れ平均0.27% (0.05-0.86%)、平均1.09% (0.07-2.63%)を示した。また、SIV特異的CD4陽性、CD8陽性Tリンパ球の多機能性レベルはそれぞれ平均1.57 (1.03-2.23)、平均2.88 (1.27-3.97)を示した。

SIV特異的細胞頻度と慢性持続感染期の血中ウイルス量との相関を解析した結果、CD4陽性、CD8陽性Tリンパ球ともに有意な相関は認められなかった（CD4陽性Tリンパ球：R = -0.096, P = 0.724；CD8陽性Tリンパ球：R = -0.407, P = 0.118）。一方、多機能性レベルと血中ウイルス量との間には、CD4陽性、CD8陽性Tリンパ球ともに有意な逆相関が認められた（CD4陽性Tリンパ球：R = -0.538, P = 0.032；CD8陽性Tリンパ球：R = -0.622, P = 0.010）（図1）。

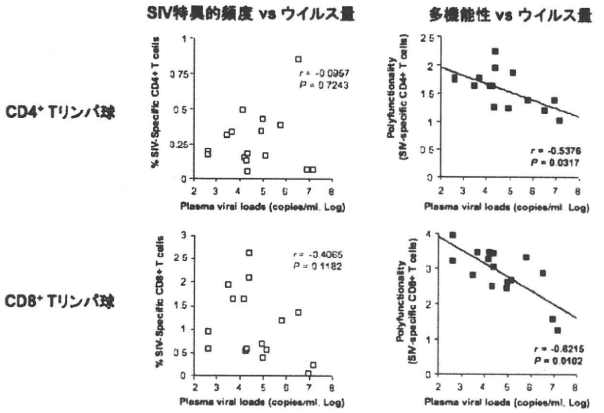

図1. 血中ウイルス量に対するSIV特異的細胞頻度および多機能性の関係

さらに、CD4陽性、CD8陽性Tリンパ球両者の関連を解析した結果、SIV特異的細胞頻度については有意な相関が認められなかったが（R = 0.312, P = 0.240）、多機能性レベルについては有意な相関が認められた（R = 0.632, P < 0.001）（図2）。

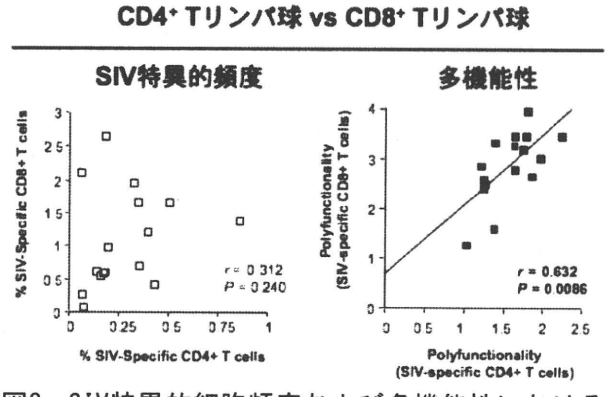

図2. SIV特異的細胞頻度および多機能性におけるCD4陽性Tリンパ球とCD8陽性Tリンパ球の関係

2) 各ハプロタイプ共有群間でのHTL多機能性レベルの違い

SIV抵抗性に対する各ハプロタイプ共有群の特徴として、(1)ハプロタイプA共有群はSeV-Gagワクチンの有効性が顕著であり、ワクチン非接種においてもSIV抵抗性を有する、(2) ハプロタイプE共有群におけるSeV-Gagワクチンの有効性、ワクチン非接種におけるSIV抵抗性は、ハプロタイプA共有群と比較して中程度である、(3)ハプロタイプJ共有群はSeV-Gagワクチンの有効性が殆ど認められずSIV抵抗性を示さない、ことが知られている。

各ハプロタイプ共有群のCD4陽性Tリンパ球の多機能性レベルを解析した結果、ハプロタイプA共有群で最も高く（1.84 ± 0.23）、続いてハプロタイプE共有群（1.58 ± 0.28）、ハプロタイプB共有群（1.43 ± 0.30）、そしてハプロタイプJ共有群で最も低かった（1.17 ± 0.12）。同様に、CD8陽性Tリンパ球の多機能性レベルもハプロタイプA共有群で最も高く（3.52 ± 0.27）、ハプロタイプJ共有群で最も低かった（2.11 ± 0.73）（図3）。

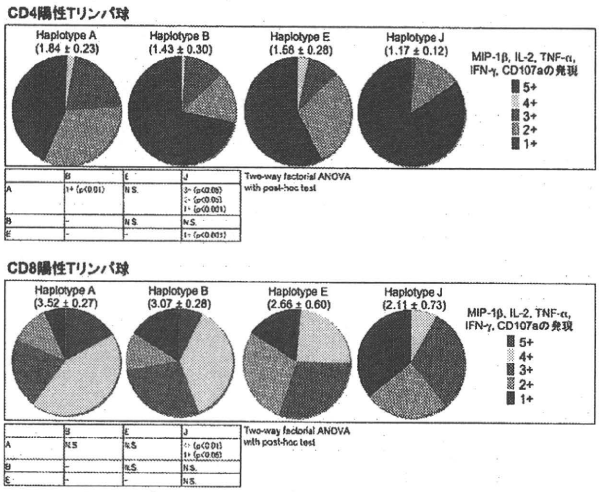

図 3. 各ハプロタイプ共有群における CD4 陽性 T リンパ球と CD8 陽性 T リンパ球の多機能性

D．考察

本年度の実験結果から、HTL の多機能性レベルは、ウイルス複製制御レベル、ならびに CTL の多機能性レベルと関連すること、つまり、SIV 複製制御がみられ多機能性レベルの高い CTL を有する個体では HTL の多機能性レベルも高いことが示された。さらに、SIV 抵抗性の異なるハプロタイプ共有群間の比較から、HTL の多機能性レベルは SIV 抵抗性の高いハプロタイプ A 共有群で高く、SIV 抵抗性の無いハプロタイプ J 共有群で低いことが示された。

昨年度（平成 21 年度）は SIV 導入 BLCL を用

いた抗原刺激により、検出感度の高い多機能性評価系を確立した。このことにより、ハプロタイプJ共有群に特徴されるようなSIV複製制御が出来ず、リンパ球の疲弊度が高いと考えられる慢性持続感染期試料においても、SIV特異的Tリンパ球を効率的に検出できたものと考えられる。

HIV/SIV複製制御におけるHTLの役割として、感染急性期におけるウイルス特異的CTLの誘導には必要でないことが示されている。さらに、ウイルス特異的HTLは他のHTLと比べてウイルスの標的となりやすいことから、感染急性期におけるウイルス特異的HTLの増大は逆に感染プールの拡大に結びつく可能性も指摘されている。このことから、慢性持続感染期において観察される高レベルの多機能性HTLは、CTLによるSIV複製制御の結果として誘導されたものと推測される。この多機能性HTLが慢性持続感染期におけるCTLの維持に役立っているかどうかについては今後の検討課題である。

本研究ではMHC-IIハプロタイプの分類に基づく解析は行っていない。しかしながら、MHC-IIハプロタイプは今回の分類によるMHC-Iハプロタイプとほぼ相関していることが知られている。このことから、多機能性HTLの誘導には、ある特定のMHC-IIハプロタイプが関与することも十分考えられる。

E．結論

HTLの多機能性レベルは、ウイルス複製制御レベル、ならびにCTLの多機能性レベルと関連することが示された。

F．研究発表

1　論文発表

(1) Terahara K, Nochi T, Yoshida M, Takahashi Y, Goto Y, Hatai H, Kurokawa S, Jang MH, Kweon M-N, Domino SE, Hiroi T, Yuki Y, Tsunetsugu-Yokota Y, Kobayashi K, Kiyono H. Distinct fucosylation of M cells and epithelial cells by Fut1 and Fut2, respectively, in response to intestinal environmental stress. Biochem. Biophys. Res. Commun. 404: 822-828, 2011.

2　学会発表

(1) Terahara K, Ishige M, Mitsuki Y, Shibusawa K, Watanabe S, Okada S, Kobayashi K, Tsunetsugu-Yokota Y. Characteristic activation/differentiation phenotype of $CD4^+$ T cells and their distinct susceptibility to X4-type and/or R5-type HIV-1 infection in humanized NOD/SCID/Jac3-null mice. 14th International Congress of Immunology, Kobe, Japan, 8/22-27/2010.

(2) Mitsuki Y, Shibusawa K, Terahara K, Kobayashi K, Takeda M, Yanagi Y, Tsunetsugu-Yokota Y. HIV-1 infection enhances the susceptibility of T cells to measles virus infection by upregulating signaling lymphocyte activation molecule (SLAM) expression. 14th International Congress of Immunology, Kobe, Japan, 8/22-27/2010.

(3) Shibusawa K, Mitsuki Y, Terahara K, Yanagi Y, Kobayashi K, Tsunetsugu-Yokota Y. Inhibition of HIV-1 replication by engineered lentivirus vectors: a potential advantage of SLAM-mediated virus entry. 14th International Congress of Immunology, Kobe, Japan, 8/22-27/2010.

(4) Shibusawa K, Mitsuki Y, Terahara K, Ishige M, Yanagi Y, Kobayashi K, Tsunetsugu-Yokota Y. Inhibition of HIV-1 replication by envelope-engineered lentivirus vectors: efficient gene delivery using envelope protein of measles virus. The 10th Awaji International Forum on Infection and Immunity, Awaji, Japan, Sep 7-10, 2010.

(5) 石毛真行、寺原和孝、光木裕也、渋沢謙太郎、小林和夫、岡田誠治、横田（恒次）恭子：HIV-1感染モデルとしてのヒト化マウスの妥当性とX4およびR5 HIV-1感染、第58回日本ウイルス学会学術集会、徳島、11/7-9/2010.

(6) 渋沢謙太郎、光木裕也、寺原和孝、石毛真行、柳雄介、小林和夫、横田（恒次）恭子：麻疹ウイルスエンベロープをもちいたHIV-1増殖抑制性レンチウイルスベクターの開発とその有効性、第58回日本ウイルス学会学術集会、徳島、11/7-9/2010.

G．知的財産権の出願・登録状況（予定を含む。）

1　特許取得

なし。

2　実用新案登録

なし。

3　その他

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

中和抗体誘導に関する研究

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研究要旨

HIV-1 Env gp120 の抗 V3 抗体中和における V1/V2 ループの影響を知るためには、gp120 における V1/V2 ループの配置をもとに、その役割を理解することが必要である。CD4 結合前および結合後の V1/V2 ループを含む HIV-1 Env gp120 分子モデルの構築を行い、V1/V2 ループの役割について検討した。CD4 結合前の V1/V2 ループは、他の部位に比べて gp120 コアから最も離れて配置され、gp120 コアと V1/V2 ループの間に V3 が配置される。ゆえに、V1/V2 ループは V3 への抗 V3 中和抗体のアクセスを制限する可能性が考えられる。CD4 結合後は、CD4 結合前に比べて、V1/V2 ループが大きく変化して CD4 近傍に配置される。その結果、V3 がウイルス表面に突出する配置となり、コレセプタ結合部位を露出する。以上より、V1/V2 ループは、V3 との相対的な配置に依存して、抗 V3 中和抗体感受性やコレセプタ結合に重要な役割を担う部位であると考えられる。

A．研究目的

HIV-1 Env gp120 の V3 は、感染受容体との相互作用に中心的役割を担う。そのため本来は、機能的制約が強く作用し、アミノ酸変異は抑制されるはずである。ところが V3 は高変異領域として知られる。これは、V3 は免疫原性が高く、持続感染には抗原変異を必要とするため、とされる。

V3 配列を糖鎖付加部位の有無と荷電量の違いで分類して多様性解析すると、V3 の荷電量の低下によって、V3 配列の多様性が低下することが示された。次に、V3 配列の荷電量の gp120 構造への影響を調べると、gp120 コアのアミノ酸配列が同じでも、V3 配列の荷電量の変化に伴い、V3 の配置や gp120 コアの構造を変えた。さらに、V3 荷電量の低下に伴い、抗 V3 結合抗体および CD4 結合部位を認識する中和抗体による中和感受性は低下した。ゆえに、HIV-1 Env gp120 V3 は荷電量に基づきウイルスの中和感受性と細胞指向性を司る機能領域と考えられる。

本研究は、抗原部位構造を推定することで、ウイルスが抗 V3 抗体中和を逃避するしくみを解析し、抗 V3 抗体の中和能を人為的に高める方法を開発することを目的とする。成果をワクチン開発に還元することを目指す。

今年度は HIV-1 Env gp120 の抗 V3 抗体中和における V1/V2 ループの影響を知るために、CD4 結合前および結合後の V1/V2 ループを含む HIV-1 Env gp120 分子モデルの構築を行い、V1/V2 ループの役割について検討した。

B．研究方法

（1）HIV-1 Env gp120分子モデルの構築

主にホモロジーモデリング法により、CD4結合前および結合後のV1/V2ループを含むHIV-1 Env gp120分子モデルを構築した。gp120ではV1/V2ループ以外の構造は、X線結晶構造解析により部分的に決定されている。複数の構造を鋳型として用いて、全体構造の分子モデルを構築した。未だ構造が決定されていないV1/V2ループは、スレッディング法により構築し、分子動力学計算により平衡構造を得た。

（2）分子モデルの評価

蛋白質の構造評価プログラムであるVerify3Dを用いて、蛋白質のアミノ酸残基が置かれている構造環境を評価することで、構築した分子モデルの妥当性を検討した。

（倫理面への配慮）

本研究では、特定の研究対象者は存在せず、倫理面への配慮は不要である。

C．研究結果

（1）CD4結合前のV1/V2ループを含むHIV-1 Env gp120の構造解析

CD4結合前のV1/V2ループを含まない分子モデ

ルを、ホモロジーモデリング法により構築した。鋳型に用いたgp120構造はb12抗体との複合体であるが、b12抗体を取り除いて分子モデルを構築した。CD4結合ループを正面の方向から見ると、左上にgp120のN末端とC末端が配置され、右下にV3が配置される。Inner domain上部はβ-sandwich構造、下部は構造的に可塑性のレイヤー構造となっている。左から２つ目のレイヤー（レイヤー２）のV1/V2 stemが下方に伸び、V3とほぼ平行に配置されている。

次に、CD4結合前のV1/V2ループを含む分子モデルを構築した。前述の分子モデルのV1/V2 stemに、後述の分子モデルで構築したV1/V2ループを付加した後、分子動力学計算により平衡構造を得た。その結果、CD4結合前ではV1/V2ループは他の部位に比べてgp120コアから最も離れて配置され、gp120コアとV1/V2ループの間にV3が配置されることが予測された。Verify3Dを用いて分子モデルの評価を行うと、ゆらぎの大きいV1/V2 stem付近やV4を除いて、アミノ酸残基が置かれている構造環境は適してた。ゆえに、一部のゆらぎの大きい部位を除いて、構築した分子モデルは妥当な構造であると考えられる。

（２）CD4結合後のV1/V2ループを含むHIV-1 Env gp120の構造解析

CD4 結合後の V1/V2 ループを含まない分子モデルを、ホモロジーモデリング法により構築した。CD4 D1-D2 domain が結合している gp120 構造を鋳型に用いて、CD4 D1-D2 domain との複合体の分子モデルを構築した。CD4 結合ループを正面の方向から見ると、左上に gp120 の N 末端と C 末端が配置され、右下に V3 が配置される。Inner domain のレイヤー２の V1/V2 stem が、β20-β21 loop と bridging sheet を形成している。

次に、CD4 結合後の V1/V2 ループを含む分子モデルを構築した。前述の V1/V2 ループを含まない分子モデル上で、スレッディング法により V1/V2 ループの最適なフォールディングを検索することで、分子モデルを構築した。その結果、CD4 結合後では V1/V2 ループは CD4 近傍に配置されることが予測された。この V1/V2 loop の配置は既存の極低温電子顕微鏡で観察された結果と一致している。また、Verify3D を用いて分子モデルの評価を行うと、ゆらぎの大きい N 末端や V1/V2 stem 付近を除いて、アミノ酸残基が置かれている構造環境は適してた。ゆえに、一部のゆらぎの大きい部位を除いて、構築した分子モデルは妥当な構造であると考えられる。

D．考察

HIV-1 Env gp120分子モデルにおけるV1/V2ループとV3の相対的な配置をもとに、V1/V2ループの役割について検討する。CD4結合前のV1/V2ループを含むgp120分子モデルから、CD4結合前ではV1/V2ループは他の部位に比べて、gp120コアから最も離れて配置され、V3がgp120コアとV1/V2ループの間に配置されることが予測された。これはV1/V2ループがV3への抗V3中和抗体のアクセスを制限する可能性があることを示唆している。一方、CD4結合前後のV1/V2ループを含むgp120分子モデルを比較すると、CD4結合前後で大きくV1/V2ループの配置が変化すると予測された。CD4結合前ではV3がgp120コアとV1/V2ループの間に配置されていたが、CD4結合後では、コレセプタ結合部位を露出するために、V1/V2ループが大きく変化し、V3がウイルス表面に突出すると考えられる。したがって、V1/V2ループは、V3との相対的な配置に依存して、抗V3中和抗体感受性やコレセプタ結合に重要な役割を担う部位であると考えられる。

E．結論

CD4 結合前および結合後の V1/V2 ループを含む HIV-1 Env gp120 分子モデルの構築を行い、V1/V2 ループの役割について検討した。その結果、V1/V2 ループは、V3 との相対的な配置に依存して、抗 V3 中和抗体感受性やコレセプタ結合に重要な役割を担う部位であると考えられる。

F．研究発表

1　論文発表

(1) Onyango, C.O., Leligdowicz, A., Yokoyama, M., Sato, H., Song, H., Nakayama, E.E., Shioda, T., de Silva, T., Townend, J., Jaye, A., Whittle, H., Rowland-Jones, S., Cotton, M. HIV-2 Capsids Distinguish High and Low Virus Load Patients in a West African Community Cohort. *Vaccine* 28 Suppl 2:B60-B67, 2010.

(2) Kono, K., Song, H., Yokoyama, M., Sato, H., Shioda, T., Nakayama, E.E. Multiple sites in the N-terminal half of simian immunodeficiency virus capsid protein contribute to evasion from rhesus monkey TRIM5α-mediated restriction. *Retrovirology* 7:72, 2010.

(3) Inagaki, N., Takeuchi, H., Yokoyama, M., Sato, H., Ryo, A., Yamamoto, H., Kawada, M., Matano, T.. A structural constraint for functional interaction between N-terminal and C-terminal domains in simian immunodeficiency virus capsid proteins. *Retrovirology* 7:90, 2010.

2　学会発表

(1) Yokoyama, M., Naganawa, S., Kitamura, K., Sato, H.Influence of Net Positive Charge in V3 Sequence on Conformation of HIV-1 gp120 outer domain. 5TH GERMAN JAPANESE HIV SYMPOSIUM, Tokyo, Japan, 5/10-11/2010.

(2) 横山　勝. ゲノミクス, 計算科学, 実験科学の手法に基づく HIV の中和抗体逃避機構の研究. 第 58 回日本ウイルス学会学術集会, シンポジウム, 徳島, 11/7-9/2010.

(3) 稲垣奈津子, 武内寛明, 横山　勝, 佐藤裕徳, 梁　明秀, 俣野哲朗. SIV CA の N ドメインと C ドメインの機能的相互作用に関わるアミノ酸残基の同定. 第 58 回日本ウイルス学会学術集会, 徳島, 11/7-9/2010.

(4) 河野　健, 横山　勝, 佐藤裕徳, 塩田達男, 中山英美. アカゲザル TRIM5α は HIV-2/SIVmac キャプシドの複数領域を認識する. 第 58 回日本ウイルス学会学術集会, 徳島, 11/7-9/2010.

(5) 土肥直哉, 齊藤　暁, 明里宏文, 藤原佐知, 三宅在子, 横山　勝, 大出裕高, 佐藤裕徳, 足立昭夫, 野間口雅子. サル指向性 HIV-1 CA の 1 アミノ酸変異はサル細胞での増殖を促進する. 第 58 回日本ウイルス学会学術集会, 徳島, 11/7-9/2010.

(6) 野間口雅子, 土肥直哉, 藤原佐知, 三宅在子, 横山　勝, 大出裕高, 佐藤裕徳, 足立昭夫. アカゲザルに存在する抗 HIV-1 因子 TRIM5α と tetherin を回避するサル細胞指向性 HIV-1 の構築. 第 58 回日本ウイルス学会学術集会, 徳島, 11/7-9/2010.

(7) 野間口雅子, 齊藤　暁, 明里宏文, 土肥直哉, 藤原佐知, 三宅在子, 横山　勝, 大出裕高, 佐藤裕徳, 足立昭夫. サル細胞で効率良く増殖する HIV-1 の構築—アカゲザル TRIM5α と tetherin による抑制の回避— 第 24 回日本エイズ学会学術集会・総会, 東京, 11/24-26/2010.

(8) 泉　泰輔, 横山　勝, 篠原正信, 松井道志, 井尾克宏, 佐藤裕徳, 高折晃史. 抗 HIV-1 宿主因子 APOBEC3G の N 末端ポケット構造の重要性. 第 24 回日本エイズ学会学術集会・総会, 東京, 11/24-26/2010.

(9) Izumi, T., Yokoyama, M., Shinohara, M., Matsui, M, Io, M, Sato, H., Takaori-Kondo, A. Model structure of APOBEC3G N-terminal region reveals a binding pocket modulating HIV-1 Vif interaction and RNA required for encapsidation. 第 33 回日本分子生物学会年会・第 83 回日本生化学会大会合同大会（BMB2010）, 神戸, 12/7-10/2010.

G. 知的財産権の出願・登録状況（予定を含む。）

1　特許取得

なし。

2　実用新案登録

なし。

3　その他

なし。

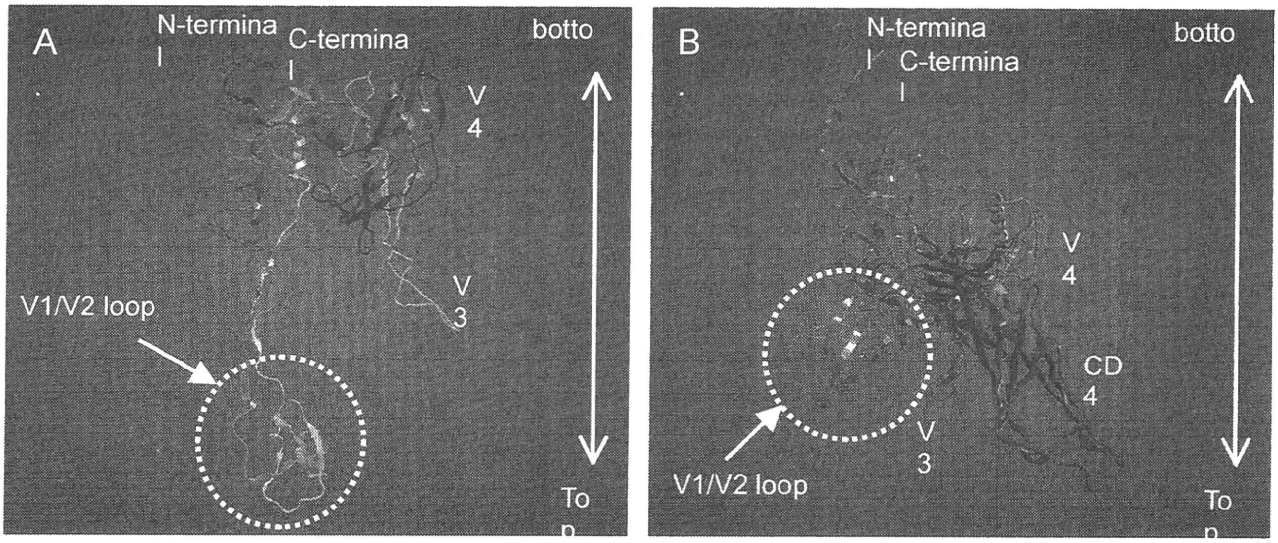

図 1. ホモロジーモデリング法および分子動力学計算により構築した V1/V2 ループを含む HIV-1 Env gp120 分子モデル。CD4 結合前(A) と CD4 結合後 (B)。

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

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
Yamamoto H, Matano T	Neutralizing antibodies in SIV control: co-impact with T cells	Vaccine	28S	B13-B17	2010
Naruse TK, Chen Z, Yanagida R, Yamashita T, Saito Y, Mori K, Akari H, Yasutomi Y, Miyazawa M, Matano T, Kimura A	Diversity of MHC class I genes in Burmese-origin rhesus macaques	Immunogenetics	62	601-611	2010
Iwamoto N, Tsukamoto T, Kawada M, Takeda A, Yamamoto H, Takeuchi H, Matano T	Broadening of $CD8^+$ cell responses in vaccine-based simian immunodeficiency virus controllers	AIDS	24	2777-2787	2010
Inagaki N, Takeuchi H, Yokoyama M, Sato H, Ryo A, Yamamoto H, Kawada M, Matano T	A structural constraint for functional interaction between N-terminal and C-terminal domains in simian immunodeficiency virus capsid proteins	Retrovirology	7	90	2010
Saito A, Nomaguchi M, Iijima S, Kuroishi A, Yoshida T, Lee Y-J, Hayakawa T, Kono K, Nakayama EE, Shioda T, Yasutomi Y, Adachi A, Matano T, Akari H	Improved capacity of a monkey-tropic HIV-1 derivative to replicate in cynomolgus monkeys with minimal modifications	Microbes Infect	13	58-64	2011
YoshidaT, Saito A, Iwasaki Y, Iijima S, Kurosawa T, Katakai Y, Yasutomi Y, Reimann KA, Hayakawa T, Akari H	Characterization of natural killer cells in tamarins: a technical basis for studies of innate immunity	Frontiers Microbiol		in press	
Xing Li, Wang JC, Li T-C, Yasutomi Y, Lara J, Khurdyakaov Y, Schofield D, Emerson S, Purcell R, Takeda N, Miyamura T, Holland RC	Spatial configuration of hepatitis E virus antigenic domain	J Virol	85	1117-1124	2011
Chono H, Matsumoto K, Tsuda H, Saito N, Lee, K, Kim S, Shibata H, Ageyama N, Terao K, Yasutomi Y, Mineno J, Kim S, Inoue M, Kato I	Acquistion of HIV-1 resistance in T lymphocytes using an ACA-specific E.coli mRNA interferase	Human Gene Ther	22	35-43	2011
Okabayashi S, Uchida K, Nakayama H, Ohno C, Hanari K, Goto I, Yasutomi Y	Periventricular Leucomalacia (PVL)-like lesions in two neonatal cynomolgus monkeys (macaca fascicularis)	J Comp Pathol		in press	

Yasutomi Y	Establishment of Specific Pathogen-Free Macaque Colonies in Tsukuba Primate Research Center of Japan for AIDS research	Vaccine	28S	B75-B77	2010
Fujimoto K, Takano J, Narita T, Hanari K, Shimozawa N, Sankai T, Yoshida T, Terao K, Kurata T, Yasutomi Y	Simian Retrovirus type D infection in a colony of cynomolgus monkeys	Comp Med	60	51-53	2010
Cueno ME, Karamatsu K, Yasutomi Y, Laurena AC, Okamoto T	Preferential expression and immunogenicity of HIV-1 Tat fusion protein expressed in tomato plant	Transgenic Res	19	889-895	2010
Brumme ZL, Li C, Miura T, Sela J, Rosato PC, Brumme CJ, Markle T, Martin E, Block BL, Trocha T, Kadie CM, Allen TM, Pereyra F, Heckerman D, Walker BD, Brockman MA	Reduced replication capacity of NL4-3 recombinant viruses encoding RT-Integrase sequences from HIV-1 elite controllers	J Acquir Immune Defic Syndr	56(2)	100-108	2011
Nakamura H, Miyazaki N, Hosoya N, Koga M, Odawara T, Kikuchi T, Koibuchi T, Kawana-Tachikawa A, Fujii T, Miura T, Iwamoto A	Long-term successful control of super-multidrug-resistant human immunodeficiency virus type 1 infection by a novel combination therapy of raltegravir, etravirine, and boosted-darunavir	J Infect Chemother	17(1)	105-110	2011
Gesprasert G, Wichukchinda N, Mori M, Shiino T, Auwanit W, Sriwanthana B, Pathipvanich P, Sawanpanyalert P, Miura T, Auewarakul P, Thitithanyanont A, Ariyoshi K	HLA-associated immune pressure on Gag protein in CRF01_AE-infected individuals and its association with plasma viral load	PLoS One	5(6)	e11179	2010
Miura T, Brumme ZL, Brockman MA, Rosato P, Sela J, Brumme CJ, Pereyra F, Kaufmann DE, Trocha A, Block BL, Daar ES, Connick E, Jessen H, Kelleher AD, Rosenberg E, Markowitz M, Schafer K, Vaida F, Iwamoto A, Little S, Walker BD	Impaired replication capacity of acute/early viruses in persons who become HIV controllers	J Virol	84	7581-7591	2010

Wright JK, Brumme ZL, Carlson JM, Heckerman D, Kadie CM, Brumme CJ, Wang B, Losina E, Miura T, Chonco F, van der Stok M, Mncube Z, Bishop K, Goulder PJ, Walker BD, Brockman MA, Ndung'u, T	Gag-protease-mediated replication capacity in HIV-1 subtype C chronic infection: associations with HLA type and clinical parameters	J Virol	84	10820-10831	2010
Julg B, Pereyra F, Buzon MJ, Piechocka-Trocha A, Clark MJ, Baker BM, Lian J, Miura T, Martinez-Picado J, Addo MM, Walker BD	Infrequent recovery of HIV from but robust exogenous infection of activated CD4(+) T cells in HIV elite controllers	Clin Infect Dis	51	233-238	2010
Brockman MA, Brumme ZL, Brumme CJ, Miura T, Sela J, Rosato PC, Kadie CM, Carlson JM, Markle TJ, Streeck H, Kelleher AD, Markowitz M, Jessen H, Rosenberg E, Altfeld M, Harrigan PR, Heckerman D, Walker BD, Allen TM	Early selection in Gag by protective HLA alleles contributes to reduced HIV-1 replication capacity that may be largely compensated in chronic infection	J Virol	84(22)	11937-11949	2010
Urano E, Ichikawa R, Morikawa Y, Yoshida T, Koyanagi Y, Komano J	T cell-based functional cDNA library screening identified SEC13-like 1a carboxy-terminal domain as a negative regulator of human immunodeficiency virus replication	Vaccine	28S	B68-B73	2010
Haraguchi H, Sudo S, Noda T, Momose F, Kawaoka Y, Morikawa Y	Intracellular localization of human immunodeficiency virus type 1 Gag and GagPol products and virus particle release: Relationship with the Gag-to-GagPol ratio	Microbiol Immunol	54(12)	734-746	2010
Yamamoto SP, Okawa K, Nakano T, Sano K, Ogawa K, Masuda T, Morikawa Y, Koyanagi Y, Suzuki Y	Huwe1, a novel cellular interactor of Gag-Pol through integrase binding, negatively influences HIV-1 infectivity	Mcirobes Infect		in press	
Terahara K, Nochi T, Yoshida M, Takahashi Y, Goto Y, Hatai H, Kurokawa S, Jang MH, Kweon M-N, Domino SE, Hiroi T, Yuki Y, Tsunetsugu-Yokota Y, Kobayashi K, Kiyono H	Distinct fucosylation of M cells and epithelial cells by Fut1 and Fut2, respectively, in response to intestinal environmental stress	Biochem Biophys Res Commun	404	822-828	2011

Onyango CO, Leligdowicz A, Yokoyama M, SatoH, Song, H, Nakayama EE, Shioda T, de Silva T, Townend J, Jaye A, Whittle H, Rowland-Jones S, Cotton M	HIV-2 Capsids Distinguish High and Low Virus Load Patients in a West African Community Cohort	Vaccine	28S	B60-B67	2010
Kono K, Song H, Yokoyama M, Sato H, Shioda T, Nakayama EE	Multiple sites in the N-terminal half of simian immunodeficiency virus capsid protein contribute to evasion from rhesus monkey TRIM5α-mediated restriction	Retrovirology	7	72	2010

Ⅳ.　研究成果の刊行物・別刷

Vaccine 28S (2010) B13–B17

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Neutralizing antibodies in SIV control: Co-impact with T cells

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ARTICLE INFO

Article history:
Received 15 May 2009
Received in revised form 19 August 2009
Accepted 18 September 2009

Keywords:
AIDS vaccine
Neutralizing antibody
Polyfunctional T-cell response
HIV-1
SIV

ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) and pathogenic simian immunodeficiency virus (SIV)-infected naïve hosts experience a characteristic absence of early and potent virus-specific neutralizing antibody (NAb) responses preceding establishment of persistent infection. Yet conversely, we have recently shown that NAbs passively immunized in rhesus macaques at early post-SIV challenge are capable of playing a critical role in non-sterile viremia control with implications of antibody-enhanced antigen presentation. In a current follow-up study we have further reported that NAbs mediate rapid elicitation of polyfunctional virus-specific CD4+ T-cells in vivo. The NAb-immunized macaques mounting these responses exhibited sustained viremia control for over 1 year, accompanied with robust anti-SIV cellular immunity. Perspectives obtained from the results are discussed.

1. Introduction: NAb absence in HIV-1/SIV acute infection

Absence of potent neutralizing antibody (NAb) responses in the very acute phase of human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV) infections is one major manifestation of defective adaptive immune responses in naïve hosts, generally failing in containment of virus replication unless privileged with certain genetic polymorphisms. HIV-1-specific NAb responses are unusually delayed in orders of months and hardly detected near peak infection. The primary humoral immune responses against these viruses are instead dominated by non-neutralizing virus-specific IgMs and IgGs [1] along with signs of aberrant polyclonal B-cell activation [2]. This initial failure is followed by a discordant array of NAbs appearing in the subacute to chronic phase, each reaching considerable titers yet being permissive of continuous neutralization escape by the autologous virus [3–6]. A preferential and possibly consequent exhaustion of HIV-1-specific B-cell responses has also been indicated in the chronic phase [7]. With these backgrounds a prophylactic induction of NAbs, particularly via pursuit of an optimal immunogen design eliciting a broadly neutralizing spectrum, has been a major aim in AIDS vaccine development [8].

Along with molecular analyses of NAbs and the HIV-1/SIV envelope proteins known for their skewed antigenicity, protective activities of monoclonal and polyclonal NAbs in vivo have been assessed by passive immunization mainly in nonhuman primates. To date, vaccine regimens inducing satisfactory NAb titers even against homologous challenge strains have not yet been developed. Passive immunization currently is a first choice surrogate for NAb analysis, but they do hold certain advantageous aspects, such as being suited for examining their impact within a certain time zone of interest. Initial studies showed that NAbs reaching a sufficient pre-challenge (or very early post-challenge) plasma or mucosal neutralizing titer typically render complete protection from chimeric simian/human immunodeficiency virus (SHIV) challenged via the same route [9–12], whereas titers to be attained for the viral inoculum sterilization had been a demanding one. On the other hand, it had been rather difficult to reach a consensus in determining whether NAbs can exhibit anti-HIV-1/SIV activity in established infections. This was partly because the rapid memory CD4+ T-cell destructive nature of CCR5-tropic HIV-1 and pathogenic SIV had been clarified only recently [13,14], which turned out to differentially validate the moments of NAb infusion in each study retrospectively. For example, NAbs passively administered in the chronic phase of HIV-1/SIV infection did not exert any impact on disease course even as a sequel to antiretroviral therapy in humans [15–16], while anti-SIV IG infusion at day 1 and day 14 post-SIVsmE660 challenge provided divergent viremia outcomes in infected macaques [17]. In HIV-1-inoculated human peripheral blood leukocyte-reconstituted SCID mice (hu-PBL-SCID mice), no suppressive effect was observed by NAb cocktail infusion past day 6 infection [18]. A common niche of these studies did however exist, which was the evaluation of the direct impact of NAbs on pre-peak viral replication and what we had designed to assess in our system.

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0264-410X/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2009.09.080

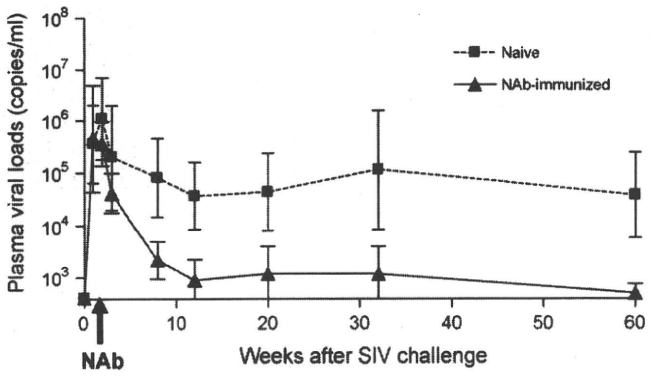

Fig. 1. Plasma viral loads in naïve controls and NAb-immunized macaques. Changes in geometric mean plasma viral loads in naïve controls (squares with dotted lines) and NAb-immunized macaques (triangles with bold lines) are shown. Error bars show the 95% confidence interval at each time point. The geometric mean plasma viral loads between weeks 12 and 60 were 5×10^4 copies/ml in naïve controls and $\leq 1 \times 10^3$ copies/ml NAb-immunized macaques.

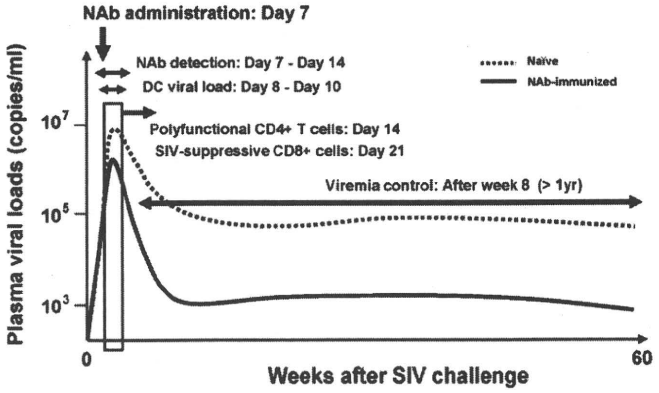

Fig. 2. Time-course events in NAb-mediated SIV control. The limited detection of plasma NAb titers between days 7 and 14 are concurrent with the rise in DC-viral loads (days 8–10), which is followed by appearance of Gag-specific CD4+ T cells with higher polyfunctionality at day 14 and CD8+ cells possessing higher anti-SIV efficacy at day 21 post-challenge. Plasma viral loads in the two groups start to show significant differences at week 8, and this is withheld up to week 60 post-challenge shown in Fig. 1. The box shows the critical time zone (weeks 1–2 post-challenge) to prime SIV-specific cellular immunity via NAb coexistence. Viral loads up to week 2 are drawn in a wider scale to ease visualization.

2. Non-sterile SIV control via NAbs

As an answer to this delineated question, we recently provided evidence for the clear potency of NAbs to control established immunodeficiency virus replication in vivo by performing a post-infection NAb passive immunization in SIVmac239-challenged rhesus macaques [19]. While most SIVmac239-infected naïve macaques usually fail to elicit NAb responses during the early phase of infection, some acquire detectable levels of NAbs against the challenge strain in the late phase. IgG purified from plasma of such SIVmac239-infected macaques with NAb induction, showing in vitro SIVmac239-specific neutralizing activity, was used for passive immunization as polyclonal anti-SIV NAbs. These NAbs were administered intravenously at day 7 post-SIVmac239 challenge, just before peak replication. The NAb passive immunization resulted in significant reduction of set-point viral loads (Fig. 1); this suppressive effect on viral replication became apparent (after week 5) past the limited duration (<1 week) of detectable NAb titers (Fig. 2). A notable observation in the NAb-immunized macaques was an accumulation of viral RNA in peripheral lymph node dendritic cells (DCs) within 24 h after the NAb infusion. Pulsing of DCs with NAb-bound SIV activated virus-specific CD4+ T cells in vitro with Fc-dependence, pointing out to a possibility of antibody-mediated virion uptake to DCs and facilitation of T-cell priming. This study thus unraveled that the existence of sub-sterile NAbs near peak infection can indeed render significant suppressive activity against establishment of immunodeficiency virus persistent infection.

In our second follow-up study [20], the functional phenotypes of virus-specific T-cell responses in NAb-immunized macaques and naïve controls were further evaluated. Peripheral blood mononuclear cells (PBMCs) from both groups were pulsed with recombinant SIV Gag proteins in vitro, and SIV Gag-specific CD4+ T cells were assessed of their polyfunctionality via measurement of antigen-specific interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), macrophage inflammatory protein-1β (MIP-1β), and CD107a expression. The frequencies of polyfunctional Gag-specific CD4+ T cells, defined here as the upregulation of ≥3 of these five markers, were significantly elevated in the NAb-immunized macaques at day 14 post-challenge compared with naïve controls. Frequencies of these polyfunctional Gag-specific CD4+ T cells showed an inverse correlation with plasma viral loads at week 5, implying that early induction of these effectors was involved in the subsequent reduction of viremia.

In the chronic phase of infection (around week 30), Gag-specific CD4+ T-cell responses were detected in the NAb-immunized animals with higher polyfunctionality. These cells also showed their enhanced proliferative capacity as determined by SIV Gag-specific BrdU uptake. Accompanying these Gag-specific CD4+ T-cell responses, viral replication in the chronic phase remained significantly contained in the NAb-immunized macaques (Table 1 and Fig. 1). Three out of five animals exhibited undetectable plasma

Table 1
Summary of the passive NAb immunization experiment.

Animal	MHC-I haplotype	Ab-Tx at week 1 post-challenge[a]	Set-point VL (copies/ml)
Naïve controls			
R01-011	*90-010-Ie*	–	2×10^4 to 2×10^5
R01-012	*90-010-Id*	–	2×10^4 to 2×10^5
R03-005	*90-030-Ig*	–	1×10^3 to 2×10^4
R02-004	*90-088-Ij*	–	5×10^4 to 5×10^5
R02-021	ND[b]	Control Ab	3×10^5 to 6×10^6
R06-038	*90-010-Ie*	Control Ab	1×10^4 to 2×10^5
NAb-immunized			
R03-011	*90-010-Ie*	Anti-SIV NAb	$<4 \times 10^2$
R06-023	*90-010-Id*	Anti-SIV NAb	$<4 \times 10^2$
R03-020	ND	Anti-SIV NAb	1×10^3 to 2×10^4
R02-020	ND	Anti-SIV NAb	1×10^3 to 2×10^4
R03-013	*90-030-Ih*	Anti-SIV NAb	$<2 \times 10^3$

[a] Macaques received no immunization (–) or passive immunization with control Abs or anti-SIVmac239 NAbs intravenously at week 1 post-SIVmac239 challenge.
[b] Not determined.

viral loads up to 60 weeks post-challenge, while the other two also maintained them at low levels lacking any palpable sign of control failure. De novo NAbs were not detected in the NAb-immunized group, together suggesting that a single administration of NAbs in acute SIV infection can result in long-term viremia control with appearance of robust virus-specific CD4+ T-cell responses.

Gag-specific CD8+ T-cell frequencies at day 14 post-challenge were simultaneously assessed in both groups, which revealed no significant differences in polyfunctionality between the two. The caveat here may have been the stimulation protocol, relying on cross-presentation of the pulsed recombinant SIV Gag protein which left room for a possibility of suboptimal virus-specific CTL examination. Therefore we alternatively attempted to assess their direct anti-SIV efficacy by performing an in vitro viral suppression assay (VSA). In this assay, CD8+ effector cells positively selected from PBMCs at week 3 post-infection were cocultured with autologous CD8-negative target cells pre-infected with SIV and peak virus production in the culture supernatant was measured [20]. CD8+ cells from three out of four examined NAb-immunized macaques showed nearly complete suppression of progeny virus production, a phenomenon not observed in any of the examined naïve controls. Notably, none of the NAb-immunized macaques possessed an MHC class I (MHC-I) haplotype *90-120-Ia* which has been previously shown to mount potent anti-SIV CTL responses. These data suggest that the NAb administration may help de novo induction of CD8+ cells exerting enhanced anti-SIV efficacy in vivo.

3. Significance of T-cell induction in NAb-mediated SIV control

Collectively these findings have depicted a previously undocumented pattern of immunodeficiency virus control, in that the early existence of NAbs preceding peak SIV replication was followed by de novo appearance of polyfunctional Gag-specific CD4+ T cells (at week 2) and subsequent robust viremia control (after week 5) (Fig. 2). While the direct virus-neutralizing activity of the antibodies, as well as other effector functions such as antibody-dependent cell-mediated virus inhibition (ADCVI) [21,22] and/or complement activation [23] may have additively provided the protection of induced virus-specific CD4+ T cells from events such as DC trans-infection [24], the above sequence also coheres with our proposed possibility of NAb-mediated antigen presentation [19,25]. Antibody binding to soluble antigens is known to facilitate Fc-mediated uptake and resultant MHC class II antigen presentation in DCs [26,27], which renders the appearance of virus-specific polyfunctional CD4+ T cells in NAb-immunized macaques likely being a result of direct induction via NAb-mediated virion uptake into DCs.

An enigmatic role for HIV-1/SIV-specific CD4+ T cells has overall been posed in this regard of their potential vulnerability. While having been found of their presence as an inverse correlate of chronic phase viremia in HIV-1-infected humans [28], memory CD4+ T-cell subsets themselves (which likely include the virus-specific effectors) have been later determined as the primary target of CCR5-tropic primary AIDS-virus infections [15,29–32]. Detectable HIV-1-specific effector CD4+ T-cell responses in humans show an agreeable decline as viremia progresses [33], which may be driven by selective infection to some extent [34]. These are also in agreement with one study which documents prophylactic induction of Env-specific CD4+ T cells exerting a detrimental influence on the otherwise self-remitting course of SIVsmE660 viremia within that system [35]. In CTL-based prophylactic vaccines, acute and long-term preservation of (total and central) memory CD4+ T-cell counts has accordingly been defined as a passive correlate of protection status [36–38]. Hence the entity of truly contributable HIV-1/SIV-specific CD4+ T-cell responses has overall not been clarified, although implications of their enrollment exist such as in relatively benign HIV-2 infection [39]. The current study newly supports two possibilities. One is that de novo polyfunctional Gag-specific effector CD4+ T cells, if induced, may be potent of actively driving primary viremia control; in other words, their existence can be taken as a cause rather than a result of protection. The other is that the antigen-specific activation potential of central memory CD4+ T cells is reinforced as a detailed protective correlate during chronic infection as in humans [28], in addition to the quantitative preservation of the subset. These two hallmarks in NAb-mediated SIV control sharply contrast Gag-specific CD4+ T-cell responses in naïve controls which, importantly, exhibit neither of the two.

It is important to question how these Gag-specific polyfunctional CD4+ T cells had exerted anti-SIV activity. Possibilities include roles such as conventional helper cells providing cognate assistance in priming CTLs for viremia clearance [40], effectors directly suppressing infected cells with [41,42] or without cytolytic activity, or both. Results of the CD8+ cell VSA in NAb-immunized macaques do not contradict participation of SIV-specific CTLs in acute phase viremia control, partially supporting the first candidate. When we performed VSA with CD8+ cells from the chronic phase (around week 20 post-challenge) in NAb-immunized macaques, no comparable in vitro virus suppression had been observed (unpublished results). This may also concur with the contracting levels of SIV-specific CTLs at low viral replication levels in vivo. Augmented virus-specific CTLs being a major, though maybe not the only, determinant of viremia suppression in the acute phase may be a reasonable explanation; if so, how their commitment may differ from viremia control in vaccinated and naïve SIV elite controllers (ECs) [43,44], or HIV-1 ECs in humans [45], is still unknown. Another factor to consider may be the Gag-specificity of these CD4+ T cells. As in CTLs, where importance of their Gag-biased induction in HIV-1/SIV viremia control has been emphasized both in vitro and in vivo [46,47], inducing CD4+ T-cell responses with a preference for Gag epitopes may also be advantageous in HIV-1/SIV control.

Regardless of the precise mechanism, there certainly lies a limitation to directly extrapolating results of this post-infection NAb passive immunization study to the patterns of protection likely afforded by endogenous NAb-inducing regimens. Nevertheless it is still important to recognize that the concordance of primary SIV control with de novo induction of polyfunctional Gag-specific CD4+ T cells, near the normal peak of systemic memory CD4+ T-cell destruction [14], does strengthen the rationale for prophylactic NAb induction. Even if induced NAbs do not prevent the initial establishment of HIV-1/SIV infection, they may well exhibit their potential against systemic infection by a non-sterile protective mechanism. This narrow 1-week window around peak plasma viremia (Box in Fig. 2) is indeed some watershed for the HIV-1/SIV-infected host to impact viremia thereafter.

4. Perspectives: humoral and cellular immune concert for HIV-1 control

Whether or how cellular and humoral immune responses may collectively exert their effect against CCR5-tropic HIV-1/SIV acute infections had been unexplored, while systems involving other retroviruses have given some implications for this question. In Friend murine leukemia virus infection, high doses of administered pre-exposure NAbs have provided an augmentation to memory T-cell-based control [48]. A similar augmentation does not, however, appear to happen in CXCR4-tropic SHIV89.6P infection [49]. Turning to a self-remitting benign SHIV infection (SHIV DH12R clone 8), the negative effect of CD20-depletion had only become apparent in macaques lacking a highly protective MHC-I allele

(*Mamu*-A*01), suggesting a seemingly interdependent rather than synergistic cooperation [50].

It is noteworthy that CD20+ B-cell depletion in the SIV/SHIV-macaque model is an interesting but intrinsically sensitive approach. What is problematic is that the outcome is complicated in a virus strain-dependent manner. In a pathogenic virus challenge (e.g., SIVmac) model the set-point viremia is significantly high. This seemingly blunts the effect of transient B-cell depletion from an incompetent baseline [51], while an impact on disease course appears to be partially observed in the subacute phase by constitutive B-cell depletion [52]. On the contrary, in models where relatively benign SIV (or SHIV) strains are challenged, host CTL responses reaching a certain threshold of potency may readily control viremia by themselves. The need of humoral immunity assistance is sufficiently compensated here, which again dampens the impact of B-cell depletion [53]. The propensity in such models seems to be that B-cell depletion effects only become appreciable in non-elite cellular immunity-eliciting controllers [50,54]. Since no detectable NAbs are elicited in acute to subacute SIV infection, it likely follows that the cases of accelerated pathology via B-cell depletion may derive from the deprival of non-NAb-mediated effects, such as ADCVI. Taken together, the endogenous existence of antiviral non-neutralizing antibodies may change the outcome from rapid progression to persistent infection, whereas the exogenous administration of NAbs may change the outcome from persistent infection to relative viremia control.

The entity of humoral immunity modulation against cellular immunity in HIV-1/SIV infection is hence still unclear; nevertheless one thing is becoming evident which is that, at least, NAbs and T cells do not diminish each other in mere competitive coexistence for target elimination. Especially near peak SIV replication, NAb-dependent modification of T-cell immunity does exert a significant impact on viremia control.

In conclusion, there appears to be a preference of a balance, perhaps a temporal one, between induction of HIV-1/SIV-specific CD8+, CD4+ T cells and NAbs. Determining the requisites for NAb-triggered T-cell immunity-based SIV replication control shall further reveal rational endpoints for prophylactic HIV-1 vaccines.

Conflict of interest statement

The authors state that they have no conflict of interest.

References

[1] Tomaras GD, Yates NL, Liu P, Qin L, Fouda GG, Chavez LL, et al. Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. J Virol 2008;82(24):12449–63.

[2] De Milito A, Nilsson A, Titanji K, Thorstensson R, Reizenstein E, Narita M, et al. Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection. Blood 2004;103(6):2180–6.

[3] Kwong PD, Doyle ML, Casper DJ, Cicala C, Leavitt SA, Majeed S, et al. HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature 2002;420(6916):678–82.

[4] Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc Natl Acad Sci USA 2003;100(7):4144–9.

[5] Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. Nature 2003;422(6929):307–12.

[6] Gray ES, Moore PL, Choge IA, Decker JM, Bibollet-Ruche F, Li H, et al. Neutralizing antibody responses in acute human immunodeficiency virus type 1 subtype C infection. J Virol 2007;81(12):6187–96.

[7] Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med 2008;205(8):1797–805.

[8] Burton DR, Desrosiers RC, Doms RW, Koff WC, Kwong PD, Moore JP, et al. HIV vaccine design and the neutralizing antibody problem. Nat Immunol 2004;5(3):233–6.

[9] Shibata R, Igarashi T, Haigwood N, Buckler-White A, Ogert R, Ross W, et al. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. Nat Med 1999;5(2):204–10.

[10] Mascola JR, Lewis MG, Stiegler G, Harris D, VanCott TC, Hayes D, et al. Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. J Virol 1999;73(5):4009–18.

[11] Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, Hanson CE, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 2000;6(2):207–10.

[12] Nishimura Y, Igarashi T, Haigwood NL, Sadjadpour R, Donau OK, Buckler C, et al. Transfer of neutralizing IgG to macaques 6 h but not 24 h after SHIV infection confers sterilizing protection: implications for HIV-1 vaccine development. Proc Natl Acad Sci USA 2003;100(25):15131–6.

[13] Nishimura Y, Igarashi T, Donau OK, Buckler-White A, Buckler C, Lafont BA, et al. Highly pathogenic SHIVs and SIVs target different CD4+ T cell subsets in rhesus monkeys, explaining their divergent clinical courses. Proc Natl Acad Sci USA 2004;101(33):12324–9.

[14] Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature 2005;434(7037):1093–7.

[15] Trkola A, Kuster H, Rusert P, Joos B, Fischer M, Leemann C, et al. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat Med 2005;11(6):615–22.

[16] Binley JM, Clas B, Gettie A, Vesanen M, Montefiori DC, Sawyer L, et al. Passive infusion of immune serum into simian immunodeficiency virus-infected rhesus macaques undergoing a rapid disease course has minimal effect on plasma viremia. Virology 2000;270(1):237–49.

[17] Haigwood NL, Watson A, Sutton WF, McClure J, Lewis A, Ranchalis J, et al. Passive immune globulin therapy in the SIV/macaque model: early intervention can alter disease profile. Immunol Lett 1996;51(1–2):107–14.

[18] Poignard P, Sabbe R, Picchio GR, Wang M, Gulizia RJ, Katinger H, et al. Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo. Immunity 1999;10(4):431–8.

[19] Yamamoto H, Kawada M, Takeda A, Igarashi H, Matano T. Post-infection immunodeficiency virus control by neutralizing antibodies. PLoS ONE 2007;2(6):e540.

[20] Yamamoto T, Iwamoto N, Yamamoto H, Tsukamoto T, Kuwano T, Takeda A, et al. Polyfunctional CD4+ T-Cell induction in neutralizing antibody-triggered control of simian immunodeficiency virus infection. J Virol 2009;83(11):5514–24.

[21] Forthal DN, Landucci G, Daar ES. Antibody from patients with acute human immunodeficiency virus (HIV) infection inhibits primary strains of HIV type 1 in the presence of natural-killer effector cells. J Virol 2001;75(15):6953–61.

[22] Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, et al. Fc receptor but not complement binding is important in antibody protection against HIV. Nature 2007;449(7158):101–4.

[23] Huber M, Fischer M, Misselwitz B, Manrique A, Kuster H, Niederöst B, et al. Complement lysis activity in autologous plasma is associated with lower viral loads during the acute phase of HIV-1 infection. PLoS Med 2006;3(11):e441.

[24] Frankel SS, Steinman RM, Michael NL, Kim SR, Bhardwaj N, Pope M, et al. Neutralizing monoclonal antibodies block human immunodeficiency virus type 1 infection of dendritic cells and transmission to T cells. J Virol 1998;72(12):9788–94.

[25] Yamamoto H, Matano T. Anti-HIV adaptive immunity: determinants for viral persistence. Rev Med Virol 2008;18(5):293–303.

[26] Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 1994;179(4):1109–18.

[27] Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. Nature 1997;388(6644):782–7.

[28] Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science 1997;278(5342):1447–50.

[29] Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, et al. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science 1998;280(5362):427–31.

[30] Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 2004;200(6):749–59.

[31] Picker LJ, Hagen SI, Lum R, Reed-Inderbitzin EF, Daly LM, Sylwester AW, et al. Insufficient production and tissue delivery of CD4+ memory T cells in rapidly progressive simian immunodeficiency virus infection. J Exp Med 2004;200(10):1299–314.

[32] Li Q, Duan L, Estes JD, Ma ZM, Rourke T, Wang Y, et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature 2005;434(7037):1148–52.

[33] Zaunders JJ, Munier ML, Kaufmann DE, Ip S, Grey P, Smith D, et al. Early proliferation of CCR5(+) CD38(+++) antigen-specific CD4(+) Th1 effector cells during primary HIV-1 infection. Blood 2005;106(5):1660–7.

[34] Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nature 2002;417(6884):95–8.
[35] Staprans SI, Barry AP, Silvestri G, Safrit JT, Kozyr N, Sumpter B, et al. Enhanced SIV replication and accelerated progression to AIDS in macaques primed to mount a CD4 T cell response to the SIV envelope protein. Proc Natl Acad Sci USA 2004;101(35):13026–31.
[36] Letvin NL, Mascola JR, Sun Y, Gorgone DA, Buzby AP, Xu L, et al. Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys. Science 2006;312(5779):1530–3.
[37] Mattapallil JJ, Douek DC, Buckler-White A, Montefiori D, Letvin NL, Nabel GJ, et al. Vaccination preserves CD4 memory T cells during acute simian immunodeficiency virus challenge. J Exp Med 2006;203(6):1533–41.
[38] Wilson NA, Reed J, Napoe GS, Piaskowski S, Szymanski A, Furlott J, et al. Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. J Virol 2006;80(12):5875–85.
[39] Duvall MG, Jaye A, Dong T, Brenchley JM, Alabi AS, Jeffries DJ, et al. Maintenance of HIV-specific CD4+ T cell help distinguishes HIV-2 from HIV-1 infection. J Immunol 2006;176(11):6973–81.
[40] Castellino F, Germain RN. Cooperation between CD4+ and CD8+ T cells: when, where, and how. Annu Rev Immunol 2006;24:519–40.
[41] Gauduin MC, Yu Y, Barabasz A, Carville A, Piatak M, Lifson JD, et al. Induction of a virus-specific effector-memory CD4+ T cell response by attenuated SIV infection. J Exp Med 2006;203(12):2661–72.
[42] Casazza JP, Betts MR, Price DA, Precopio ML, Ruff LE, Brenchley JM, et al. Acquisition of direct antiviral effector functions by CMV-specific CD4+ T lymphocytes with cellular maturation. J Exp Med 2006;203(13):2865–77.
[43] Matano T, Kobayashi M, Igarashi H, Takeda A, Nakamura H, Kano M, et al. T lymphocyte-based control of simian immunodeficiency virus replication in a preclinical AIDS vaccine trial. J Exp Med 2004;199(12):1709–18.
[44] Friedrich TC, Valentine LE, Yant LJ, Rakasz EG, Piaskowski SM, Furlott JR, et al. Subdominant CD8+ T-cell responses are involved in durable control of AIDS virus replication. J Virol 2007;81(7):3465–76.
[45] Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 2006;107(12):4781–9.
[46] Sacha JB, Chung C, Rakasz EG, Spencer SP, Jonas AK, Bean AT, et al. Gag-specific CD8+ T lymphocytes recognize infected cells before AIDS-virus integration and viral protein expression. J Immunol 2007;178(5):2746–54.
[47] Kiepiela P, Ngumbela K, Thobakgale C, Ramduth D, Honeyborne I, Moodley E, et al. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med 2007;13(1):46–53.
[48] Messer RJ, Dittmer U, Peterson KE, Hasenkrug KJ. Essential role for virus-neutralizing antibodies in sterilizing immunity against Friend retrovirus infection. Proc Natl Acad Sci USA 2004;101(33):12260–5.
[49] Mascola JR, Lewis MG, VanCott TC, Stiegler G, Katinger H, Seaman M, et al. Cellular immunity elicited by human immunodeficiency virus type 1/simian immunodeficiency virus DNA vaccination does not augment the sterile protection afforded by passive infusion of neutralizing antibodies. J Virol 2003;77(19):10348–56.
[50] Mao H, Lafont BA, Igarashi T, Nishimura Y, Brown C, Hirsch V, et al. CD8+ and CD20+ lymphocytes cooperate to control acute simian immunodeficiency virus/human immunodeficiency virus chimeric virus infections in rhesus monkeys: modulation by major histocompatibility complex genotype. J Virol 2005;79(23):14887–98.
[51] Schmitz JE, Kuroda MJ, Santra S, Simon MA, Lifton MA, Lin W, et al. Effect of humoral immune responses on controlling viremia during primary infection of rhesus monkeys with simian immunodeficiency virus. J Virol 2003;77(3):2165–73.
[52] Miller CJ, Genescà M, Abel K, Montefiori D, Forthal D, Bost K, et al. Antiviral antibodies are necessary for control of simian immunodeficiency virus replication. J Virol 2007;81(10):5024–35.
[53] Gaufin T, Gautam R, Kasheta M, Ribeiro R, Ribka E, Barnes M, et al. Limited ability of humoral immune responses in control of viremia during infection with SIVsmmD215 strain. Blood 2009;113(18):4250–61.
[54] Johnson WE, Lifson JD, Lang SM, Johnson RP, Desrosiers RC. Importance of B-cell responses for immunological control of variant strains of simian immunodeficiency virus. J Virol 2003;77(1):375–81.

Immunogenetics (2010) 62:601–611
DOI 10.1007/s00251-010-0462-z

ORIGINAL PAPER

Diversity of MHC class I genes in Burmese-origin rhesus macaques

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Received: 2 April 2010 / Accepted: 24 June 2010 / Published online: 17 July 2010

Abstract Rhesus macaques (*Macaca mulatta*) are widely used in developing a strategy for vaccination against human immunodeficiency virus by using simian immunodeficiency virus infection as a model system. Because the genome diversity of major histocompatibility complex (MHC) is well known to control the immune responsiveness to foreign antigens, MHC loci in Indian- and Chinese-origin macaques used in the experiments have been characterized, and it was revealed that the diversity of MHC in macaques was larger than the human MHC. To further characterize the diversity of *Mamu-A* and *Mamu-B* loci, we investigated a total of 73 different sequences of *Mamu-A*, 83 sequences of *Mamu-B*, and 15 sequences of *Mamu-I* cDNAs isolated from Burmese-origin macaques. It was found that there were one to five expressing genes in each locus. Among the *Mamu-A*, *Mamu-B*, and *Mamu-I* sequences, 44 (60.2%), 45 (54.2%), and 8 (53.3%), respectively, were novel, and most of the other known alleles were identical to those reported from Chinese- or Indian-origin macaques, demonstrating a genetic mixture between the geographically distinct populations of present day China and India. In addition, it was found that a *Mamu* haplotype contained at least two highly transcribed *Mamu-A* genes, because multiple *Mamu-A1* cDNAs were obtained from one haplotype. These findings further revealed the diversity and complexity of MHC locus in the rhesus macaques.

Keywords Rhesus macaque · MHC · *Mamu-A* · *Mamu-B* · *Mamu-I* · Haplotype

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Introduction

The rhesus macaque (*Macaca mulatta*) is a member of the old world monkey. It is estimated that the ancestor of macaques was diverged from the human-chimpanzee ancestor approximately 25 million years ago (Stewart and Disotell 1998). The habitat of the rhesus macaque extends from Pakistan and India to the southern part of China

(Timmins et al. 2008), wider than that of the other nonhuman primates. It is known that the genome diversity in rhesus macaques is quite unique, because more than 60% of the rhesus macaque-specific expansions are found in the protein coding sequences (Gibbs et al. 2007). The increase in the gene copy number in the rhesus macaque, relative to that in humans, can also be observed in the major histocompatibility complex (MHC) locus (Gibbs et al. 2007).

The rhesus macaque is widely used as a nonhuman primate species model in biomedical researches for human diseases including acquired immunodeficiency syndrome (AIDS). Particularly, the development of vaccines against the human immunodeficiency virus (HIV) in part depends on the results of experiments using macaques, because the simian immunodeficiency virus (SIV) infection causes AIDS-like syndrome (Barouch et al. 2000; Schmitz et al. 1999; Yasutomi et al. 1993). Previous SIV challenge studies indicated association of MHC class I genotypes with rapid or delayed AIDS progression in rhesus macaques like HIV-1 infection in humans (Mothe et al. 2003; Yant et al. 2006; Loffredo et al. 2008; Reynolds et al. 2008).In addition, effective vaccination was associated with specific MHC class I alleles called as "elite controller" alleles, by which prevention of viral replication could be achieved in macaques challenged by SIVmac239 (Loffredo et al. 2007; Maness et al. 2008). In these experiments, macaques of Indian or Chinese origin have been widely used, and macaques from different regions such as Burma have also been used recently.

To evaluate the efficacy of SIV vaccination, it is necessary to characterize the MHC alleles because the presentation of antigenic peptides by MHC molecules to T cells, more specifically the binding of antigenic peptide to the MHC molecule, depends on the structure of the MHC allele. We have previously developed a reference strand conformation analysis-based typing system for *Mamu* class I genes and reported that the number of expressing genes varies among macaques of Burmese or Laotian origin; we could identify at least 16 different *Mamu* class I locus haplotypes that were composed of different numbers of *Mamu* class I genes (Tanaka-Takahashi et al. 2007). In addition, we reported that a haplotype of *Mamu* class I genes, *90-120-Ia*, exerted a protective vaccination against SIVmac239 challenge (Matano et al. 2004). Furthermore, it was revealed that one of highly expressed *Mamu-A* allele of the *90-120-Ia* haplotype, *Mamu-A1*065:01* (previously designated as *Mamu-A*90120-5*), encoded a Mamu-A molecule that could efficiently present a SIV-derived $Gag_{241-249}$ peptide to cytotoxic T cells from the vaccinated macaques (Tsukamoto et al. 2008).

The aim of present study was to define the allelic polymorphisms and haplotype diversity of the *Mamu* class I gene from Burmese-origin macaques.

Materials and methods

Animals

A total of 100 rhesus macaques from breeding colonies maintained in Japan were enrolled. Founders of colonies were captured in Myanmar or Laos, and the colonies were separately maintained. Macaque colonies were classified into seven groups based on their paternal lineages (90-120, 90-010, 90-030, 90-088, 89-002, 89-075, and 91-010F1) (Tanaka-Takahashi et al. 2007). The animal 91-010F1 was an offspring of 89-075.

Sequencing analysis of cDNAs from *Mamu* class I genes

Total cellular RNA was extracted from B lymphoblastoid cell lines established from the macaques by using RNAiso reagent (TaKaRa, Shiga, Japan). Oligo (dT)-primed cDNA was synthesized using Transcriptor reverse high fidelity transcriptase (Roche, Mannheim, Germany) according to the manufacturer's recommendations. Full-length cDNAs for *Mamu* class I genes were amplified by polymerase chain reaction (PCR) using locus-specific primer pairs, as described previously (Tanaka-Takahashi et al. 2007), with a modification of primer pairs to those reported by Karl et al. (Karl et al. 2008): 5'MHC_UTR (5'-GGACTCAGAAT CTCCCCAGACGCCGAG) and 3'MHC_UTR_A (5'-CAG GAACAYAGACACATTCAGG) for *Mamu-A* locus and 5'MHC_UTR and 3'MHC_UTR_B (5'-GTCTCTCCAC CTCCTCAC) for *Mamu-B*, *-I* loci, using Phusion Flash DNA polymerase (Finzymes, Espoo, Finland). The PCR

Table 1 *Mamu* class I alleles found in Burmese-origin macaques

Loci	Number of analyzed macaques	Number of observed alleles	Novel alleles (number, %)		Known alleles (number, %)	
Mamu-A	100	73	44	60.2	29	39.8
Mamu-B	93	83	45	54.2	38	45.8
Mamu-I	93	15	8	53.3	7	46.7
Others (AG, F)	93	2	0	-	2	100
Total		173	97	56.1	76	43.9

Table 2 Alleles of *Mamu-A* locus identified in Burmese-origin macaques

Locus	Allele name	Novelty[a]	Accession Number[b]	Shared allele[c]	Number of animals	Identity to *Mafa* or *Mane* alleles[d]
A1	A1*003:01:03	Novel	AB496714		1	
A1	A1*003:08		AB444903	C	7	
A1	A1*003:10	Novel	AB444904		1	
A1	A1*004:01:02		AB444866	C	19	*Mafa-A1*004:02*
A1	A1*007:06:01	Novel	AB540211		2	
A1	A1*008:01:02	Novel	AB430443		11	
A1	A1*008:01:03	Novel	AB496711		1	
A1	A1*008:02	Novel	AB477383		2	
A1	A1*015:01		AB551785		2	
A1	A1*018:05		AB444927	I	1	
A1	A1*018:07	Novel	AB444928		11	
A1	A1*018:08	Novel	AB444926		6	
A1	A1*019:02		AB444900	C	2	
A1	A1*019:05		AB444901	C	1	
A1	A1*019:07	Novel	AB444899		2	
A1	A1*022:01		AB444895	C	1	
A1	A1*022:03	Novel	AB444894		7	
A1	A1*023:02	Novel	AB444874		4	
A1	A1*026:03		AB477385	C	1	
A1	A1*028:06	Novel	AB444924		1	
A1	A1*028:07:01	Novel	AB444923		3	
A1	A1*032:02	Novel	AB444933		13	
A1	A1*032:03	Novel	AB444934		4	
A1	A1*040:01		(AM295910)		1	
A1	A1*041:01		AB444931	C	1	
A1	A1*041:02		(EU429608)	C	1	
A1	A1*042:01	Novel	AB444868	C	2	
A1	A1*043:01		AB444869	C	7	
A1	A1*049:03		AB444880	C	2	
A1	A1*049:04	Novel	AB444881		2	
A1	A1*050:01		AB444889	C	7	
A1	A1*052:01		AB444890	C	3	*Mafa-A1*052:02*
A1	A1*056:02		AB477384	C	6	
A1	A1*056:02:02	Novel	AB444935		3	
A1	A1*065:01		AB444921	C	6	*Mafa-A1*065:04*
A1	A1*066:01	Novel	AB444888		14	
A1	A1*074:04:01	Novel	AB540213		1	
A1	A1*105:01	Novel	AB444898		1	
A1	A1*105:02	Novel	AB444896		11	
A1	A1*105:03	Novel	AB496716		2	
A1	A1*105:04	Novel	AB496709		1	
A1	A1*106:01	Novel	AB444875		1	
A1	A1*107:01	Novel	AB444887		9	*Mafa-A1*096:01*
A1	A1*108:01	Novel	AB444925		1	
A1	A1*109:01	Novel	AB444902		7	*Mafa-A1*097:01*
A1	A1*110:01	Novel	AB444884		4	
A1	A1*111:01	Novel	AB444886		1	
A1	A1*112:01	Novel	AB496717		1	
A1	A1*117:01:01	Novel	AB540212		2	

Table 2 (continued)

Locus	Allele name	Novelty[a]	Accession Number[b]	Shared allele[c]	Number of animals	Identity to *Mafa* or *Mane* alleles[d]
A1	A1*118:01:01	Novel	AB540214		1	
A2	A2*01:03	Novel	AB444917		15	
A2	A2*05:03:02		AB444910	C	2	
A2	A2*05:10		AB444907	I	2	
A2	A2*05:11		AB444909	I	7	
A2	A2*05:13		(AM295927)	C	1	
A2	A2*05:14		(AM295928)	C	1	
A2	A2*05:15:04	Novel	AB444914		3	
A2	A2*05:22		AB444911	C	1	*Mane-A2*05:18*
A2	A2*05:26		AB496715	C	2	
A2	A2*05:31	Novel	AB444908		2	
A2	A2*05:32:02	Novel	AB444920		2	
A2	A2*05:44	Novel	AB444912		1	
A2	A2*05:45	Novel	AB444915		2	
A2	A2*05:46	Novel	AB444913		4	*Mane-A2*05:03:01*
A3	A3*13:13	Novel	AB496712		4	
A4	A4*01:02:02	Novel	AB444879		3	
A4	A4*14:03		AB444876	C, I	15	
A4	A4*14:04		AB444878	C	1	
A5	A5*30:01:01		(AM295945)	C	1	
A5	A5*30:01:02		AB444882	C	1	
A5	A5*30:06	Novel	AB444883		2	
A6	A6*01:01		AB444938	C	1	
A6	A6*01:05	Novel	AB444937		4	

[a] New alleles are indicated as novel

[b] Nucleotide sequences were submitted to public database and can be obtained with the indicated accession number. The accession numbers in the parentheses indicated that the Mamu class I sequences were identical to those numbers which had been deposited previously by other investigators.

[c] Alleles found in Burmese-origin macaques were shared with macaques originated from the other region. *C* Chinese-origin macaques, *I* Indian-origin macaques

[d] Identical sequences found in *Mafa* or *Mane* alleles

program was composed of the following steps: denaturation at 98°C for 10 s; 25 cycles at 98°C for 1 s, 63°C for 5 s, 72°C for 20 s; and additional extension at 72°C for 1 min. The PCR products were cloned into pSTBlue-1 Perfectly Blunt vector (Novagen, WI, USA) according to the manufacturer's instructions. Both strands from 30 to 90 independent cDNA clones obtained from each macaque for each locus were sequenced by BigDye Terminator cycling system and analyzed in an ABI 3730 automated sequence analyzer (Applied Biosystems, CA, USA).

Data analyses and nomenclature for *Mamu* class I alleles

Nucleotide sequences of cDNAs were analyzed and aligned using Genetyx Ver. 8 software package (Genetyx Corp., Japan). When at least three clones from independent PCR or from different individuals showed identical sequences, we submitted the sequences to DNA Data Bank of Japan database and to the Immuno Polymorphism Database for nonhuman primate MHC (http://www.ebi.ac.uk/ipd/mhc/submit.html; Robinson et al. 2003) to obtain official nomenclature for novel alleles of *Mamu-A* and *-B* genes. Phylogenetic analysis of *Mamu-A* sequences corresponding to exon 2, 3 and a part of exon 4 obtained in this study was done by using Gentetyx Ver. 8 software package. *Mamu-A1* 001:01* was included in the analysis as a reference. Neighbor-joining trees were constructed with the Kimura 2 parameter method. Bootstrap values were based on 5,000 replications.

Results

Identification of *Mamu* class I alleles in Burmese-origin macaques

We analyzed cDNA clones obtained by RT-PCR for *Mamu-A* locus and *Mamu-B* locus (Table 1). When at least three