confer highly sensitive to anti-V3 monoclonal antibody. 10th Kumamoto AIDS Seminar GCOE Joint International Symposium. 2009.9.28-29, Kumamoto

5. Hatada M. Yoshimura K., Harada S, and Matsushita S.:Mechanism of maintaining a glycan-insertion in HIV-1 gp120 V2 region under pressure of a potent neutralizing antibody in vitro. 10th Kumamoto AIDS Seminar GCOE Joint International Symposium. 2009.9.28-29, Kumamoto.
6. Matsushita S. Narahara C, Nishida Y, Honda A., Harada S., Yoshimura K.: Mechanism of maintaining a glycan-insertion in HIV-1 gp120 V2 region under pressure of a potent neutralizing antibody in vitro. 10th Kumamoto AIDS Seminar GCOE Joint International Symposium. 2009.9.28-29, Kumamoto.
7. Yoshimura K, Matsushita S: In vitro induction of HIV-1 resistant to a CCR5. Kumamoto AIDS Seminar GCOE Joint International Symposium- Satellite Symposium. 2009.9.30, Aso, Kumamoto.
8. Matsushita S: Accumulation of multiple functional mutations in HIV-1 gp120 is involved in the development of neutralization escape under pressure of neutralizing antibody in vitro. Kumamoto AIDS Seminar GCOE Joint International Symposium-Satellite Symposium, 2009.9.30, Aso, Kumamoto.
9. 畑田万紀子、吉村和久、原田惠嘉、松下修三：抗 HIV-1V3 抗体からの逃避過程で挿入される V2 領域の糖鎖が保存されるメカニズム-HIV-1 の進化における耐性度と増殖能のバランスに関する考察- 第 57 回日本ウイルス学会学術集会. 2009.10.25-27 東京.
10. 松下修三：エンベロープの進化と中和抗体. シンポジウム HIV 細胞侵入とその防御機構. 第 23 回に本エイズ学会学術集会・総会. 2009.11.26-28 名古屋.
11. 吉村和久: ケモカインレセプター阻害剤の臨床的研究～臨床分離株を用いたマラビロック耐性誘導～. 第 23 回に本エイズ学会学術集会・総会. 2009.11.26-28 名古屋.
12. 原田 恵嘉, 吉村和久, 松下修三： 最近分離した 7 種の臨床 HIV-1 株を用いた in vitro ラルテグラビル耐性ウイルス誘導. 第 23 回に本エイズ学会学術集会・総会. 2009.11.26-28 名古屋.
13. 石川哲也, 畑田万紀子, 原田 恵嘉, 吉村和久, 松下修三: 実験室 HIV-1 R5 株を用いた in vitro CCR5 阻害薬 (maraviroc) 耐性ウイルス誘導の試み第 23 回に本エイズ学会学術集会・総会. 2009.11.26-28 名古屋.
14. Matsushita, S., Mouri, S., Harada, S., Yamada, Y., Tamamura, H., Yoshimura, K.: Strategy to overcome neutralization of HIV-1 primary isolates. 11th GCOE joint international Symposium Kumamoto AIDS Seminar, Oct.6-8, 2010.Kumamoto.
15. Harada, S., Hamaji A., Matsushita, S., Yoshimura, K.: Evalution and selection of the env gene of HIV-1 primary isolates during in vitro selection of raltegravir. 11th GCOE joint international Symposium Kumamoto AIDS Seminar, Oct.6-8, 2010.Kumamoto.
16. 吉村和久、原田恵嘉、濱治有希、松下修三：CCR5 阻害剤 maraviroc(MVC)耐性誘導による Env の変異が中和抗体感受性に及ぼす影響. 第 24 回日本エイズ学会学術集会・総会. 2010.11.24 -26. 東京.
17. 原田恵嘉、濱治有希、松下修三、吉村和久：ラルテグラビルは HIV-1 の in vitro 馴化における Env 選択に影響する. 第２４回日本エイズ学会学術集会・総会. 2010.11.24 -26.東京.
18. 松下修三：共催シンポジウム２ 最新の情報を明日の臨床に生かす-Year in review 2010-「病態の研究と治療薬開発の未来」.

第 24 回日本エイズ学会学術集会・総会. 2010.11.24 -26.東京.

19. 松下修三：共催セミナー8　HIV 陽性者のメンタルヘルスへのアプローチ　その２　メンタルヘルス問題の「今」を考える：どのように捉え、どうアプローチすることが可能だろうか？～うつと依存症（薬物）を中心に。第 24 回日本エイズ学会学術集会・総会. 2010.11.24 -26.　東京.
20. Yoshimura K., Harada S., Hamji A., Matsushita S. Two-step escape pathway of the HIV-1 primary isolates induced by the in vitro selection of maraviroc. 12th Kumamoto AIDS Seminar GCOE Joint International Symposium, 2011.10.19-21, Aso, Kumamoto, Japan.
21. Harada S., Ishikawa T., Hamji A., Matsushita S., Yoshimura K. Impact of raltegravir pressure on the selection of HIV-1 envelope sequences in vitro. 12th Kumamoto AIDS Seminar GCOE Joint International Symposium, 2011.10.19-21, Aso, Kumamoto, Japan.
22. Maruta, S, Ramirez, K, Kuwata, T., Matsushita, S.：Construction of neutralizing antibody fragments for efficient access to V3 epitope. 12th Kumamoto AIDS Seminar GCOE Joint International Symposium, 2011.10.19-21, Aso, Kumamoto, Japan.
23. Ramirez, K, Maruta, Y., Kuwata, T., Yoshimura, K, Tamamura, H., Matsuhsita, S ： Novel CD4-induced monoclonal antibodies (MAbs) with cross-neutralizing activity against primary isolates of HIV-1 B and C subtypes. 12th Kumamoto AIDS Seminar GCOE Joint International Symposium, 2011.10.19-21, Aso, Kumamoto, Japan.
24. Kuwata, T, Takaki, K., Matsushita, S: Biased induction of neutralizing antibodies with particular specificity and gene usage in SIVsmH635FC-infected macaques. 12th Kumamoto AIDS Seminar GCOE Joint International Symposium, 2011.10.19-21, Aso, Kumamoto, Japan.
25. 桑田岳夫：SIVsmH635FC 感染サルにおける特定のエピトープと遺伝子に偏った中和抗体の誘導. 第 25 回日本エイズ学会学術集会・総会. 2011.11.30-12.2.　東京.
26. Ramirez, K, Maruta, Y., Kuwata, T., Yoshimura, K, Tamamura, H., Matsuhsita, S ： Cross-reactivity and cross-neutralizing activity of monoclonal antibody(MAbs) to CD4-induced epitope of gp120 against HIV-1B, C, CRF_01 subtype viruses. 第 25 回日本エイズ学会学術集会・総会. 2011.11.30-12.2.　東京.
27. 丸田泰広、桑田岳夫、クリステル.パオラ.ラミレス.バルデス、松下修三：HIV-1 の V3 領域に結合する中和抗体の遺伝子組換えによる小型化の試み.第 25 回日本エイズ学会学術集会・総会. 2011.11.30-12.2. 東京.

H．知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（エイズ対策研究事業）

分担研究報告書

NK細胞受容体を制御する効果的なHIVペプチド ワクチン開発のための研究

研究分担者　前仲勝実　北海道大学大学院薬学研究院　教授

研究要旨　ウイルス感染を防御するヒトナチュラルキラー(NK)細胞や細胞傷害性 T 細胞(CTL)に発現する細胞表面受容体 Killer cell Ig-like receptor (KIR)群は、標的細胞上のヒト主要組織適合性抗原(MHC)であるヒト白血球抗原(HLA)分子を認識し、NK 細胞や CTL の機能を制御する。特に抑制型 KIR 群はこれらの細胞の不活性化に関与すると考えられている。HIV-1 gp120 由来ペプチドの変異が抑制型 KIR 群との親和性を変化させ、免疫不全へと導くことを示唆する事実に基づき、我々は HIV の２重免疫逃避機構という新しい説を提唱している。本研究では、この説に基づき、HIV 由来ペプチドの同定とその KIR 群に対する親和性を網羅的に検証することにより、KIR 群を正確に制御できる効果的な HIV ペプチド候補分子を見いだすことを目指している。事業に参加後の２年間では、HIV ゲノム配列から設計した HLA - C 結合モチーフを持つ 9 アミノ酸ペプチドライブラリーから簡易巻き戻し系と微量ゲルろ過解析法などを組み合せて、実際に HLA-Cw4 に結合する複数の HIV ペプチドを同定した。また、他の HLA-Cw12 について同様の同定法を検討したところ、HLA-Cw12 は巻き戻し効率が悪いものの、ペプチド量を上げることで解析可能であることがわかった。多種類のペプチドを提示する HLA - C 分子を作製し、これと NK 細胞受容体 KIR2DL1（抑制型）と KIR2DS1(活性型)との結合解析を表面プラズモン共鳴法により行った。HLA-Cw4 については、いずれも抑制型が活性型に比べて結合が強かった。他方、HLA-Cw12 については、モデルペプチドを用いて、KIR 群との結合解析を行った。

A. 研究目的

ヒトNK細胞受容体Killer cell Ig-like receptor (KIR)群はHLAを認識し、ウイルス感染を防御するNK細胞や細胞傷害性T細胞(CTL)の制御に関与すると考えられている。これまでに我々は、HIV-1 gp120由来ペプチドの変異が抑制型KIR群との親和性を向上させることに見出し、これが免疫不全を引き起こす一因であることを示唆する事実に基づき、NK細胞もCTLも機能不全に陥らせるHIVの新規の免疫逃避機構を提唱した(AIDS 2009)。そこで、HIVゲノム配列から結合モチーフ情報を基にバイオインフォマティックスの方法により候補ペプチドを絞り、網羅的に合成を行い、HLA分子に提示されるかを実験的に検証する。さらに、提示される場合には、実際にペプチドを提示したHLA分子を用いて抑制型および活性型のKIR群に対する親和性を網羅的に検証することにより、KIR群を適切に制御できる効果的なHIVペプチドワクチン候補分子を見いだすことを目的とする。

B. 研究方法

HIV ゲノム配列から設計した HLA - Cw4 結合モチーフ（アンカー残基を基準とする）を持つ 80 種類強からなる 9 アミノ酸ペプチドライブラリーを合成する。これらのペプチドを用いて、我々が実績を有する HLA-Cw4 の巻き戻し法により、組換え蛋白質を作成する。これを微量ゲルろ過解析により、少量の巻き戻しサンプルから、正しくペプチドの結合した HLA-Cw4 分子が得られるかどうかを判定することにより、HLA-Cw4 結合ペプチドを同定する。同様に HLA-Cw12 についても巻き戻し・ゲルろ過分析系を確立するために、HLA-Cw4 の系を参考に進める。

次に、多種類のペプチドを提示する組換え HLA-Cw4 分子を利用して、これと NK 細胞受容体 KIR2DL1（抑制型）と KIR2DS1(活性型)との結合解析を表面プラズモン共鳴法により行う。さらに、同定済みのペプチドを提示する HLA - Cw12 分子を作製し、これと NK 細胞受容体 KIR2DL1（抑制型）と KIR2DS1(活性型)との結合解析を表面プラズモン共鳴法により行う。

(倫理面での配慮)

基礎的研究であり該当しない。

C. 研究結果

簡易巻き戻し系と微量ゲルろ過解析により、80 種の候補ペプチドから HLA-Cw4 に結合する数種類の HIV ペプチドを同定することができた。いずれもこれまでに報告のないペプチド配列であり、新規の HIV ペプチドワクチン候補と言え、この同定法の有用性を示唆する結果となった。

これらのペプチドを提示した HLA-Cw4 を作成し、これを表面プラズモン共鳴法による相互作用解析を行うために、チップ状に固定した。このチップの上に、抑制型および活性型の NK 細胞受容体 KIR2D の細胞外ドメインである可溶型分子を流すことにより、結合解析を行った。いずれもμM オーダーの弱い結合であったが、抑制型 KIR と活性型 KIR を比べると、いずれも抑制型 KIR の方がより強い結合を示した。

次に、同定済みの HLA-Cw1202 結合モデルペプチドを用いて、微量巻き戻し法による網羅的スクリーニング条件を検討した。HLA-Cw4 と同様の条件で行ったところ、巻き戻し効率が低く、ペプチドの量が多く必要であることがわかった。また、ゲルろ過分析には、溶出条件に工夫が欠かせないことも明らかとなった。これらの条件を基に、HLA-Cw12 を作成し、これを表面プラズモン共鳴法による相互作用解析を行った。HLA-Cw12 を固定化したチップの上に、抑制型 KIR2DL1 および活性型 KIR2DS1 の可溶型分子を流すことにより、初期の結合解析を行った結果、いずれもこれまでの報告がある KIR-HLA の相互作用の中では、弱い結合にあたることが明らかとなった。

D. 考察

HLA-Cw4 結合能を有する HIV 由来ペプチドが 80 種類以上の候補から、ほんの数種類しかえられず、事前の予想よりも少なかった。このため、より広い範囲でペプチドライブラリーの作成が必要であることがわかった。これは今後の課題である。他の HLA である HLA-Cw12 に結合するペプチドの網羅的同定に向けたスクリーニングの目処もついたが、まだ条件検討のできる余地があ

るので、より効率の良いスクリーニング系に改良していくことを目指し、HLA 結合ペプチド同定法としての汎用性を高めるように進める必要がある。次に、KIR 群の抑制型は活性型よりも親和性が高いことが言われてきたが、これまでの結果から活性型が親和性の強くなるペプチドの存在する確率は高くない事が考えられた。まだ、個々のペプチドを丹念に調べれば、候補ペプチドを見つけることができることを期待しているが、数種類程度の結果では結論を出せないので、より広い範囲でのライブラリーの作成を進める必要がある。

また、NK 細胞受容体 KIR2D の活性化型と抑制化型との相互作用解析については、HLA-Cw4 と HLA-Cw12 との活性の違いを明らかにすることで、HLA ごとの NK 細胞制御の特徴を見いだせる可能性が十分にある。

E. 結論

(1)HLA に結合する HIV ペプチドの同定方法および、抑制型および活性型の NK 細胞受容体 KIR との結合解析法を確立した。

(2) HLA-Cw4 に結合する HIV ペプチドの初期スクリーニングを完了し、KIR との結合解析も行うことができた。

(3) HIV 感染細胞で提示される HIV ペプチドの種類は多くない可能性が示唆され、ペプチドワクチン開発に重要な知見を得た。

(4) HLA-C 結合能を有する HIV ペプチドをより多く同定するために 10mer や 8mer ライブライリーを作製する必要があることがわかった。

(5)数種類のペプチドに対する KIR 分子の反応性を確認した段階であるが、これまでのところ、抑制型 KIR の方が活性型 KIR よりも強い結合を示すことがわかった。

(6) HLA-Cw12 について、巻き戻し条件を検討したが、HLA-Cw4 に比べ、ペプチド量を増やす必要があった。これにより、結合する HIV ペプチドのスクリーニング法としてある程度利用できる目処がついた。

(7) HLA-Cw12 結合モデルペプチドを用いて、KIR2D の抑制型と活性化型との初期の結合解析を行った。

G. 研究発表

1.論文発表

1. Long-term control of HIV-1 in hemophiliacs carrying slow-progressing allele HLA-B*5101.
Kawashima Y, Kuse N, Gatanaga H, Naruto T, Fujiwara M, Dohki S, Akahoshi T, Maenaka K, Goulder P, Oka S, Takiguchi M.
J Virol. 2010 Jul;84(14):7151-60.

2. Molecular basis for LLT1 protein recognition by human CD161 protein (NKRP1A/KLRB1).
Kamishikiryo J, Fukuhara H, Okabe Y, Kuroki K, Maenaka K.
J Biol Chem. 2011 Jul 8;286(27):23823-30.

3. Differential but competitive binding of Nogo protein and class i major histocompatibility complex (MHCI) to the PIR-B ectodomain provides an inhibition of cells.
Matsushita H, Endo S, Kobayashi E, Sakamoto Y, Kobayashi K, Kitaguchi K, Kuroki K, Söderhäll A, Maenaka K, Nakamura A,

Strittmatter SM, Takai T.
J Biol Chem. 2011 Jul 22;286(29):25739-47.

4. Molecular basis for herpesvirus entry mediator recognition by the human immune inhibitory receptor CD160 and its relationship to the cosignaling molecules BTLA and LIGHT.
Kojima R, Kajikawa M, Shiroishi M, Kuroki K, Maenaka K.
J Mol Biol. 2011 Nov 4;413(4):762-72.

5. 黒木喜美子、北辻千展、前仲勝実　NK レセプターと HLA の最前線　MHC 18(3), 1-19.

2.学会発表

1. 黒木喜美子、福永裕子、上敷領淳、白石充典、尾瀬農之、前仲勝実.　クラスⅠ認識受容体の構造とリガンド認識. 日本組織適合性学会、2010 年、東京.
2. 前仲勝実　表面タンパク質の不安定な複合体の分子解析、日本蛋白質科学会・ワークショップ、招待講演　大阪 2011.6

H. 知的財産権の出願・登録状況

1. 特許取得

無し

2. 実用新案登録

無し

3. その他

無し

Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

研究分担者　滝口 雅文

雑誌

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
Murakoshi H, Kitano M, Akahoshi T, Kawashima Y, Dohki, S, Oka S, Takiguchi M.	Identification and characterization of 2 HIV-1 Gag immunodominant epitopes restricted by Asian HLA allele HLA-B*4801.	Hum. Immunol.	70	170-174	2009
1 Kawashima Y, Pfafferott K, Frater J, Matthews P, Payne R, Addo M, Gatanaga H, Fujiwara M, Hachiya A, Koizumi H, Kuse N, Oka S, 他 29 名, Takiguchi M*, Goulder P.* (*equally contributed).	Adaptation of HIV-1 to HLA I.	Nature	458	641-645	2009
Koizumi H, Iwatani T, Tanuma J, Fujiwara M, Izumi T, Oka S, Takiguchi M.	Escape mutation selected by Gag28-36-specific cytotoxic T cells in HLA-A*2402-positive HIV-1-infected donors.	Microbes Infect.	11	198-204	2009
2 Zheng N, Fujiwara M, Ueno T, Oka S, Takiguchi, M.	Strong ability of Nef-specific CD4+ cytotoxic T cells to suppress HIV-1 replication in HIV-1-infected CD4+ T cells and macrophages.	J. Virol.	83	7668-7677	2009
Hashimoto M, Kitano M, Honda K, Koizumi H, Dohki S, Oka S, Takiguchi, M.	Selection of escape mutation by Pol154-162-specific cytotoxic T cells among chronically HIV-1-infected HLA-B*5401-positive individuals.	Hum. Immunol.	71	123-127	2010
Gatanaga H, Ode H, Hachiya A, Hayashida T, Sato H, Takiguchi M, Oka S.	Impact of human leukocyte antigen-B*51-restricted cytotoxic T-lymphocyte pressure on mutation patterns of nonnucleoside reverse transcriptase inhibitor resistance.	AIDS	24	F15-22	2010
Sakai K, Gatanaga H, Takata H, Oka S, Takiguchi M.	Comparison of $CD4^+$ T-cell-subset distribution in chronically infected HIV^+ patients with various CD4 nadir counts.	Microbes Infect.	12	374-381	2010

研究成果の刊行に関する一覧表

研究分担者　滝口 雅文

雑誌　　（前頁つづき）

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
Koizumi H, Hashimoto M, Fujiwara M, Murakoshi H, Chikata T, Borghan MA, Hachiya A, Kawashima Y, Takata H, Ueno T, Oka S, Takiguchi, M.	Different *in vivo* effects of HIV-1 immunodominant epitope-specific CTLs on selection of escape mutant viruses.	J. Virol.	84	5508-5519	2010
Kawashima Y, Kuse N, Gatanaga H, Naruto T, Fujiwara M, Dohki S, Akahoshi T, Maenaka K, Goulder P, Oka S, Takiguchi M.	Long-term control of HIV-1 in hemophiliacs carrying slow-progressing allele HLA-B*5101.	J. Virol.	84	7151-7160	2010
Honda K, Zheng N, Murakoshi H, Hashimoto M, Sakai K, Borghan MA, Chikata T, Koyanagi M, Tamura Y, Gatanaga H, Oka S, Takiguchi M.	Selection of escape mutant by HLA-C-restricted HIV-1 Pol-specific cytotoxic T lymphocytes carrying strong ability to suppress HIV-1 replication.	Eur J Immunol.	41	97-106	2011
Watanabe T, Murakoshi H, Gatanaga H, Koyanagi M, Oka S, Takiguchi M.	Effective recognition of HIV-1-infected cells by HIV-1 integrase-specific HLA-B*4002-restricted T cells.	Microbes Infect.	13	160-166	2011
Naruto T, Murakoshi H, Chikata T, Koyanagi M, Kawashima Y, Gatanaga H, Oka S, Takiguchi M.	Selection of HLA-B57-associated Gag A146P mutant by HLA-B*48:01-resticted Gag140-147-specific CTLs in chronically HIV-1-infected Japanese.	Microbes Infect.	13	766-770	2011
Zhang Y, Peng Y, Yan H, Xu K, Saito M, Wu H, Chen X, Ranasinghe S, Kuse N, Powell T, Zhao Y, Li W, Zhang X, Feng X, Li N, Leligdowicz A, Xu X, John M, Takiguchi M, McMichael A, Rowland-Jones S, Dong T.	Multilayered defense in HLA-B51-associated HIV viral control.	J Immunol.	187	684-91	2011
Iglesias MC, Almeida JR, Fastenackels S, van Bockel DJ, Hashimoto M, Venturi V, Gostick E, Urrutia A, Wooldridge L, Clement M, Gras S, Wilmann PG, Autran B, Moris A, Rossjohn J, Davenport MP, Takiguchi M, Brander C, Douek DC, Kelleher AD, Price DA, Appay V.	Escape from highly effective public CD8+ T cell clonotypes by HIV.	Blood	118	2138-2149	2011
Akahoshi T, Chikata T, Tamura Y, Gatanaga H, Oka S, Takiguchi M.	Selection and accumulation of an HIV-1 escape mutant by three types of HIV-1-specific CTLs recognizing wild-type and/or escape mutant epitopes.	J Virol.	86	1971-1981	2012

研究成果の刊行に関する一覧表

研究分担者　潟永博之

雑誌

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
Kawashima Y, Pfafferott K, Frater J, Matthews P, Payne R, Addo M, Gatanaga H, Fujiwara M, Hachiya A, Koizumi H, Kuse N, Oka S, Duda A, Prendergast A, Crawford H, Leslie A, Brumme Z, Brumme C, Allen T, Brander C, Kaslow R, Tang J, Hunter E, Allen S, Mulenga J, Branch S, Roach T, John M, Mallal S, Ogwu A, Shapiro R, Prado JG, Fidler S, Weber J, Pybus OG, Klenerman P, Ndung'u T, Phillips R, Heckerman D, Harrigan PR, Walker BD, Takiguchi M, Goulder P.	Adaptation of HIV-1 to human leukocyte antigen class I.	Nature	458	641-645	2009
Tsukada K, Teruya K, Tasato D, Gatanaga H, Kikuchi Y, Oka S.	Raltegravir-associated perihepatitis and peritonitis: a single case report.	AIDS	24	160-161	2010
Watanabe K, Honda M, Watanabe T, Tsukada K, Teruya K, Kikuchi Y, Oka S, Gatanaga H.	Emergence of raltegravir -resistant HIV-1 in the central nervous system.	Int J STD AIDS	21	840-841	2010
Gatanaga H, Ode H, Hachiya A, Hayashida T, Sato H, Takiguchi M, Oka S.	Impact of human leukocyte antigen-B*51-restricted cytotoxic T-lymphocyte pressure on mutation patterns of nonnucleoside reverse transcriptase inhibitor resistance.	AIDS	24	F15-22	2010
Gatanaga H, Ode H, Hachiya A, Hayashida T, Sato H, Oka S.	Combination of V106I and V179D polymorphic mutations in human immunodeficiency virus type 1 reverse transcriptase confer resistance to efavirenz and nevirapine but no to etravirine.	Antimicrob Agents Chemother	54	1596-1602	2010
Watanabe K, Gatanaga H, Escueta-de Cadiz A, Tanuma J, Nozaki T, Oka S.	Amebiasis in HIV-1-infected Japanese men: clinical features and response to therapy.	PLoS Negl Trop Dis.	5	e1318	2011
Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, Kinai E, Honda H, Tanuma J, Yazaki H, Tsukada K, Honda M, Teruya K, Kikuchi Y, Oka S.	Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients.	PLoS One	6	e22661	2011
Nakamura H, Teruya K, Takano M, Tsukada K, Tanuma J, Yazaki H, Honda H, Honda M, Gatanaga H, Kikuchi Y, Oka S.	Clinical symptoms and courses of primary HIV-1 infection in recent years in Japan.	Intern Med.	50	95-101	2011

6

7

研究成果の刊行に関する一覧表

研究分担者　天野将之

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Arun K. Ghosh, Bruno D. Chapsal, Melinda Steffey, Johnson Agniswamy, Yuan-Fang Wang, Masayuki Amano, Irene T. Weber, Hiroaki Mitsuya.	Substituent effects on P2-cyclopentyltetrahydrofuranyl urethanes: Design, synthesis, and X-ray studies of potent HIV-1 protease inhibitors.	Bioorganic & Medicinal Chemistry Letters.	22	2308-2311	2012
Arun K. Ghosh, Bruno D. Chapsal, Garth L. Parham, Melinda Steffey, Johnson Agniswamy, Yuan-Fang Wang, Masayuki Amano, Irene T. Weber, Hiroaki Mitsuya.	Design of HIV-1 Protease Inhibitors with C3-Substituted Hexahydrocyclopentafuranyl Urethane as P2-Ligands: Synthesis, Biological Evaluations, and Protein-Ligand X-ray Crystal Structure.	Journal of Medicinal Chemistry.	54	5890-5901	2011
Yasuhiro Koh, Manabu Aoki, Matthew L. Danish, Hiromi Aoki-Ogata, Masayuki Amano, Debananda Das, Robert W. Shafer, Arun K. Ghosh, Hiroaki Mitsuya.	Loss of Protease Dimerization Inhibition Activity of Darunavir Is Associated with the Acquisition of Resistance to Darunavir by HIV-1.	Journal of Virology.	85	10079-10089	2011
Arun K. Ghosh, Cuthbert D. Martyr, Melinda Steffey, Yuan-Fang Wang, Johnson Agniswamy, Masayuki Amano, Irene T. Weber, Hiroaki Mitsuya.	Design, Synthesis, and X-ray Structure of Substituted Bis-tetrahydrofuran (Bis-THF)-Derived Potent HIV-1 Protease Inhibitors	ACS Medicinal Chemistry Letters.	2	298-302	2011
Ide K, Aoki M, Amano M, Koh Y, Yedidi RS, Das D, Leschenko S, Chapsal B, Ghosh AK, Mitsuya H	Novel HIV-1 Protease Inhibitors (PIs) Containing a Bicyclic P2 Functional Moiety, Tetrahydropyrano-Tetrahydrofuran, That Are Potent against Multi-PI-Resistant HIV-1 Variants.	Antimicrob Agents Chemother.	55	1717-1727	2011
Ghosh AK, Chapsal BD, Baldridge A, Steffey MP, Walters DE, Koh Y, Amano M, Mitsuya H.	Design and synthesis of potent HIV-1 protease inhibitors incorporating Hexahydrofuropyranol-derived high affinity P(2) ligands: structure-activity studies and biological evaluation.	J Med Chem.	54	622-34	2011
Ghosh AK, Xu CX, Rao KV, Baldridge A, Agniswamy J, Wang YF, Weber IT, Aoki M, Miguel SG, Amano M, Mitsuya H	Probing multidrug-resistance and protein-ligand interactions with oxatricyclic designed ligands in HIV-1 protease inhibitors.	ChemMedChem.	5	1850-1854	2010
Koh Y, Amano M, Towata T, Danish M, Leshchenko-Yashchuk S, Das D, Nakayama M, Tojo Y, Ghosh AK, Mitsuya H.	In vitro selection of highly darunavir-resistant and replication-competent HIV-1 variants using a mixture of clinical HIV-1 isolates resistant to multiple conventional protease inhibitors.	J Virol.	84	11961–11969	2010
Tojo Y, Koh Y, Amano M, Aoki M, Das D, Kulkarni S, Anderson DD, Ghosh AK, Mitsuya H.	Novel protease inhibitors (PIs) containing macrocyclic components and 3(R),3a(S),6a(R)-bis-tetrahydrofuranylurethane that are potent against multi-PI-resistant HIV-1 variants in vitro.	Antimicrob Agents Chemother.	54	3460-3470	2010
Ghosh AK, Gemma S, Simoni E, Baldridge A, Walters DE, Ide K, Tojo Y, Koh Y, Amano M, Mitsuya H.	Synthesis and biological evaluation of novel allophenylnorstatine-based HIV-1 protease inhibitors incorporating high affinity P2-ligands.	Bioorg Med Chem Lett.	20	1241-1246	2010
Ghosh AK, Kulkarni S, Anderson DD, Hong L, Baldridge A, Wang YF, Chumanevich AA, Kovalevsky AY, Tojo Y, Amano M, Koh Y, Tang J, Weber IT, Mitsuya H.	Design, Synthesis, Protein-Ligand X-ray Structure, and Biological Evaluation of a Series of Novel Macrocyclic Human Immunodeficiency Virus-1 Protease Inhibitors to Combat Drug Resistance.	J. Med. Chem.	52	7689-7705	2009
Koh Y, Das D, Leschenko S, Nakata H, Ogata-Aoki H, Amano M, Nakayama M, Ghosh AK and Mitsuya H.	A Novel Nonpeptidic Protease Inhibitor (PI) Containing A Stereochemically Defined Fused Cyclopentanyltetrahydrofuran (Cp-THF) Potent Against Multi-PI-Resistant HIV-1 In Vitro.	Antimicrob Agents Chemother.	53	997-1006	2009

研究成果の刊行に関する一覧表

研究分担者　満屋裕明

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakata, H., Kruhlak, M., Kamata, W., Ogata-Aoki, H., Li, J., Maeda, K., Ghosh, AK., and Mitsuya, H.	Effects of CC chemokine receptor 5 (CCR5) inhibitors on the dynamics of CCR5 and CC-chemokine-CCR5 interactions.	Antiviral Therapy	15	321-31	2010
Ghosh AK, Leshchenko-Yashchuk S, Anderson DD, Baldridge A, Noetzel M, Miller HB, Tie Y, Wang YF, Koh Y, Weber IT, Mitsuya H.	Design of HIV-1 protease inhibitors with pyrrolidinones and oxazolidinones as novel P1'- ligands to enhance backbone- binding interactions with protease: synthesis, biological evaluation, and protein-ligand X-ray studies.	J. Med. Chem.	52	3902-14	2009
Fujimoto H, Higuchi M, Watanabe H, Koh Y, Ghosh AK, Mitsuya H, Tanoue N, Hamada A, and Saito H.	P-glycoprotein mediates efflux transport of darunavir in human intestinal Caco-2 and ABCB1 gene-transfected renal LLC-PK1 cell lines.	Biol. Pharm. Bull.	32	1588-93	2009
Ghosh AK, Sarang Kulkarni S, Anderson DD, Hong L, Baldridge A, Wang Y-F, Chumanevich AA, Kovalevsky AY, Tojo Y, Koh Y, Tang J, Weber IT, and Mitsuya H.	Design, synthesis, protein- ligand X-ray structures and biological evaluation of a series of novel macrocyclic HIV-1 protease inhibitors to combat drug- resistance.	J. Med. Chem.	52	7689-705	2009
Hattori S, Ide K, Nakata H, Harada H, Suzu S, Ashida N, Kohgo S, Hayakawa H, Mitsuya H, and Okada S.	Potent activity of a nucleoside reverse transcriptase inhibitor,4'-ethynyl-2-fluoro-2'-deoxyadenosine, against HIV-1 infection in Hu-PBMC-NOD/ SCID/JAK3null (NOJ) mouse model.	Antimicrob. Agents Chemother.	53	3887-93	2009
Das D, Koh Y, Tojo Y, Ghosh AK, and Mitsuya H.	Prediction of Potency of Protease Inhibitors Using Free Energy Simulations with Polarizable Quantum Mechanics-Based Ligand Charges and a Hybrid Water Model.	J. Chem. Inf. Model	49	2851-62	2009
Michailidis E, Bruno Marchand B, Ei-Ichi Kodama E-I, Kamlendra Singh K, Matsuoka M, Ashida N, Kirby K, Ryan EM, Sawani AM, 1, Eva Nagy E, Mitsuya H, Parniak MP, and Sarafianos SG.	Mechanism of inhibition of HIV-1 reverse transcriptase by 4'-ethynyl-2-fluoro-deoxyadenosine triphosphate, a translocation defective reverse transcriptase inhibitor.	J. Biol. Chem.	284	35681-91	2009
Aoki M, Venzon DJ, Koh Y, Aoki-Ogata H, Miyakawa T, Yoshimura K, Maeda K, Mitsuya H.	Non-cleavage Site Gag Mutations in Amprenavir-resistant HIV-1 Predispose HIV-1 to Rapid Acquisition of Amprenavir Resistance But Delays Development of Resistance to Other Protease Inhibitors.	J Virol.	83	3059-68	2009

1 1

研究成果の刊行に関する一覧表

研究分担者　馬場昌範

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Baba M	Entry inhibitors of human immuno-deficiency virus	Robert L. LaFemina	Antiviral Research: Strategy in Antiviral Drug Discovery	ASM Press	Washington, DC	2009	19-32

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Shi M, Wang X, Okamoto M, Takao S, Baba M	Inhibition of porcine endogenous retrovirus (PERV) replication by HIV-1 gene expression inhibitors	Antiviral Research	83	201-204	2009
馬場昌範, 中田浩智 朝光かおり, 駒野　淳 岡本実佳, 杉浦　亙	シンポジウム「これからの抗 HIV 薬研究の進むべき方向」	日本エイズ学会誌	12	74-80	2010
馬場昌範	HIV 感染症／AIDS 治療の進歩の展望－HIV のアキレス腱は他にもあるか－	血液フロンティア	20	2167-2172	2010
Nishioka H, Uesugi K, Ueda N, Kondo Y, Ysuji M, Abe H, Harayama T, Hamasaki T, Baba M, Takeuchi Y	Synthesis and anti-human immunodeficiency virus activity of the skeleton isomers of 3',4'-di-(*O*)-(-)-camphanoyl-(+)-khellactone	Chem. Pharm. Bull.	59	1075-1076	2011
Isono Y, Sakakibara N, Ordonez P, Hamasaki T, Baba M, Ikejiri M, Maruyama T	Synthesis of 1-benzyl-3-(3,5-dimethylbenzyl)uracil derivatives with potential anti-HIV activity.	Antiviral Chem. Chemother.	22	57-65	2011
Haraguchi K, Shimada H, Kimura K, Akutsu G, Tanaka H, Abe H, Hamasaki T, Baba M, Gullen EA, Dutschman GE, Cheng Y-C, Balzarini J.	Synthesis of 4'-ethynyl-2'-deoxy-4'-thioribonucleosides and discovery of a highly potent and less toxic NRTI.	ACS Med. Chem. Lett.	2	692-697	2011

12

研究成果の刊行に関する一覧表

研究分担者　松岡 雅雄

雑誌

	発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
13	Izumi K, Kodama E, Shimura K, Sakagami Y, Watanabe K, Ito S, Watabe T, Terakawa Y, Nishikawa H, Sarafianos S. G, Kitaura K, Oishi S, Fujii N, Matsuoka M.	Design of peptide-based inhibitors of HIV-1 strains resistant to T-20.	J. Biol. Chem.	284(8)	4914-4920	2009
	Ueno M, Kodama E.N, Shimura, K, Sakurai Y, Kajiwara K, Sakagami, Y, Oishi S, Fujii N, Matsuoka M.	Synonymous mutations in stem-loop III of Rev responsive elements enhance HIV-1 replication impaired by primary mutations for resistance to enfuvirtide.	Antiviral Res.	82(1)	67-72	2009
	Naito T, Izumi K, Kodama E, Sakagami Y, Kajiwara K, Nishikawa H, Watanabe K, Sarafianos S.G, Oishi S, Fujii N, Matsuoka M.	SC29EK, a peptide fusion inhibitor with enhanced α-helicity, inhibits replication of human immunodeficiency virus type 1 mutants resistant to enfuvirtide.	Antimicrob. Agents Chemother.	53(3)	1013-1018	2009
14	Michailidis E, Marchand B, Kodama EN, Singh K, Matsuoka M, Kirby KA, Ryan EM, Sawani AM, Nagy E, Ashida N, Mitsuya H, Parniak MA, Sarafianos SG.	Mechanism of inhibition of HIV-1 reverse transcriptase by 4'-ethynyl-2-fluoro-2'-deoxyadeno sine triphosphate, a translocation defective reverse transcriptase inhibitor.	J. Biol. Chem.	284	35681-32691	2009
	Nishikawa H, Nakamura S, Kodama E, Ito S, Kajiwara K, Izumi K, Sakagami Y, Oishi S, Ohkubo T, Kobayashi Y, Otaka A, Fujii N, Matsuoka M.	Electrostatically constrained alpha-helical peptide inhibits replication of HIV-1 resistant to enfuvirtide.	Int J Biochem Cell Biol.	41(4)	891-899	2009
	Zhao T, Yasunaga J, Satou Y, Nakao M, Takahashi M, Fujii M, Matsuoka M.	Human T-cell leukemia virus type 1 bZIP factor selectively suppresses the classical pathway of NF-κB.	Blood	113 (12)	2755-2764	2009
	Oishi S, Ito S, Nishikawa H, Tanaka M, Ohno H, Otaka A, Izumi K, Kodama E, Matsuoka M, Fujii N.	Development of a novel fusion inhibitor against T-20-resistant HIV-1.	Adv Exp Med Biol.	611	389-391	2009
	Oishi S, Kamitani H, Kodera Y, Watanabe K, Kobayashi K, Narumi T, Tomita K, Ohno H, Naito T, Kodama E, Matsuoka M, Fujii N.	Peptide bond mimicry by (E)-alkene and (Z)-fluoroalkene peptide isosteres: synthesis and bioevaluation of alpha-helical anti-HIV peptide analogues.	Org Biomol Chem.	7	2872-2877	2009
	Oishi S, Kodera Y, Nishikawa H, Kamitani H, Watabe T, Ohno H, Tochikura T, Shimane K, Kodama E, Matsuoka M, Mizukoshi F, Tsujimoto H, Fujii N.	Design and synthesis of membrane fusion inhibitors against the feline immunodeficiency virus.	Bioorg Med Chem.	17	4916-4920	2009

研究成果の刊行に関する一覧表

研究分担者　松岡 雅雄

雑誌　　（前頁つづき）

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Watabe T, Terakawa Y, Watanabe K, Ohno H, Nakano H, Nakatsu T, Kato H, Izumi K, Kodama E, Matsuoka M, Kitaura K, Oishi S, Fujii N.	X-ray crystallographic study of an HIV-1 fusion inhibitor with the gp41 S138A substitution.	J Mol Biol.	392	657-665	2009
Kajiwara K, Watanabe K, Tokiwa R, Kurose T, Ohno H, Tsutsumi H, Hata Y, Izumi K, Kodama E, Matsuoka M, Oishi S, Fujii N.	Bioorganic synthesis of a recombinant HIV-1 fusion inhibitor, SC35EK, with an N-terminal pyroglutamate capping group.	Bioorg Med Chem.	17	7964-7970	2009
Tanaka M, Kajiwara K, Tokiwa R, Watanabe K, Ohno H, Tsutsumi H, Hata Y, Izumi K, Kodama E, Matsuoka M, Oishi S, Fujii N.	Bioorganic synthesis of end-capped anti-HIV peptides by simultaneous cyanocysteine-mediated cleavages of recombinant proteins.	Bioorg Med Chem.	17	7487-7492	2009
Izumi K, Nakamura S, Nakano H, Shimura K, Sakagami Y, Oishi S, Uchiyama S, Ohkubo T, Kobayashi Y, Fujii N, Matsuoka M, Kodama E.	Characterization of HIV-1 resistance to a fusion inhibitor, N36, derived from the gp41 amino terminal heptad repeat.	Antiviral Res.	87	179-186	2010
Shimane K, Kodama EN, Nakase I, Futaki S, Sakurai Y, Sakagami Y, Li X, Hattori T, Sarafianos SG, Matsuoka M.	Rev-derived peptides inhibit HIV-1 replication by antagonism of Rev and a co-receptor, CXCR4.	Int J Biochem Cell Biol.	42	1482-1488	2010
Shimura K, Nameki D, Kajiwara K, Watanabe K, Sakagami Y, Oishi S, Fujii N, Matsuoka M, Sarafianos SG, Kodama E.	Resistance profiles of novel electrostatically HIV-1 fusion inhibitors.	J Biol Chem.	285	39471-39480	2010
Izumi K, Watanabe K, Oishi S, Fujii N, Matsuoka M, Sarafianos SG, Kodama EN.	Potent anti-HIV-1 activity of N-HR-derived peptides including a deep pocket-forming region without antagonistic effects on T-20.	Antivir. Chem. Chemother.	22(1)	51-5	2011
Sugata K, Satou Y, Yasunaga JI, Hara H, Ohshima K, Utsunomiya A, Mitsuyama M, Matsuoka M.	HTLV-1 bZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines.	Blood	119(2)	434-444	2011
Inokuchi E, Oishi S, Kubo T, Ohno H, Shimura K, Matsuoka M, Fujii N.	Potent CXCR4 antagonists containing amidine type peptide bond isosteres.	ACS Med. Chem. Lett	2(6)	477-480	2011
Zhao T, Satou Y, Sugata K, Miyazato P, Green PL, Imamura T, Matsuoka M.	HTLV-1 bZIP factor enhances TGF-β signaling through p300 coactivator.	Blood	118	1865-1876	2011

研究成果の刊行に関する一覧表

研究分担者　松下修三

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Narumi, T., Arai, H., Yoshimura, K., Harada, S., Nomura, W. Matsushita, S., Tamamura, H.	Small Molecular CD4 Mimics as HIV Entry Inhibitors.	Bioorg. Med. Chem.	19	6735-6742	2011
Honda M, Ishisaka M, Ishizuka N, Satoshi Kimura S, Oka S and behalf of Japanese Anti-HIV-1 QD Therapy Study Group.	Open-Label Randomized Multicenter Selection Studyof Once Daily Antiretroviral Treatment RegimenComparing Ritonavir-Boosted Atazanavir to Efavirenz with Fixed-Dose Abacavir and Lamivudine.	Intern Med	50	699-705	2011
Narumi, T., Ochiai, C., Yoshimura, K., Harada, S., Tanaka,T., Nomura,W., Arai, H., Ozaki, T., Ohashi, N., Matsushita, S., Tamamura, H.	CD4mimics targeting the HIV entry mechanism and their hybrid molecules with a CXCR4 antagonist.	Bioorganic & Medicinal Chemistry Letters	20	5853-5858	2010
Yoshimura, K., Harada, S., Shibata, J., Hatada, M., Yamada, Y., Ochiai, C., Tamamura, H., Matsushita, S.	Enhanced exposure of human immunodeficiency virus type 1 primary isolate neutralization epitopes through binding of CD4 mimetic compounds.	J Virol	84	7558-7568	2010
Hatada, M., Yoshimura, K., Harada, S., Kawanami, Y., Shibata, J., Matsushita, S.	HIV-1 evasion of a neutralizing anti-V3 antibody involves acquisition of a potential glycosylation site in V2.	J Gen Virol	91	1335-1345	2010
Yamada, Y., Ochiai, C., Yoshimura, K., Tanaka, T., Ohashi, N., Narumi, T., Nomura, W., Harada, S., Matsushita, S., Tamamura, H.	CD4 mimics targeting the mechanism of HIV entry.	Bioorganic & Medicinal Chemistry Letters	20	354–358	2010

研究成果の刊行に関する一覧表

研究分担者　前仲 勝実

雑誌

発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
Kawashima Y, Kuse N, Gatanaga H, Naruto T, Fujiwara M, Dohki S, Akahoshi T, Maenaka K, Goulder P, Oka S, Takiguchi M.	Long-term control of HIV-1 in hemophiliacs carrying slow-progressing allele HLA-B*5101.	J Virol.	84	7151-60	2010
Kamishikiryo J, Fukuhara H, Okabe Y, Kuroki K, Maenaka K.	Molecular basis for LLT1 protein recognition by human CD161 protein (NKRP1A/KLRB1).	J Biol Chem.	286	23823-30	2011
Matsushita H, Endo S, Kobayashi E, Sakamoto Y, Kobayashi K, Kitaguchi K, Kuroki K, Söderhäll A, Maenaka K, Nakamura A, Strittmatter SM, Takai T.	Differential but competitive binding of Nogo protein and class i major histocompatibility complex (MHCI) to the PIR-B ectodomain provides an inhibition of cells.	J Biol Chem.	286	25739-47	2011
Kojima R, Kajikawa M, Shiroishi M, Kuroki K, Maenaka K.	Molecular basis for herpesvirus entry mediator recognition by the human immune inhibitory receptor CD160 and its relationship to the cosignaling molecules BTLA and LIGHT.	J Mol Biol.	413	762-72	2011

16

Ⅳ. 研究成果の刊行物・別刷

※成果多数のため、別刷は刊行一覧より一部抜粋のみ編纂

LETTERS

Adaptation of HIV-1 to human leukocyte antigen class I

Yuka Kawashima[1], Katja Pfafferott[3,6], John Frater[4,5], Philippa Matthews[3], Rebecca Payne[3], Marylyn Addo[7], Hiroyuki Gatanaga[2,8], Mamoru Fujiwara[1], Atsuko Hachiya[1,8], Hirokazu Koizumi[1], Nozomi Kuse[1], Shinichi Oka[2,8], Anna Duda[4,5], Andrew Prendergast[3], Hayley Crawford[3], Alasdair Leslie[3], Zabrina Brumme[7], Chanson Brumme[7], Todd Allen[7], Christian Brander[7,9], Richard Kaslow[10], James Tang[10], Eric Hunter[11], Susan Allen[12], Joseph Mulenga[12], Songee Branch[13], Tim Roach[13], Mina John[6], Simon Mallal[6], Anthony Ogwu[14], Roger Shapiro[14], Julia G. Prado[3], Sarah Fidler[15], Jonathan Weber[15], Oliver G. Pybus[16], Paul Klenerman[4,5], Thumbi Ndung'u[17], Rodney Phillips[4,5], David Heckerman[19], P. Richard Harrigan[18], Bruce D. Walker[7,17,20], Masafumi Takiguchi[1] & Philip Goulder[3,6,17]

The rapid and extensive spread of the human immunodeficiency virus (HIV) epidemic provides a rare opportunity to witness host–pathogen co-evolution involving humans. A focal point is the interaction between genes encoding human leukocyte antigen (HLA) and those encoding HIV proteins. HLA molecules present fragments (epitopes) of HIV proteins on the surface of infected cells to enable immune recognition and killing by $CD8^+$ T cells; particular HLA molecules, such as HLA-B*57, HLA-B*27 and HLA-B*51, are more likely to mediate successful control of HIV infection[1]. Mutation within these epitopes can allow viral escape from $CD8^+$ T-cell recognition. Here we analysed viral sequences and HLA alleles from >2,800 subjects, drawn from 9 distinct study cohorts spanning 5 continents. Initial analysis of the HLA-B*51-restricted epitope, TAFTIPSI (reverse transcriptase residues 128–135), showed a strong correlation between the frequency of the escape mutation I135X and HLA-B*51 prevalence in the 9 study cohorts ($P = 0.0001$). Extending these analyses to incorporate other well-defined $CD8^+$ T-cell epitopes, including those restricted by HLA-B*57 and HLA-B*27, showed that the frequency of these epitope variants ($n = 14$) was consistently correlated with the prevalence of the restricting HLA allele in the different cohorts (together, $P < 0.0001$), demonstrating strong evidence of HIV adaptation to HLA at a population level. This process of viral adaptation may dismantle the well-established HLA associations with control of HIV infection that are linked to the availability of key epitopes, and highlights the challenge for a vaccine to keep pace with the changing immunological landscape presented by HIV.

The extent to which HIV is evolving at the population level in response to immune selection pressure is under debate[2–6]. Resolving the impact of HLA class I alleles on viral evolution is problematic because it can be obscured by other influences, such as founder effect[6] (polymorphisms present within the early strains establishing the epidemic in a group). In addition, most HLA alleles do not drive significant selection pressure on HIV, a proportion of escape mutations revert to wild type after transmission, and different HLA alleles may drive the identical escape mutation[7].

To test the hypothesis that the frequency of escape mutations in a given population is correlated with the prevalence of the relevant HLA allele in that population, we studied nine distinct cohorts from North America, the Caribbean, Europe, sub-Saharan Africa, Australia and Japan, in which we performed HLA typing, and defined the viral mutations arising within $CD8^+$ T-cell epitopes. We focused initially on a well-characterized mutation, I135X, within the HLA-B*51-restricted epitope, TAFTIPSI (RT 128–135)[8], because it arises in acute infection, non-HLA-B*51 alleles do not also select this mutation[7,9], and it does not revert to Ile 135 after transmission to HLA-B*51-negative subjects[9]. Thus, if highly prevalent HLA alleles drive a high frequency of escape mutations in the population, this would be most obvious in relation to HLA-B*51 and the escape mutant I135X. We then considered an additional 13 well-defined escape mutations, including those known to reduce viral fitness and therefore liable to revert after transmission.

I135X was selected in 205 of 213 (96%) HLA-B*51-positive individuals analysed (Figs 1 and 2, and Supplementary Fig. 1). The I135X variants do not significantly affect viral replicative capacity *in vitro*, other than the rare I135V mutation. This was the only variant observed to revert to wild-type *in vivo* during a 3-year follow-up of 38 HLA-B*51-negative subjects identified during acute HIV infection who carried I135X mutant viruses at transmission (Fig. 1e). The I135X mutants substantially affect HLA binding, and therefore also recognition by $CD8^+$ T cells (Fig. 1f–h). Thus, HIV transmission from HLA-B*51-positive subjects would probably involve transmission of I135X, which would persist in the new host. Newly infected HLA-B*51-positive subjects receiving an I135X mutant would be unable to generate an HLA-B*51-TAFTIPSI-specific response.

To test the hypothesis that the population frequency of I135X is correlated with HLA-B*51 prevalence, HIV sequence and HLA data were collated from the nine study cohorts. One cohort comprised subjects with acute/early HIV infection; the remaining cohorts comprised chronically infected subjects. In all cohorts the odds ratio strongly favoured I135X in the HLA-B*51-positive subjects, even in the acute cohort where I135X was selected sufficiently early to be already over-represented in HLA-B*51-positive subjects (odds ratio 1.65, $P = 0.07$, Fig. 2a). In Japan, where HLA-B*51 is highly

[1]Divisions of Viral Immunology and [2]Infectious Disease, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan. [3]Department of Paediatrics, [4]Nuffield Department of Clinical Medicine and [5]The James Martin 21st Century School, Peter Medawar Building for Pathogen Research, South Parks Road, Oxford OX1 3SY, UK. [6]Centre for Clinical Immunology and Biomedical Statistics, Royal Perth Hospital and Murdoch University, Western Australia 6000, Australia. [7]Partners AIDS Research Center, Massachusetts General Hospital, 13th Street, Building 149, Charlestown, Boston, Massachusetts 02129, USA. [8]AIDS Clinical Center, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. [9]Fundació IrsiCaixa-HIVACAT, Hospital Germans Trias i Pujol, Badalona and Institucio Catalana de Recerca i Estudis Avancats (ICREA), Barcelona 08916, Spain. [10]University of Alabama at Birmingham, Birmingham, Alabama 35294, USA. [11]Emory University Vaccine Center and Yerkes National Primate Research Center, Atlanta, Georgia 30329, USA. [12]Zambia Emory HIV Research Project, and the Zambia Blood Transfusion Service, Lusaka, Zambia. [13]Ladymeade Reference Unit, University of West Indies, Bridgetown BB11156, Barbados. [14]Botswana-Harvard School of Public Health AIDS Initiative Partnership, Gaborone, Botswana. [15]Division of Medicine, Wright Fleming Institute, Imperial College, St Mary's Hospital, Norfolk Place, Paddington, London W2 1PG, UK. [16]Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3SY, UK. [17]HIV Pathogenesis Programme, The Doris Duke Medical Research Institute, University of KwaZulu-Natal, Durban 4013, South Africa. [18]Miscrosoft Research, One Microsoft Way, Redmond, Washington 9805, USA. [19]BC Centre for Excellence in HIV/AIDS, Vancouver, British Columbia V6Z 1Y6, Canada. [20]Howard Hughes Medical Institute, Chevy Chase, Maryland 20185, USA.

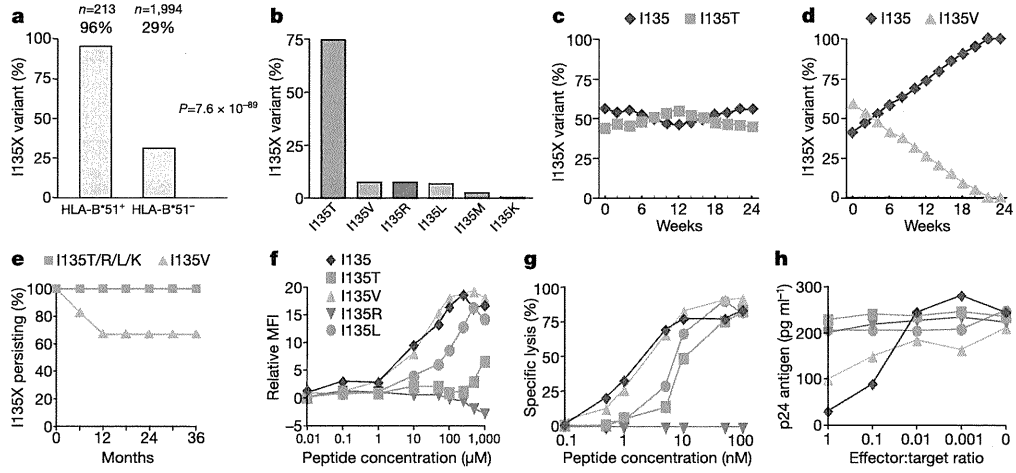

Figure 1 | Selection and fitness cost of I135X escape variants and recognition by the HLA-B*51–TAFTIPSI (RT 128–135)-specific CD8$^+$ T cells. **a**, Association between I135X and HLA-B*51 in all study cohorts. **b**, Ile 135 variation in HLA-B*51-positive subjects. **c**, **d**, *In vitro* competition assays between NL4-3 wild-type virus and I135X viral variants (I135T (**c**) and I135V (**d**)). I135R and I135L showed no fitness cost (not shown). **e**, Persistence of I135X mutants in 38 HLA-B*51-negative subjects followed from acute infection. **f**, TAFTIPSI variant binding to HLA-B*51 (see Methods). MFI, mean fluorescence intensity. **g**, **h**, Recognition of peptide-pulsed HLA-B*51-matched targets and viral variants by representative TAFTIPSI-specific CD8$^+$ T-cell clones.

prevalent[10] (21.9% of the study cohort), the frequency of I135X was >50%, and overall across all cohorts the I135X frequency was strongly correlated with HLA-B*51 prevalence ($P = 0.0001$, Fig. 2b). To control for the possibility that disproportionately more virus sequences from HLA-B*51-positive subjects were analysed, the same analysis comparing I135X frequency in HLA-B*51-negative subjects only was undertaken, with similar findings (Fig. 2c, $P = 0.0006$). These data suggest that HIV may be adapting to HLA-B*51 with respect to the HLA-B*51–TAFTIPSI response in localities where HLA-B*51 is at high prevalence.

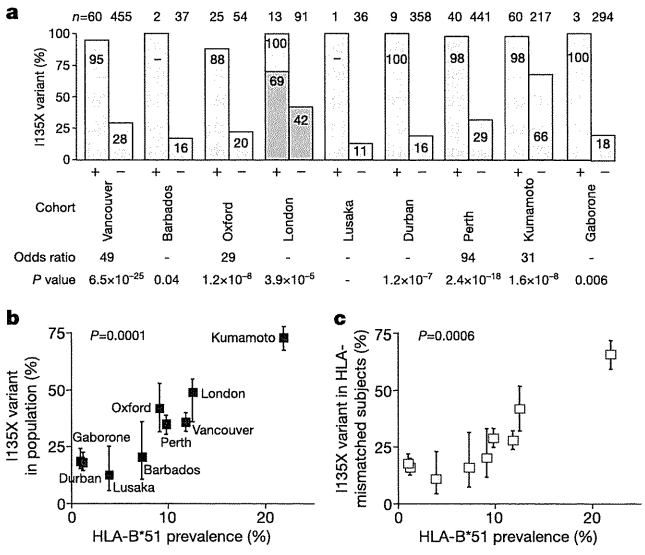

Figure 2 | Correlation between frequency of HLA-B*51-associated escape mutations and HLA-B*51 prevalence in study cohorts. **a**, Frequency of I135X mutations within TAFTIPSI (RT 128–135) in HLA-B*51-positive (+) and -negative (−) subjects within nine study cohorts. In the acute cohort (London) 69% of HLA-B*51-positive subjects expressed I135X mutant at enrolment, 100% within 2 years of baseline (Supplementary Fig. 1). **b**, Correlation between frequency of I135X mutation and HLA-B*51 prevalence in the nine study populations. Logistic regression $P = 0.0001$ (Supplementary Table 1). **c**, Correlation between I135X frequency in HLA-B*51-negative subjects and HLA-B*51 prevalence in nine study populations. Error bars represent 95% confidence limits, obtained using a binomial error distribution.

Additional evidence that I135X is accumulating in Japan comes from the observation that only 3 of 14 (21%) HLA-B*51-negative Japanese haemophiliacs infected in 1983 carried I135X, compared with 30 of 43 (70%) HLA-B*51-negative subjects infected between 1997 and 2008 ($P = 0.002$). Furthermore, HLA-B*51 does not protect against disease progression in Japanese subjects infected between 1997 and 2008, whereas HLA-B*51-positive haemophiliacs infected in 1983 had lower viraemia levels and higher CD4 counts than HLA-B*51-negative haemophiliacs (Supplementary Fig. 2). These data are consistent with fewer HLA-B*51-positive subjects targeting TAFTIPSI during 1997–2008, owing to a population-level increase in the HLA-B*51 I135X escape mutation over this 14–25-year period.

To investigate HIV adaptation to other HLA alleles, we initially examined other escape mutations shown previously to persist stably after transmission[5,7]. We selected the three non-reverting Gag polymorphisms that, from analysis of 673 study subjects in Durban, South Africa[7], were most strongly associated with the relevant restricting allele ($P < 10^{-6}$ after phylogenetic correction), namely, S357X, D260X and D312X within epitopes restricted, respectively, by HLA-B*07 (GPSHKARVL, Gag 355–363), HLA-B*35 (PPIPVGDIY, Gag 254–262) and HLA-B*44 (AEQATQDVKNW, Gag, 306–316). In addition, we analysed a non-reverting I31V variant (LPPIVAKEI, Int 28–36) previously hypothesized to increase in relation to population HLA-B*51 prevalence[5]. These additional polymorphisms show a similar relationship to that between I135X and HLA-B*51, overall showing a strongly significant correlation between variant frequency and prevalence of the restricting HLA allele (Figs 3 and 4a, and Supplementary Fig. 3).

The spectrum of HLA-associated polymorphisms also includes mutations reducing viral fitness[1]. These either revert to wild type after transmission, or persist in the presence of compensatory mutations. We extended these analyses to include epitopes restricted by HLA-B*27 and HLA-B*57, alleles strongly associated with successful immune control of HIV[11,12]. The mutations analysed themselves are associated with precipitating loss of immune control[13–16] and all inflict a documented viral fitness cost, either demonstrated by *in vitro* fitness studies and/or *in vivo* reversion[7,14,17–21] (data not shown for V168I).

Again, a strong correlation between escape mutant frequency and prevalence of the restricting HLA allele was observed (Figs 3c–f and 4b, and Supplementary Fig. 3; overall, for these nine variants affecting viral fitness, $r = 0.69$, $P < 0.0001$). Unexpectedly, this correlation