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サル免疫不全ウイルス中和抗体の感染個体レベルにおける防御機序の解析

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研究代表者　　山本　浩之

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サル免疫不全ウイルス中和抗体の感染個体レベルにおける防御機序の解析

研究代表者　山本　浩之　　国立感染症研究所　研究員

研究要旨

エイズウイルス（ヒト免疫不全ウイルス、HIV）感染症の最大の問題は、自然感染経過において T 細胞応答不全と中和抗体（NAb）反応の欠失を伴い慢性持続感染化する事であるが、その成立過程は明らかではない。これに対し筆者は近年、SIV（サル免疫不全ウイルス）感染サルエイズモデルにおいて感染急性期の NAb 受動免疫による特異的 T 細胞応答亢進を伴っ持続感染阻止効果を証明し、機序として樹状細胞（DC）への Fc 依存性 NAb-ウイルス粒子複合体取込み促進による抗原提示亢進が関わりうる可能性を見出した。本研究では、NAb 受動免疫を端緒とした、液性・細胞性免疫による相乗的なエイズウイルス防御機構の更なる機構の解析を行った。まず、NAb 抗原提示能への DC 受容体 CD64 の関与を見出し、NAb 存在下での CD8 陽性 T 細胞の特異的 CCL4 産生亢進を見出した。さらに抗体による cross-priming が成立するという知見に基づき受動免疫抗体の中和能の必要性の検証を最重要視し、非・中和抗体（nNAb）を大量精製後 ELISA 法でスクリーニングし、背景研究で使用した NAb と同じ粒子結合能を有する nNAb を選抜し、SIV 感染急性期に受動免疫する実験を行った。病態進行と宿主免疫応答を解析した結果、nNAb は試験管レベルで十分なウイルス複製抑制能を付与するにも関わらず、個体レベルでは感染成立後の受動免疫による持続感染阻止能を呈し得ないことを見出した。本研究により、SIV 感染初期の抗体受動免疫による non-sterile なウイルス複製制御時には直接的なウイルス中和能が必須であることが逆説的に初めて明らかとなり、これは中和抗体誘導型予防エイズワクチン開発への基礎情報となる重要な知見である。

A.　研究目的

エイズウイルス（ヒト免疫不全ウイルス、HIV）の感染個体レベルにおける防御に極めて重要であることを筆者が近年見出したウイルス中和抗体につき、その作用機序の詳細をサルモデルを用い網羅的に明らかとし、予防エイズワクチン開発への論理的基盤を見出すことを本研究は目標とした。

エイズウイルス感染症における、中和抗体を代表とする液性免疫応答の位置付けは近年に至るまで明らかとなっていなかった。これに対し研究代表者は SIV 感染サルモデルを用い、感染初期の中和抗体受動免疫により特異的 T 細胞の誘導亢進が生じ、持続感染成立阻止効果が著明に呈される事を初めて証明した（Yamamoto H, PLoS ONE 2007）。

本研究はその結果を踏まえ、中和抗体の個体レベル防御機構において、抗原提示亢進パターンの特徴、及び誘導される抗 SIV 細胞性免疫の保護がどの程度関わるかを中心的に評価した。

そのために、次の 2 段階で研究を進めた。

1. 最初に、中和抗体を介した SIV 抗原提示亢進を司る樹状細胞受容体の検索および中和抗体-SIV 複合体パルス樹状細胞により誘導される細胞性免疫の解析を行った。

2. 樹状細胞において NAb による cross-priming 亢進が成立するという初年度結果を重視し、NAb による SIV 制御時の中和能の必要性評価を行うために SIV 結合・非中和抗体の感染サル受動免疫実験を行い、病態評価及び各種の宿主免疫応答の解析を行った。

B. 研究方法

（H22）NAb による DC 抗原提示亢進の機序解析

SIV 複製制御サル（エフェクターT 細胞が充分誘導され、血中ウイルス量が検出限界以下に抑制された個体）由来のプレート付着単球系細胞より GM-CSF 及び IL-4 を用い 5 日間培養して試験管内誘導した樹状細胞に中和抗体と結合した SIV をパルスし、自家由来末梢血単核球（PBMC）と共培養後、抗原特異的細胞内サイトカイン産生をフローサイトメーターにて測定した。この際に各種の受容体阻害抗体を添加し、特異的 T 細胞活性化の可否に関わる受容体を検索した。

（H23）SIV 非中和抗体（nNAb）受動免疫実験準備

1：SIV 感染初期の NAb 受動免疫時における CD64 阻害抗体の共接種予備実験

アカゲサル群に SIV を接種後 7 日目に NAb 受動免疫した際 CD64 結合抗体を共接種し、血中ウイルス量と NAb 受動免疫直後の DC 分画 SIV ゲノム数、リンパ節中 SIV p27 陰性 CCR7/CD11c 陽性 DC の流入率を評価した。

2：NAb による SIV 制御時の中和能の必要性評価：SIV 感染時の抗 SIV 結合・非中和抗体受動免疫実験抗原提示アッセイで NAb が感染標的とならない特異的 CTL の CCL4 産生亢進を誘導したことを重視し、この反応のみ個体内で起こすことを目的に「粒子結合（抗原提示）能を有し」、「中和能を有さない」SIV 結合・非中和抗体（nNAb）の受動免疫実験を行うこととした。

①SIVmac239 持続感染アカゲサル群をスクリーニングし、ウイルス中和活性を示さなかった個体の血漿よりポリクローナル IgG を精製した。

②感染性 SIV 粒子を抗原とした ELISA 系を樹立した。サル T 細胞株（HSC-F）株に SIV を感染させ、上清中に産生されるウイルスをショ糖濃度勾配法により精製し、96 穴プレートに固相化した。SIV 感染あるいは非感染サルの血漿より精製したポリクローナル抗体を試料とし、界面活性剤非存在下で間接酵素抗体法により各抗体の SIV 粒子に対する結合性を評価した。

③上記の系に基づき、背景研究で使用した NAb と同様の粒子結合能を有する nNAb を選抜した。

④選抜した nNAb を用いた SIV 感染急性期の受動免疫実験を開始し、抗体に中和能がなく CD8 陽性 T 細胞誘導に偏りうる抗原提示で複製制御が得られるかを評価した。

（H24）nNAb 受動免疫実験の病態解析

アカゲサル群に SIV を接種後 7 日目に nNAb 300mg を受動免疫し、以下について群間（受動免疫群 5 頭、対照群 6 頭）で比較しつつ経時的に解析を行った。これにより受動免疫抗体に中和能がなく、CD8 陽性 T 細胞誘導に偏りうる抗原提示で SIV 複製制御が得られるかを評価した。

1．受動免疫由来の抗 SIV 抗体価：ウェスタンブロッティング法を用い血漿抗体価を解析した。

2．ADCVI（抗体依存性細胞性ウイルス複製抑制）能の評価：サル末梢血単核球（PBMC）をエフェクター細胞、サル CD4 陽性 T 細胞株を標的細胞とする抗体依存性細胞性ウイルス複製抑制（ADCVI）アッセイを行った。試験管内において HSC-F 株に SIVmac239 を感染させ、ウイルス非感染サル由来の PBMC と各種の SIV 感染サルそれぞれ単独に由来す

る抗 SIV ポリクローナル抗体の存在下で共培養を 7 日間行い、上清中ウイルス量を Gag 蛋白の ELISA 法にて測定し ADCVI 活性の定量を行った。

3．血中ウイルス量：血漿中ウイルス RNA を限界希釈したのち RT-PCR、nested PCR を行い、Reed-Muench 法を用いて算出した。

4．血中 CD4 陽性 T 細胞数中のメモリー分画比率：末梢血単核球（PBMC）の表面染色により CD4 陽性 T 細胞集団中の CD95/CD28 の発現パターンを評価した。

5．CD8 陽性 T 細胞応答：感染後の PBMC を、SIV 抗原ペプチドで刺激した自家 B リンパ芽球(B-LCL)と細胞内輸送阻害剤存在下で共培養し、特異的 IFN-γ産生を評価した。

6．ウイルス塩基配列解析：感染慢性期の血漿ウイルス Env 領域塩基配列解析をダイレクトシークエンス法で行い、選択圧の存否を評価した。

（倫理面への配慮）

当該研究における遺伝子組み換え生物等を用いる実験については、必要に応じた国立感染症研究所の機関承認および文部科学大臣承認（第二種使用等核酸防止措置確認申請承認）を取得のうえ行った。

全ての動物実験は、倫理面および動物愛護問題の観点から、国立感染症研究所、医薬基盤研究所の動物実験委員会の審査・承認のち、医薬基盤研究所霊長類医科学研究センターにて、動物実験委員会が定めたルールおよびガイドラインに沿って遂行した。

C. 研究結果

（H22）

1：中和抗体を介した SIV 抗原提示亢進を司る樹状細胞受容体：<u>FcγRI（CD64）の同定</u>

各種の受容体阻害抗体を添加して、特異的 T 細胞活性化の可否に関わる受容体を検索した結果、培養時に FcγRI（CD64）特異結合抗体を一定濃度にて加えた検体において、抗原特異的 CD4 陽性 T 細胞の IFN-γ産生能が遮断された。一方 FcγRIII（CD16）特異結合抗体を同程度に加えた検体において同じ水準の遮断は認められなかった。この傾向は後述する特異的 CD8 陽性細胞応答においてより明瞭に認められた。

2：中和抗体-SIV 複合体パルス樹状細胞により誘導される細胞性免疫の解析：<u>抗原特異的 CD8 陽性 T 細胞による CCL4（MIP-1β）産生</u>

上記の抗原提示アッセイの際各種のサイトカイン産生パターンを精査した結果、抗原特異的 CD8 陽性 T 細胞集団における CCL4（MIP-1β）産生の選択的亢進を見出した。全く同じ CD8 陽性 T 細胞集団を SIV 抗原発現 B リンパ芽球（B-LCL）と共培養させた際は IFN-γのみの選択的な産生亢進を認めたため、前者は T 細胞消耗によるものではなく、抗原提示時の共刺激シグナルのパターンの相違により CCL4 産生に応答が偏移する可能性が示唆された。

（H23）

1：SIV 感染初期の NAb 受動免疫時における CD64 阻害抗体の共接種実験

セットポイント期血中ウイルス量は CD64 抗体共接種群で対照群より増加する示唆が得られた。後述する抗体の中和能の必要性評価を最重要と認め解析を保留した。

2：NAb による SIV 制御時の中和能の必要性評価：SIV 全粒子 ELISA 法では SIV 感染個体由来の試料抗体の結合性は陰性から高度陽性まで多様であった（図 1）。評価した 10 頭由来から、背景研究で使用した NAb と同じ粒子結合能を有する 3 頭由来の nNAb を選抜した。

（H24）

<u>SIV 感染急性期 nNAb 受動免疫実験</u>：

選抜した nNAb（合計 300mg）を SIV 感染急性期（Day 7）において受動免疫した結果、下記を得た。

1．受動免疫由来の抗 SIV 抗体価：

受動免疫由来の抗 SIV 抗体価は接種 0.5 週後では nNAb 受動免疫群のみ全頭で検出され、*de novo* 抗体価は対照群では感染後 5 週前後で一様に検出を認め、群間の差は感染後 12 週時点で認められなくなった（図４下）。

２．ADCVI（抗体依存性細胞性ウイルス複製抑制）能の評価：生理的範疇の濃度（0.1～1mg/ml）の nNAb による高い ADCVI 能を確認し、受動免疫した抗体の体内濃度相当で ADCVI 活性が呈されうることが示唆された（図 4 上）。

３．血中ウイルス量：

受動免疫直後・セットポイント期、慢性期とも、中和抗体受動免疫時とは異なり、当該群は対照群と比してウイルス量の差異を認めなかった。本結果により、感染急性期の抗体受動免疫による non-sterile な SIV 複製制御には抗体の中和能が必要であることが明らかとなった（図 2）。

４．血中 CD4 陽性 T 細胞数中のメモリー分画比率：CD95 陽性 CD28 陽性セントラルメモリー（CM）分画、CD95 陽性全メモリー分画のいずれにおいても群間で差異を認めなかった（図 3A、U-test CM：p = 0.52, 全メモリー：p = 0.75）。

５．CD8 陽性 T 細胞応答：感染後約 30 週での SIV 抗原特異的 IFN-γ産生は SIV 蛋白の種類、総レベルいずれとも群間での差異を認めなかった（図 3B）。

６．ウイルス塩基配列解析： nNAb 受動免疫群では Env V1 領域における非同義置換の数が感染後 1 年で多い傾向を示したものの、差異は対照群と比べ有意ではなかった。

D. 考察

本研究では、培養系における NAb による抗原提示亢進に CD8 陽性 T 細胞の cross-priming が関わることを見出した初年度結果により、中和能のない抗 SIV 抗体受動免疫時に個体レベルのウイルス複製制御が得られる可能性を評価することを重要視して、SIVmac239 結合性・非中和抗体の新規スクリーニング・精製を行い、SIVmac239 結合性・非中和抗体（nNAb）の感染急性期の受動免疫実験を行った。nNAb による持続感染の阻止能は認められず、背景研究と併せ、抗体による non-sterile な SIV 制御における中和能の必要性が証明された。中和抗体受動免疫を行った先行研究においては①抗原提示能と②ウイルス中和能の制御への寄与が両方考えられたが、このうち②に関する必要性が本段階で見出された。理由としては、抗原取込みに続いて誘導される対象となりうる特異的 CD4 陽性 T 細胞の感染からの保護が不十分な可能性が考えられた。

本研究の結果、全身性の感染が一度成立したのちの HIV/SIV 制御に対する抗ウイルス非中和抗体の寄与は限定的であることが判ったことに加え、nNAb による個体レベルでの感染防御への寄与を報告した他の報告（Hidajat R, J Virol 2008; Florese RH, J Immunol 2009; Barouch DH, Nature 2012）中の結果は補助的なものに留まる可能性が見出された。ただ、これらでは CD8 陽性 T 細胞を主体とした複数のエフェクターが誘導されており、それが感染成立前であったことから本研究結果と異なる解析結果が得られたものと考えられる。一方、本結果はエイズ発症までの予後観察に至ったものではなく、急性期の nNAb 受動免疫が生存率に影響を与えるかを評価するのは今後の課題となる。

以上より、中和抗体と CTL 主体の細胞性免疫による相乗的で non-sterile な SIV 複製制御には、以下のステップが全て必要なものと考えられた。

①血中（液相中）でのウイルス粒子中和

②抗原提示細胞への取込み

③特異的 T 細胞誘導亢進

④上記③の内、抗体のウイルス中和能による特異的 CD4 陽性 T 細胞集団の保護

⑤上記④の担保による特異的 CD8 陽性 T 細胞の機能修飾→non-sterile な複製制御

E. 結論

本研究では、抗体による non-sterile な SIV 制御に重要な解析指標であることが認められた中和能の必要性評価のために「粒子結合（抗原提示）能を有し」、「中和能を有さない」SIV 結合・非中和抗体（nNAb）の受動免疫を行った。nNAb の大量精製を行い、SIV 粒子 ELISA 法の新規スクリーニング系に基づき背景研究で使用した NAb と同じ粒子結合能を有する

nNAb を選抜し、サルにおける nNAb 受動免疫実験を行った。試験管レベルでは十分なウイルス複製抑制能を付与する量の接種にも関わらず、宿主適応免疫応答は nNAb 受動免疫による修飾を認めず、SIV 感染成立後の nNAb 受動免疫は持続感染阻止能を呈し得ないことを見出した。本研究により、SIV 感染初期の抗 SIV 抗体受動免疫によるウイルス複製制御時には直接的なウイルス中和能が必須であることが逆説的に初めて証明され、本結果は中長期的な中和抗体誘導型予防エイズワクチン開発への論理的基盤に寄与したと考えられる。

F. 健康危険情報

特になし。

G. 研究発表

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H. 知的財産権の出願・登録状況

1　特許登録

該当なし。

2　実用新案登録

該当なし。

3　その他

該当なし。

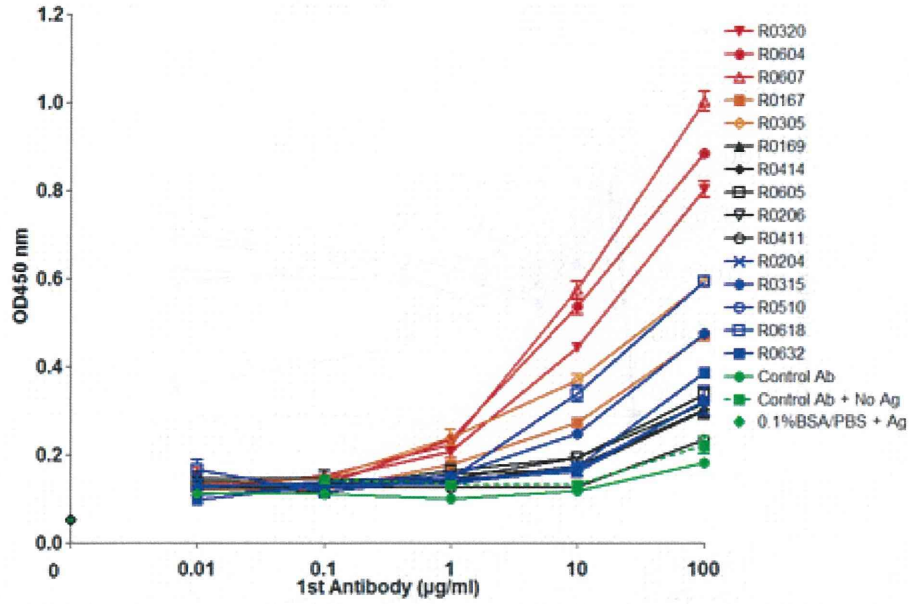


図１　ウイルス全粒子 ELISA 法を用いた SIV 結合・非中和抗体（nNAb）の結合能評価

感染性 SIV 粒子を抗原とした ELISA 系を確立した。サル T 細胞株（HSC-F）株に SIV を感染させ、上清中ウイルスをショ糖濃度勾配法により精製し、p27 量換算で 10ng を 96 穴プレートに固相化した。SIV 感染あるいは非感染サル血漿に由来するポリクローナル抗体（0.01～100 µg/ml）を試料とし、界面活性剤非存在下で間接酵素抗体法により各抗体の SIV 粒子に対する結合性を評価した。赤線は非中和抗体で高結合能、橙線は非中和抗体で中程度結合能、黒線は非中和抗体で低結合能を示した個体由来の検体を表す。青線は陽性対照としての各個体由来の中和抗体、緑線は陰性対照としての非感染個体由来コントロール抗体の結合を示す。

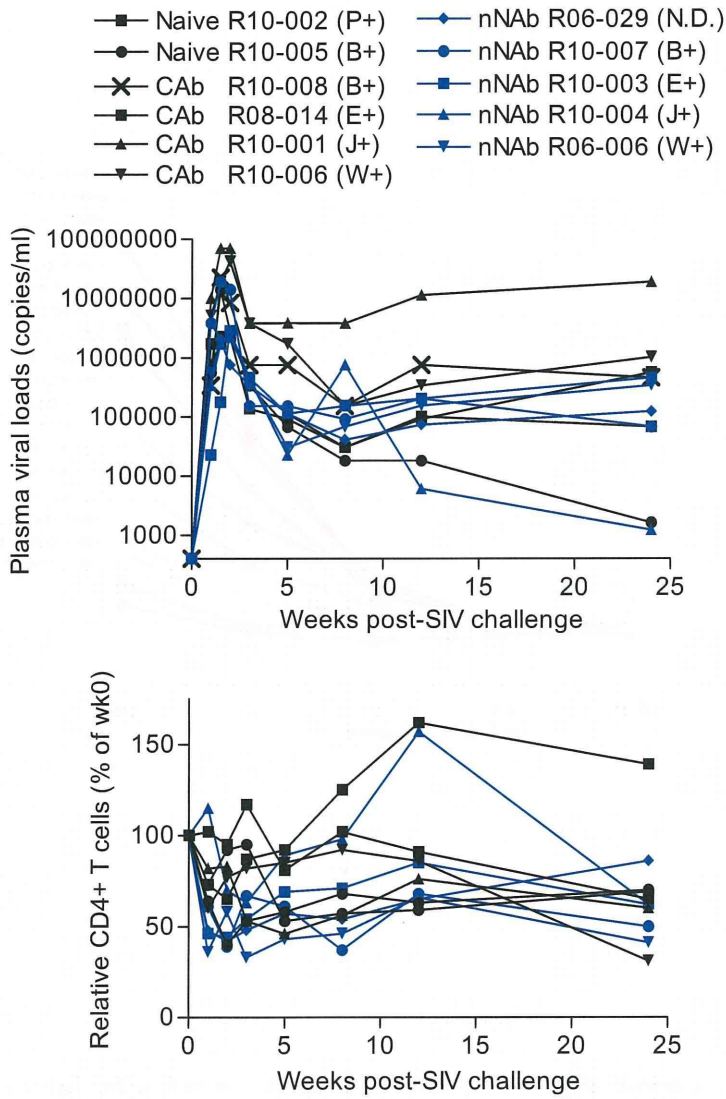
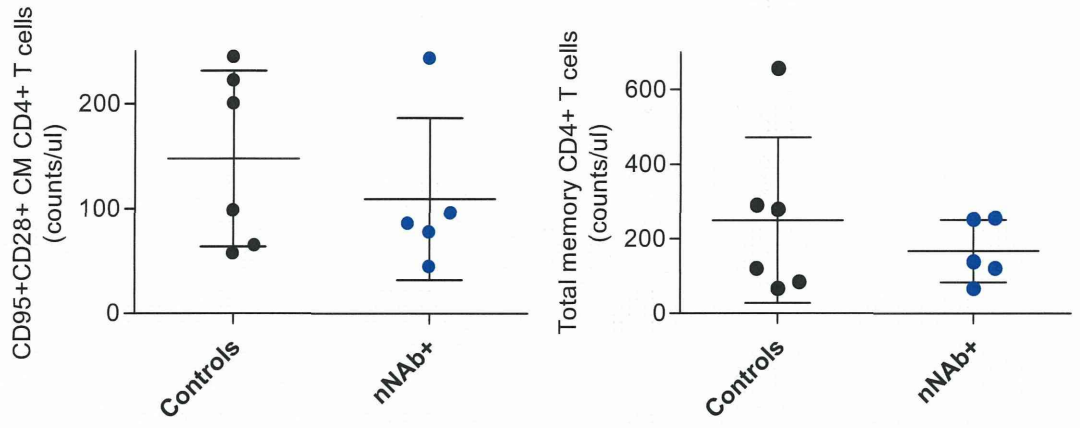


図2　アカゲサル SIV 感染急性期における非中和抗体（nNAb）受動免疫実験の経過

アカゲサルに SIVmac239 をチャレンジしたのちの血漿中ウイルス量の感染初期における経時変化を示す。黒線は対照群（n = 6）、青線は SIV 結合・非中和抗体受動免疫群（n = 5）の結果を示す。個体 ID の横の記号は MHC-I ハプロタイプ、横軸は感染後週数、上段縦軸は血中 SIV Gag RNA コピー数、下段縦軸は末梢血中 CD4 陽性 T 細胞数の経過を表す。群間の血中ウイルス量、CD4 カウントに差は認められなかった。

A



B

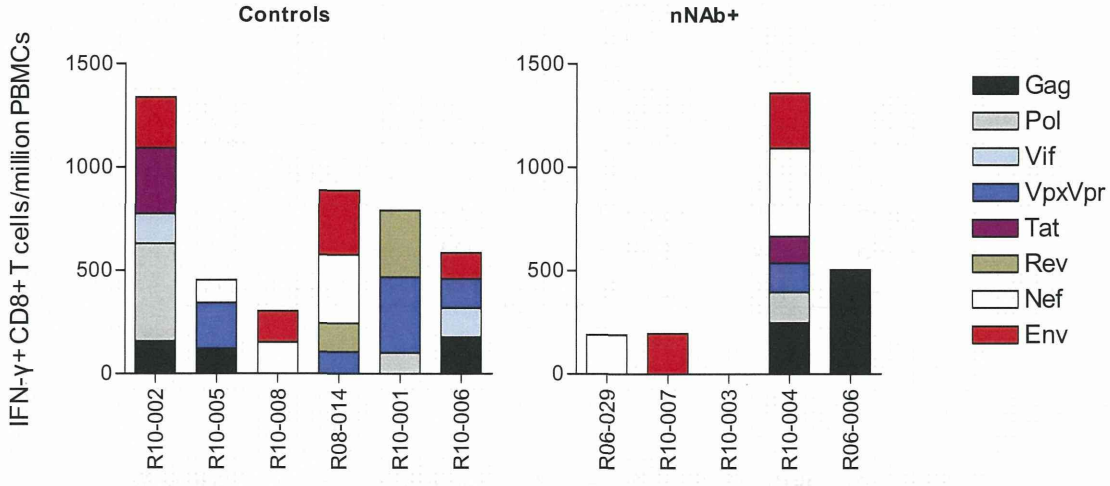
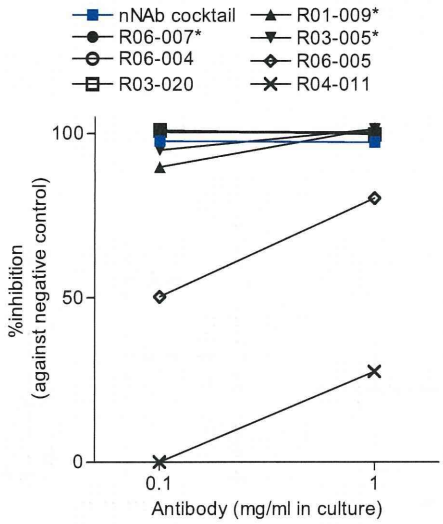


図 3　SIV 非中和抗体（nNAb）受動免疫実験における細胞性免疫応答

A.　セットポイント期における CD95 陽性 CD28 陽性セントラルメモリーCD4 陽性 T 細胞数、及び CD95 陽性全メモリーCD4 陽性 T 細胞数の群間比較を示す。黒は対照群（n = 6）、青は SIV 結合・非中和抗体受動免疫群（n = 5）を示す。いずれにおいても群間で差異を認めなかった（Mann-Whitney 検定：CM：$p = 0.52$, 全メモリー：$p = 0.75$）。

B.　両群における感染後 30 週前後の SIV 抗原特異的 CD8 陽性 T 細胞応答の比較を示す。慢性期においては、群間で CTL 誘導のパターンに差異を認めなかった。



Regimen	MHC-I Haplotype	Macaque	wk 1	1.5	3	5	12
-	P	R10-002	-	-	-	+	++++
-	B	R10-005	-	-	-	+	++++
CAb	B	R10-008	-	-	-	++	++++
CAb	E	R08-014	-	-	+	++	++++
CAb	J	R10-001	-	-	-	+	+
CAb	W	R10-006	-	-	-	+	++++
nNAb	B	R10-007	-	+++	++	++	++++
nNAb	E	R10-003	-	++++	++	++	++++
nNAb	J	R10-004	-	+++	++	+	++++
nNAb	W	R06-006	-	++++	++	+	++++
nNAb	N.D.	R06-029	-	+++	++	++	++++

図４　SIV 非中和抗体（nNAb）受動免疫実験における液性免疫応答

上段：受動免疫に用いた nNAb の各個体由来、及び接種カクテル抗体の試験管内における抗体依存性細胞性ウイルス複製抑制（ADCVI）能を示す。培養中濃度 0.1mg/ml（サル個体 3kg の 300ml 液相中に 10ml、30mg/ml の nNAb を受動免疫したときに瞬間平衡と仮定した際の濃度を表す）にて 97%程度の高値で SIV 複製抑制能が認められた。本研究では*印がついた 3 頭由来の nNAb を混合して（上記：青記号）受動免疫に用いた。

下段：血漿中抗 SIV 抗体の反応抗原の種類を定性的に示した図。+：Gag p27 陽性、++：Gag p27 及び Env gp160 陽性、+++：Gag p27 及び Env Gp160 ほか 1 抗原陽性、++++：Gag p27 及び Env Gp160 ほか 2 抗原以上陽性。受動免疫由来と考えられる抗 SIV 抗体価は感染後 3 週まで検出された。

研究成果の刊行に関する一覧表

平成２４年度

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
Nomura T, Yamamoto H, Shiino T., Takahashi N, Nakane T, Iwamoto N, Ishii H, Tsukamoto T, Kawada M, Matsuoka S, Takeda A, Terahara K., Tsunetsugu-Yokota Y, Iwata-Yoshikawa N, Hasegawa H, Sata T, Naruse TK, Kimura A, Matano, T.	Association of major histocompatibility complex class I haplotypes with disease progression after simian immunodeficiency virus challenge in Burmese rhesus macaques.	J Virol.	86	6481-90	2012

平成２３年度

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
Takahara Y, Matsuoka S, Kuwano T, Tsukamoto T, Yamamoto H, Ishii H, Nakasone T, Takeda A, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Sakawaki H, Horiike M, Miura T, Igarashi T, Naruse TK, Kimura A, Matano T.	Dominant induction of vaccine antigen-specific cytotoxic T lymphocyte responses after simian immunodeficiency virus challenge.	Biochem Biophys Res Commun.	20;408	615-19	2011

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平成２２年度

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	28S2	13-17	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

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

Hiroyuki Yamamoto, Tetsuro Matano*

International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

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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.

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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.

* Corresponding author. Tel.: +81 3 6409 2078; fax: +81 3 6409 2076.
E-mail address: matano@ims.u-tokyo.ac.jp (T. Matano).

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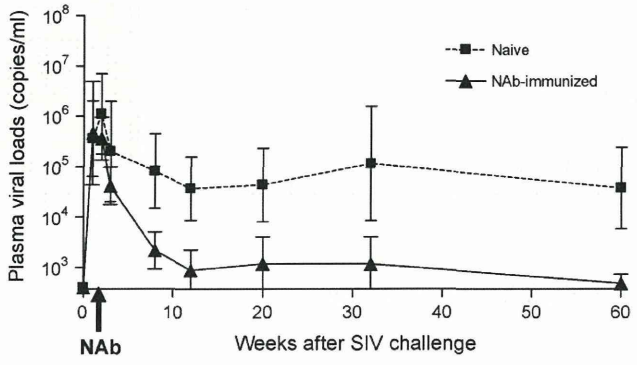

Fig. 1. Plasma viral loads in naïve controls and NAb-immunized macaques. Changes in geometric mean plasma viral loads in naïve controls (squares with dotted lines) and NAb-immunized macaques (triangles with bold lines) are shown. Error bars show the 95% confidence interval at each time point. The geometric 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.

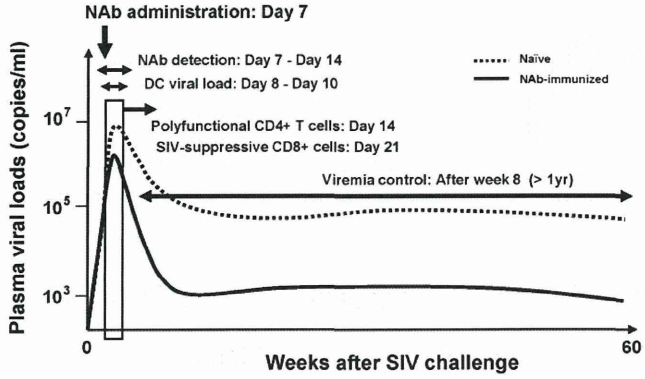

Fig. 2. Time-course events in NAb-mediated SIV control. The limited detection of plasma NAb titers between days 7 and 14 are concurrent with the rise in DC-viral loads (days 8–10), which is followed by appearance of Gag-specific CD4+ T cells with higher polyfunctionality at day 14 and CD8+ cells possessing 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.

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A structural constraint for functional interaction between N-terminal and C-terminal domains in simian immunodeficiency virus capsid proteins

Natsuko Inagaki[1], Hiroaki Takeuchi[1], Masaru Yokoyama[2], Hironori Sato[2], Akihide Ryo[3], Hiroyuki Yamamoto[1], Miki Kawada[1], Tetsuro Matano[1*]

Abstract

Background: The Gag capsid (CA) is one of the most conserved proteins in highly-diversified human and simian immunodeficiency viruses (HIV and SIV). Understanding the limitations imposed on amino acid sequences in CA could provide valuable information for vaccine immunogen design or anti-HIV drug development. Here, by comparing two pathogenic SIV strains, SIVmac239 and SIVsmE543-3, we found critical amino acid residues for functional interaction between the N-terminal and the C-terminal domains in CA.

Results: We first examined the impact of Gag residue 205, aspartate (Gag205D) in SIVmac239 and glutamate (Gag205E) in SIVsmE543-3, on viral replication; due to this difference, $Gag_{206-216}$ (IINEEAADWDL) epitope-specific cytotoxic T lymphocytes (CTLs) were previously shown to respond to SIVmac239 but not SIVsmE543-3 infection. A mutant SIVmac239, SIVmac239Gag205E, whose Gag205D is replaced with Gag205E showed lower replicative ability. Interestingly, however, SIVmac239Gag205E passaged in macaque T cell culture often resulted in selection of an additional mutation at Gag residue 340, a change from SIVmac239 valine (Gag340V) to SIVsmE543-3 methionine (Gag340M), with recovery of viral fitness. Structural modeling analysis suggested possible intermolecular interaction between the Gag205 residue in the N-terminal domain and Gag340 in the C-terminal in CA hexamers. The Gag205D-to-Gag205E substitution in SIVmac239 resulted in loss of in vitro core stability, which was recovered by additional Gag340V-to-Gag340M substitution. Finally, selection of Gag205E plus Gag340M mutations, but not Gag205E alone was observed in a chronically SIVmac239-infected rhesus macaque eliciting $Gag_{206-216}$-specific CTL responses.

Conclusions: These results present in vitro and in vivo evidence implicating the interaction between Gag residues 205 in CA NTD and 340 in CA CTD in SIV replication. Thus, this study indicates a structural constraint for functional interaction between SIV CA NTD and CTD, providing insight into immunogen design to limit viral escape options.

Background

One of the characteristics of human immunodeficiency virus (HIV) is to induce persistent viral replication resulting in AIDS progression. HIV has enormous capacity to mutate and escape from host immune recognition, driving genetic diversification of the circulating viruses [1-3]. The Gag capsid (CA), comprising the N-terminal (NTD) and the C-terminal domains (CTD) [4-6], is one of the most conserved proteins in highly-diversified HIVs [7]. Understanding structural constraints in such viral proteins could provide valuable information for immunogen design in AIDS vaccine development.

Virus-specific cytotoxic T-lymphocyte (CTL) responses play a central role in the control of immunodeficiency virus infection [7-12]. CTLs exerting strong suppressive pressure on HIV replication select for viral mutations resulting in escape from CTL recognition [13-16]. Escape mutations in viral proteins with structural constraints are often selected with viral fitness costs, possibly facilitating subsequent immune control

* Correspondence: matano@ims.u-tokyo.ac.jp
[1]International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
Full list of author information is available at the end of the article

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