二、古川恵太郎、柴田怜、柳川泰昭、谷崎隆太郎、水島大輔、西島健、青木孝弘、木内英、本田元人、田沼順子、照屋勝治、塚田訓久、潟永博之、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
DolutegravirとRilpivirineによるSmall tabletへの剤形変更がアドヒアランスの改善につながった症例･口頭	佐藤麻希、早川史織、増田純一、和泉啓司郎、潟永博之、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
免疫再構築症候群による縦隔リンパ節炎を発症し、気管・食道瘻孔形成を認めたが保存的に治療し得た非結核性抗酸菌症の1例･口頭	古川恵太郎、柴田怜、谷崎隆太郎、水島大輔、西島健、渡辺恒二、青木孝弘、本田元人、矢崎博久、田沼順子、塚田訓久、木内英、潟永博之、照屋勝治、菊池嘉、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
血友病Aに合併した狭心症に対し冠動脈形成術後の抗血小板療法2剤併用期間短縮を目的としてZotarolimus薬剤溶出ステントを用いた一例･口頭	本田元人、中川尭、山本正也、谷崎隆太郎、柴田怜、古川恵太郎、柳川泰昭、小林泰一郎、水島大輔、西島健、木内英、青木孝弘、渡辺恒二、矢崎博久、田沼順子、塚田訓久、潟永博之、照屋勝治、菊池嘉、原久男、岡慎一.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
Different effects of drug-resistant mutations on CTL recognition between HIV-1 subtype B and subtype A/E infections･口頭	Rahman Mohammad Arif、Kuse Nozomi、Murakoshi Hayato、Chikata Takayuki、Tran Van Giang、Gatanaga Hiroyuki、Oka Shinichi、Takiguchi Masafumi.	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
cART開始前のHIV感染症患者における骨密度低下の頻度と臨床マーカーの解析・口頭・	古賀一郎、妹尾和憲、若林義賢、吉野友祐、北沢貴利、太田康男	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
次世代シーケンサを用いた Human Papillomavirus の検出及び解析方法の開発	魚田慎, 今村淳治, 古川聡美, 大出裕高, 横幕能行, 杉浦亙	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内
HIV-1感染急性期におけるHIV特異的な病態バイオマーカーの探索について	重見麗, 蜂谷敦子, 松田昌和, 今村淳治, 渡邊綱正, 横幕能行, 岩谷靖雅, 杉浦亙	第28回日本エイズ学会学術集会・総会, 大阪	2014年12月	国内

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
サル指向性HIV-1の感染個体における増殖効率を上昇させる要因	芳田剛，齋藤暁，松岡和弘，大出裕高，岩谷靖雅，保富康宏，俣野哲朗，三浦智行，杉浦亙，明里宏文	第28回日本エイズ学会学術集会・総会，大阪	2014年12月	国内
Illumina MiSeqを用いたHIV-1近全長遺伝子配列解析の試み.	松田昌和，大出裕高，松岡和弘，蜂谷敦子，横幕能行，岩谷靖雅，杉浦亙	第28回日本エイズ学会学術集会・総会，大阪	2014年12月	国内
HIV-1 VifにおけるAPOBEC3C/F結合インターフェース	大出裕高，中島雅晶，河村高志，北村紳悟，長縄由里子，黒澤哲平，真野由有，粟津宏昭，松岡和弘，横幕能行，渡邉信久，杉浦亙，岩谷靖雅	第28回日本エイズ学会学術集会・総会，大阪	2014年12月	国内
Langerhans細胞における感染	川村龍吉	第28回日本エイズ学会学術集会・総会，大阪	2014年12月	国内
The Role of Langerhans Cells in Acrodermatitis Enteropathica and HIV Infection.	川村龍吉	第39回日本研究皮膚科学会	2014年12月	国内
Increased urinary NTX predicts progressive decreases in bone mineral density among HIV patients on CART・口頭	Koga I, Seo K, Wakabayashi Y, Suzuki S, Yoshino Y, Kitazawa T, Kurahashi I, Ota Y	24th ECCMID, Barcelona, Spain.	May, 2014	国外
Lack of Association between Intact/Deletion Polymorphisms of the APOBEC3B Gene and HIV-1 Risk.	Imahashi M, Izumi T, Imamura J, Matsuoka K, Ode H, Masaoka T, Sato K, Koyanagi Y, Takaori-Kondo A, Yokomaku Y, Sugiura W, Iwatani Y	Cold Spring Harbor Laboratory Meetings & Courses Program, New York, USA	May, 2014	国外
Fine-tuned HIV-1 Vif-interaction Interface of Anti-retroviral Cytidine Deaminase APOBEC3F	Nakashima M, Kitamura S, Kurosawa T, Ode H, Kawamura T, Mano Y, Naganawa Y, Yokomaku Y, Watanabe N,	Cold Spring Harbor Laboratory Meetings & Courses Program, New York, USA	May, 2014	国外
CCR3 and CCR5 Dual Ttropic HIV-1 is a Possible Major Escape Mechanism Frommaraviroc-Containing Antiretroviral Therapy	Yokomaku Y, Kito Y, Matsuoka K, Ode H, Matsuda M, Shimizu N, Iwatani Y, Sugiura W	International Workshop on Antiviral Drug Resistance (Meeting the Global Challenge), Berlin, Germany	June, 2014	国外
HIV-1 Near Full-Length Genome Analysis by Next-Generation Sequencing: Evaluation of Quasispecies and Minority Drug Resistance	Ode H, Matsuoka K, Matsuda M, Hachiya A, Hattori J, Yokomaku Y, Iwatani Y, Sugiura W	International Workshop on Antiviral Drug Resistance (Meeting the Global Challenge), Berlin, Germany	June, 2014	国外

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Japanese Drug Resistance HIV-1 Surveillance Network Molecular Epidemiology of Recent Seroconverters and Drug-Resistant HIV-1 Transmission Networks in Japan	Hattori J, Shiino T, Sugiura W	International Workshop on Antiviral Drug Resistance (Meeting the Global Challenge), Berlin, Germany	June, 2014	国外
Crystal structure of the Vif-inteaction domain of the anti-viral APOBE3F.	Nakashima M, Kitamura S, Kurosawa T, Ode H, Kawamura T, Imahashi M, Yokomaku Y, Watanabe N, Sugiura W, Iwatani Y	23rd Congress of the International Union of Crystallography (IUCr2014), Montreal, Canada	August, 2014.	国外
Exome Sequencing Identified a Novel TYK2 Compound Heterozygous Mutation in 2 Siblings with Primary Immunodeficiency	Nemoto M, Iwatani Y, Maeda N, Horibe K, Sugiura W.	Joint Meeting of the 1st Africa International Biotechnology & Biomedical Conference and the 8th International Workshop on Approaches to Single-Cell Analysis, Nairobi, Kenya	September, 2014	国外
Phylodynamic analysis of HIV-1 subtype B population in Japan: Identification of large transmission clusters and their network structure.	Shiino T, Sadamasu K, Nagashima M, Hattori J, Hachiya A, Sugiura W	9th HIV Transmission Workshop Cape Town, South Africa	October, 2014	国外
Discordant Tropism Determination For HIV-1 Isolates Of CRF01_AE From Asia.	Sugiura W, Louvel S, Pfeifer N, Matsuda M, Yokomaku Y, Kaiser R, and Klimkait T.	14th International HIV Drug Resistance Workshop. Seattle, USA	February, 2015	国外
Structural Basis of Inhibition and Resistance Mechanism to EFdA, a Highly Potent NRTI.	Li Z, Kirby K, Marchand B, Eleftherios M, Kodama E, Mitsuya H, Parniak M, and Sarafianos S.	The annual Conference on Retroviruses and Opportunistic Infections	February, 2015	国外

2. 学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Selection of TI8-8V mutant associated with long-term control of HIV-1 by cross-reactive HLA-B*51:01-restricted cytotoxic T cells.	Kuse N, Akahoshi T, Gatanaga H, Ueno T, Oka S, and Takiguchi M.	J Immunol	2014	国外
Low body weight and tenofovir use are risk factors for renal dysfunction in Vietnamese HIV-infected patients. A prospective 18-month observation study.	Mizushima D, Tanuma J, Gatanaga H, Lam NT, Dung NTH, Kinh NV, Kikuchi Y, and Oka S.	J Infect Chemothera	2014	国外
Long-term exposure to tenofovir continuously decrease renal function in HIV-1-infected patients with low body weight: results from 10 years of follow-up.	Nishijima T, Kawasaki Y, Tanaka N, Tanuma J, Tsukada K, Teruya K, Kikuchi Y, Gatanaga H, and Oka S.	AIDS	2014	国外
Single-nucleotide polymorphisms in the UDP-glucuronosyltransferase 1A-3' untranslated region are associated with atazanavir-induced nephrolithiasis in patients with HIV-1 infection: a pharmacogenetic study.	Nishijima T, Tsuchiya K, Tanaka N, Joya A, Hamada Y, Mizushima D, Aoki T, Watanabe K, Kinai E, Honda H, Yazaki H, Tanuma J, Tsukada K, Teruya K, Kikuchi Y, Oka S, and Gatanaga H.	J Antimicrob Chemothera	2014	国外
Brain magnetic resonance imaging screening is not useful for HIV-1-infected patients without neurological symptoms.	Nishijima T, Gatanaga H, Teruya K, Tajima T, Kikuchi Y, Hasuo K, Oka S.	AIDS Res Hum Retrovirus	2014	国外
Asymptomatic intestinal amebiasis in Japanese HIV-1-infected individuals.	Watanabe K, Nagata N, Sekine K, Watanabe K, Igari T, Tanuma J, Kikuchi Y, Oka S, Gatanaga H.	Am J Trop Med Hyg	2014	国外
Acute Hepatitis C in HIV-1 Infected Japanese Cohort: Single Center Retrospective Cohort Study.	Ishikane M, Watanabe K, Tsukada K, Nozaki Y, Yanase M, Igari T, Masaki N, Kikuchi Y, Oka S, and Gatanaga H.	PLoS One	2014	国外
Superimposed epitopes restricted by the same HLA molecule drive distinct HIV-specific CD8+ T cell repertoires.	Sun X, Fujiwara M, Shi Y, Kuse N, Gatanaga H, Appay V, Gao GF, Oka S, and Takiguchi M.	J Immunol	2014	国外
Low raltegravir concentration in cerebrospinal fluid in patients with ABCG2 genetic variants.	Tsuchiya K, Hayashida T, Hamada A, Kato S, Oka S, and Gatanaga H.	JAIDS	2014	国外

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Low prevalence of transmitted drug resistance of HIV-1 during 2008-2012 antiretroviral therapy scaling up in Southern Vietnam.	Tanuma J, Quang VM, Joya A, Hachiya A, Watanabe K, Gatanaga H, Chau NVV, Chinh NT, and Oka S.	JAIDS	2014	国外
Analysis of the hepatic functional reserve, portal hypertension, and prognosis of patients with human immunodeficiency virus/hepatitis C virus coinfection through contaminated blood products in Japan.	Eguchi S, Takatsuki M, SoyamaA, Hidaka M, Nakao K, ShirasakaT Yamamoto M , Tachikawa N, Gatanaga H, Kugiyama Y, Yatsuhashi H, Ichida T, and Kokudo N.	Transplant Proc	2014	国外
Raltegravir and elvitegravir-resistance mutation E92Q affects HLA-B*40:02-restricted HIV-1-specific CTL recognition.	Rahman MA, Kuse N, Murakoshi H, Chikata T, Gatanaga H, Oka S, and Takiguchi M.	Microbes and Infection	2014	国外
Clinical importance of hyper-beta-2-microglobulinuria in patients with HIV-1 infection on tenofovir-containing antiretroviral therapy.	Gatanaga H, Nishijima T, Tsukada K, Kikuchi Y, and Oka S.	JAIDS	2014	国外
Host-specific adaptation of HIV-1 subtype B in the Japanese population.	Chikata, Carlson, 73. Chikata T, Carlson J, Tamura Y, Borghan M, Naruto T, Hashimoto M, Murakoshi H, Le A, Mallal S, John M, Gatanaga H, Oka S, Brumme Z, and Takiguchi M.	J Virology	2014	国外
Lopinavir inhibits insulin signaling by promoting protein tyrosine phosphatase 1B expression	Kitazawa T, Yoshino Y, Suzuki S, Koga I, Ota Y	Exp Ther Med	2014	国外
Multifocal Cellulitis due to Disseminated Neisseria Gonorrhoeae in a Male Patient	Yoshino Y, Abe M, Seo K, Koga I, Kitazawa T, Ota Y	J Clin Med Res	2014	国外
Hyaluronic Acid concentration in pleural fluid: diagnostic aid for tuberculous pleurisy	Yoshino Y, Wakabayashi Y, Seo K, Koga I, Kitazawa T, Ota Y	J Clin Med Res	2015	国内
Clinical features of catheter-related candidemia at disease onset	Yusuke Yoshino, Yoshitaka Wakabayashi, Satoshi Suzuki, Kazunori Seo, Ichiro Koga, Takatoshi Kitazawa, Shu Okugawa, Yasuo Ota	Singapore Med J	2014	国外

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Phylodynamic Analysis Reveals CRF01_AE Dissemination between Japan and Neighboring Asian Countries and the Role of Intravenous Drug Use in Transmission	Shiino T, Hattori J, Yokomaku Y, Iwatani Y, Sugiura W	PloS one	2014	国外
The phosphorylation of HIV-1 Gag by atypical protein kinase C facilitates viral infectivity by promoting Vpr incorporation into virions	Kudoh A, Takahama S, Sawasaki T, Ode H, Yokoyama M, Okayama A, Ishikawa A, Miyakawa K, Matsunaga S, Kimura H, Sugiura W, Sato H, Hirano H, Ohno S, Yamamoto N, Ryo A	Retrovirology	2014	国外
Lack of Association between Intact/Deletion Polymorphisms of the APOBEC3B Gene and HIV-1 Risk	Imahashi M, Izumi T, Watanabe D, Imamura J, Matsuoka K, Ode H, Masaoka T, Sato K, Kaneko N, Ichikawa S, Koyanagi Y, Takaori-Kondo A, Utsumi M, Yokomaku Y, Shirasaka T, Sugiura W, Iwatani Y, Naoe T	PloS one	2014	国外
Development and Customization of a Color-Coded Microbeads-Based Assay for Drug Resistance in HIV-1 Reverse Transcriptase	Gu L, Kawana-Tachikawa A, Shiino T, Nakamura H, Koga M, Kikuchi T, Adachi E, Koibuchi T, Ishida T, Gao GF, Matsushita M, Sugiura W, Iwamoto	PloS one	2014	国外
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APOBEC3H polymorphisms associated with the susceptibility to HIV-1 infection and AIDS progression in Japanese	Sakurai, D, Iwatani, Y, Ohtani, H, Naruse, T, Terunuma, H, Sugiura, W, Kimura, A	Immunogenetics	2015 in press	国外
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IV. 研究成果の刊行物・別刷

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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Received for publication June 3, 2014. Accepted for publication September 15, 2014.

This work was supported by the Global Center of Excellence program "Global Education and Research Center Aiming at the Control of AIDS," launched as a project commissioned by the Ministry of Education, Science, Sports, and Culture (Japan), and by grants-in-aid for AIDS research from the Ministry of Health, Labor, and Welfare and from the Ministry of Education, Science, Sports, and Culture (Japan).

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The online version of this article contains supplemental material.

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

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1401419

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	0702	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	1402	10/26/1999	- - - - - - - -	100	NT[d]	<400	629	
					7/25/2002	- - - - - - - V	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	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.

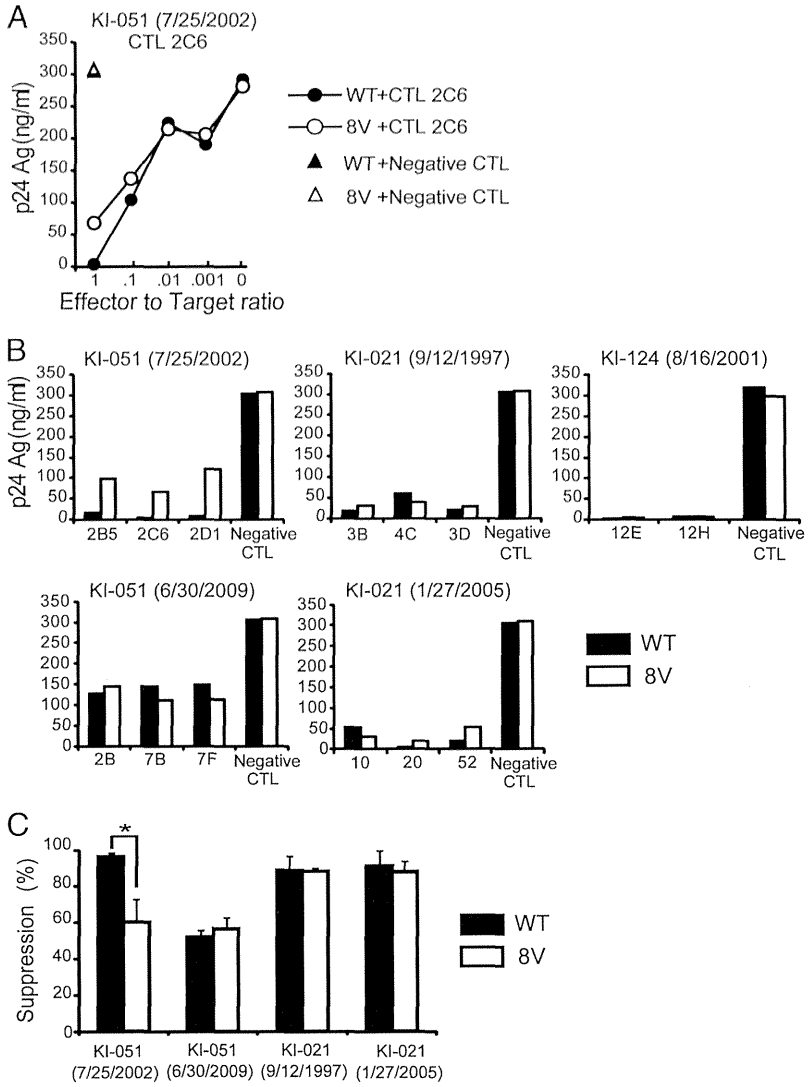

FIGURE 1. Abilities of TI8-specific CTL clones to suppress the replication of the 8Vmutant virus. (**A**) Ability of TI8-specific CTL clone 2C6, which was established from KI-051 in July 2002 when WT virus was still detectable, to suppress the replication of NL432 virus and the 8V virus. $CD4^+$ T cells 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.

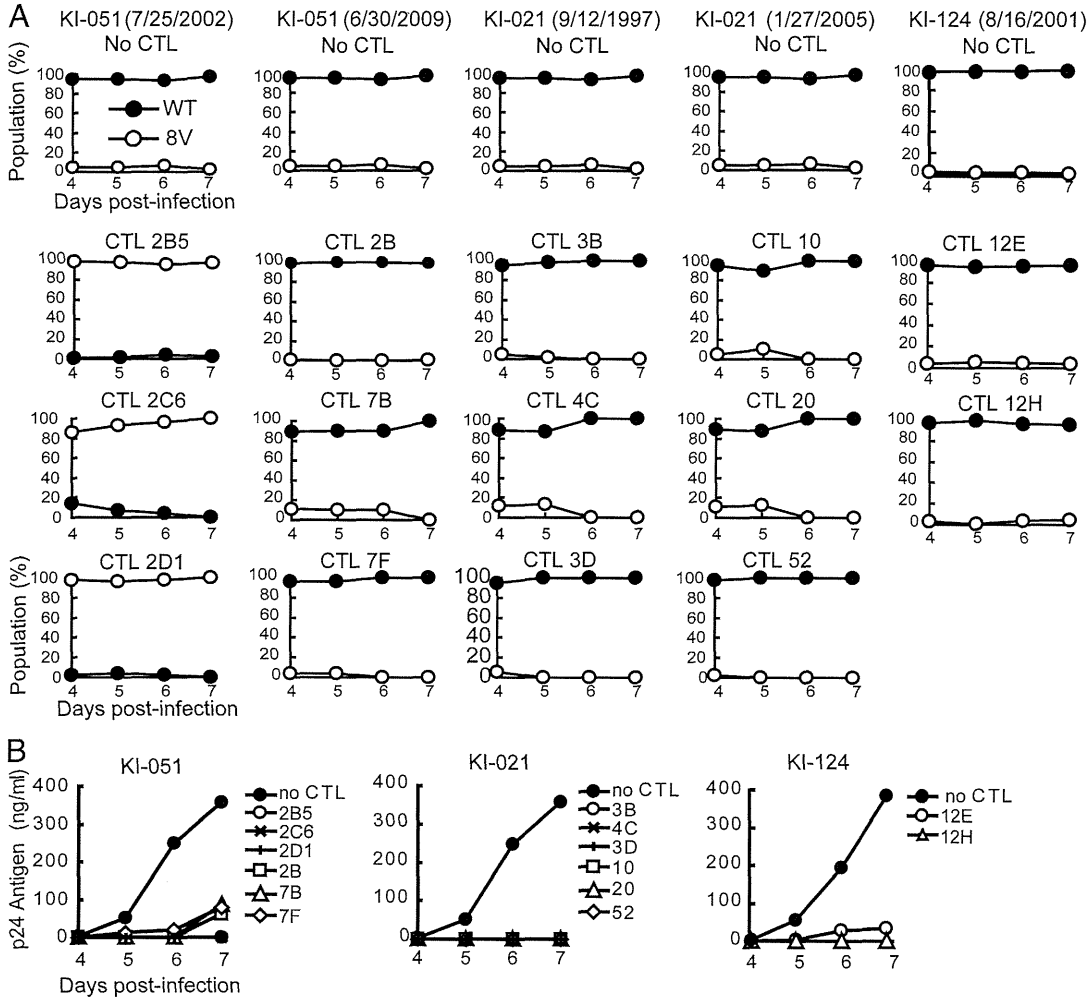

FIGURE 2. In vitro selection of the 8V mutant virus by TI8-specific CTL clones. (**A**) Ability of TI8-specific CTL clones to select the 8V mutant. T1 cells expressing HLA-B*51 and CD4 molecules were infected with NL-432 or NL432-Pol283-8V virus together at a ratio of 9:1. The infected cells were cocultured 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

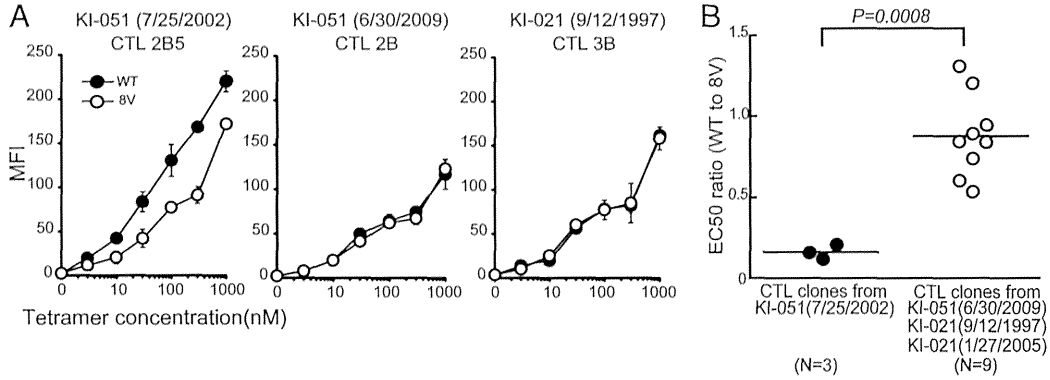

FIGURE 3. Difference in TCR affinity for HLA-B*51:01 with TV8 peptide complex among TI8-specific CTL clones. (**A**) The ability of the TCRs on TI8-specific CTL clones to bind WT or 8V tetramer was measured in terms of the mean fluorescence intensity (MFI) of each CTL clone stained with the tetramers 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

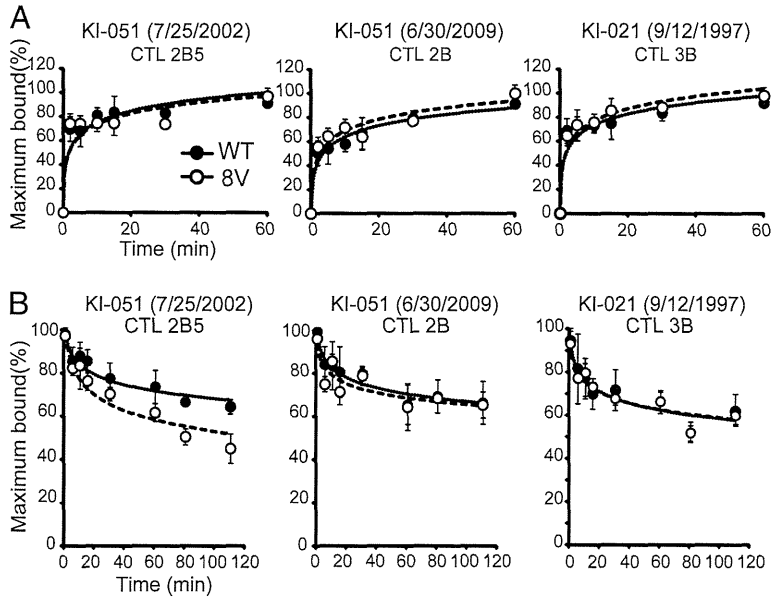

FIGURE 4. Kinetics of interaction between HLA-B*51:01–peptide complex and TI8-specific CTL clones. (**A**) Kinetics of tetramer association with TCR of TI8-specific CTL clones. The CTL clones were incubated with WT or 8V tetramer at a concentration of 1000 nM and taken periodically (2, 5, 10, 15, 30, 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

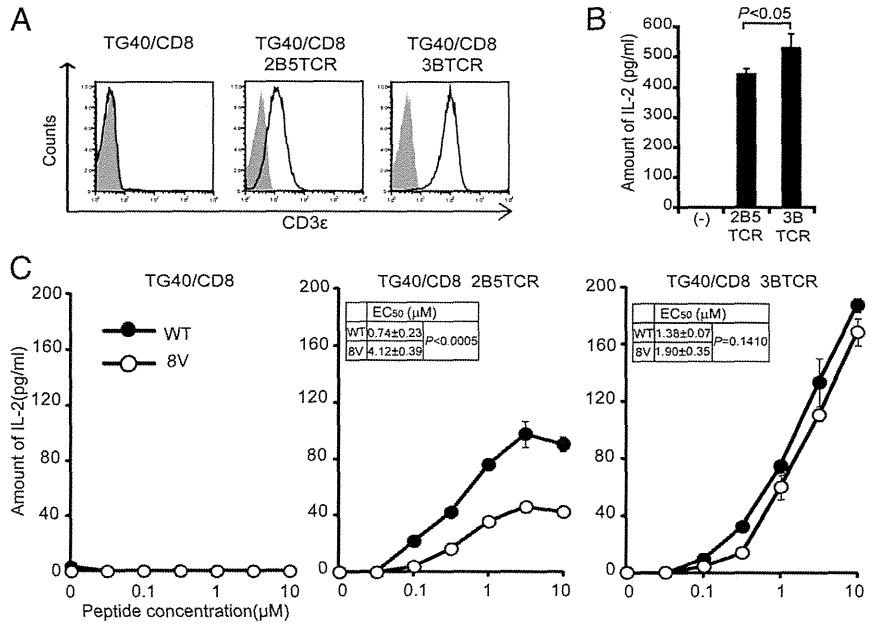

FIGURE 5. Reconstruction and recognition of TI8-specific TCRs on TCR-transfected TG40/CD8 cells. (**A**) CD3 expression in TG40/CD8 cells transfected with 2B5 TCR or 3B TCR. The TCR-transfected cells were stained with anti-CD3ε mAb (open histogram) or with an isotype control (shaded histogram), and then, 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.

Acknowledgments

We thank Dr. Chihiro Motozono for discussion and Sachiko Sakai for her secretarial assistance.

Disclosures

The authors have no financial conflicts of interest.

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