胞にFIV特異的shRNAを安定に導入することにより、明らかなFIV増殖抑制効果が得られることが示された。一方、FIV非感染ネコTリンパ球系細胞株に本レトロウイルスベクターを感染させた後、FIV Sendai-1株またはShizuoka株を接種した結果、いずれの場合においてもFIV特異的shRNA安定導入細胞におけるFIVの増殖抑制は認められなかった。また、実験用ネコの骨髄腔内にshRNA発現レトロウイルスベクターを投与した結果、ベクター遺伝子に由来するプロウイルスを有する細胞が接種後少なくとも28日目まで体内に存在していたことが示された（表1）。

DCを用いた抗ウイルス療法の開発：感染ネコおよび非感染ネコの単球由来付着細胞は、いずれもIL-4、GM-CSF存在下で樹状突起を呈し、DCに特徴的な形態を示した。また、これらの細胞はAPCに特徴的な表面形質を示し、マンノース受容体に依存するデキストラン捕食能を持ち、MLR誘導能を呈したことから、DCに分化しているものと考えられた（図2）。FIV感染ネコ由来細胞では、非感染ネコ由来細胞と比べてCD1aの発現が低下していたが、形態及び機能では両者の間に差は認められなかった。

D. 考察

本研究では、レトロウイルスベクターを用いてFIV持続感染Tリンパ系細胞に抗FIV shRNAを安定導入することによりウイルス増殖抑制効果が認められた。しかし、新たなFIV感染に対して抵抗性を示すTリンパ系細胞を作製することはできなかった。その原因として、本細胞株ではshRNAの発現量が不十分であった可能性、および導入shRNAの塩基配列と完全な相同性を持たないウイルスが存在した可能性が考えられた。本研究により、FIV感染症に対する治療的遺伝子導入法の有効性が示されたが、予防的遺伝子導入法の確立のためには発現効率やウイルス遺伝子の多様性に対する対応などについてさらなる検討が必要と考えられた。また、レトロウイルスベクターの骨髄腔内直接投与により、ネコ生体内の細胞への遺伝子導入が可能であることが明らかとなったが、その導入・発現効率に問題があることが示され、遺伝子導入法の改良を行う必要があるものと考えられた。今後は静止期の細胞にも遺伝子導入可能なレンチウイルスベクターを用いて抗FIV shRNA導入ネコリンパ球を作製し、複数のFIV株に対する持続的なウイルス増殖抑制効果を検討するとともに、FIV感染ネコへのshRNA導入法を検討する予定である。

FIV非感染ネコおよびFIV感染ネコの末梢血より単球を分離し、*in vitro*でのDCへの分化を試み、その性状解析を行った。その結果、FIV感染ネコにおいても健常ネコと同様にDCの分化誘導が可能であることが示された。よって、FIV感染症において自己のDCを移入することによって新規免疫療法を開発できる可能性が示唆された。今後、DCを用いた抗ウイルス療法の開発を目的とし、細胞障害性Tリンパ球を誘導可能なDCを誘導する予定である。

E. 結論

エイズに対する新規治療法を開発するため、猫のFIV感染モデルにおいてshRNAおよびDCを用いた新しいシステムを立ち上げた。これらシステムにおいて新規抗ウイルス療法の有効性を明らかにすることにより、ヒトのエイズに応用可能な新規治療法の有効性を検証できるものと考えられた。

F. 健康危険情報

マウス白血病ウイルス由来レトロウイルスベクターの遺伝子導入実験およびウイルスの保管は全て東京大学組替えDNA実験安全専門委員会の定める遺伝子組換え生物等の使用等実施規則に基づき、P2実験施設で行われている。

G. 研究発表

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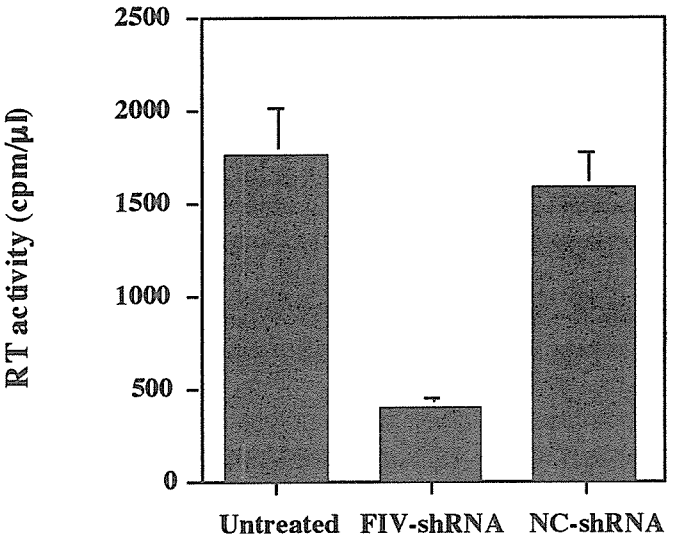
H. 知的所有権の出願・取得状況

なし

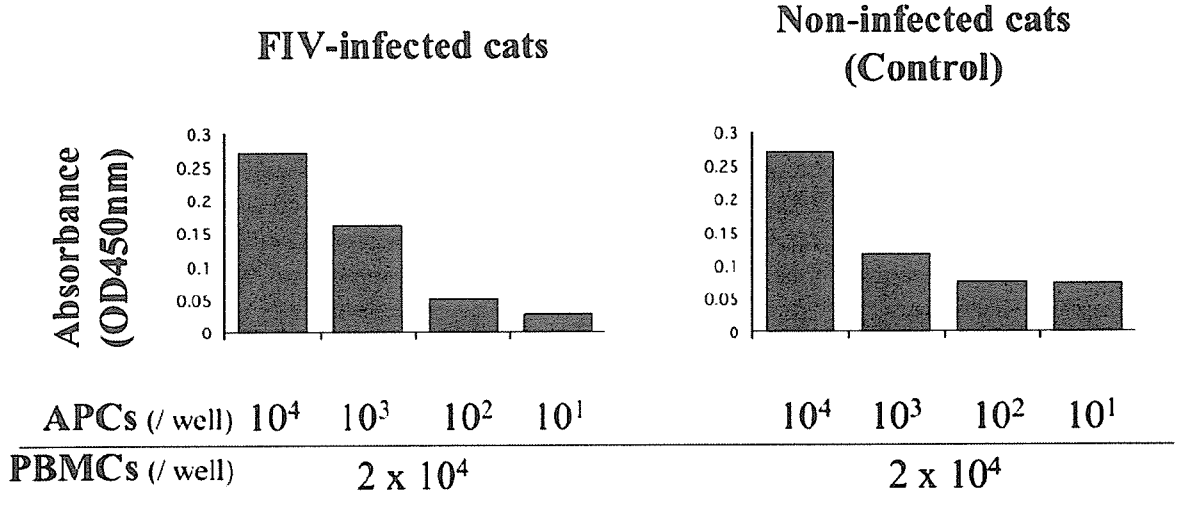
（図 1）抗 FIV shRNA 安定導入細胞における FIV の増殖抑制



（表 1）レトロウイルスベクターの骨髄腔内投与後におけるプロウイルス DNA の検出

	Peripheral blood				Bone marrow			Other tissues
(Days after infection)	3	7	14	28	7	14	28	28
Cat #1	+	+	-	-	+	-	-	Kidney
Cat #2	+	+	-	-	-	-	+	Spleen, Liver, Small intestine, Skeletal muscle
Cat #3	+	-	-	-	+	-	+	Lymph node, Spleen, Liver, Small intestine, Lung

（図 2）allogenic PBMCs に対する DCs の混合リンパ球反応



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

書籍

著者氏名	論　文 タイトル名	書籍全体の 編集者名	書 籍 名	出版社名	出版地	出版年	ページ
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IV. 研究成果の刊行物・別刷