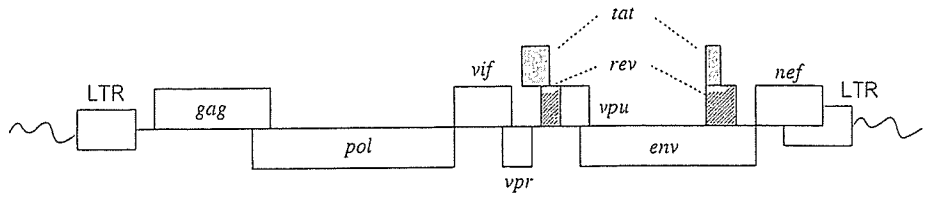

ウイルスタンパク質		機能
構造タンパク質		
Gag	マトリックス（MA：matrix）	ウイルス粒子形成・放出，脱殻・逆転写
	カプシド（CA：capsid）	ウイルス粒子形成・放出，脱殻・逆転写
	ヌクレオカプシド（NC：nucleocapsid）	脱殻・逆転写
	p6	ウイルス粒子放出，Vpr/Vpx のウイルス粒子へのターゲッティング
Pol	プロテアーゼ（PR：protease）	Gag 前駆体や Gag-Pol 前駆体の開裂による成熟ウイルス粒子（感染性ウイルス粒子）の生成
	逆転写酵素（RT：reverse transcriptase）	ウイルス DNA 合成（含 RNase H 活性）
	インテグラーゼ（IN：integrase）	ウイルス DNA の細胞染色体 DNA への組込み
Env	gp120	標的細胞受容体への結合
	gp41	標的細胞への侵入
調節タンパク質		
Tat		転写の増強
Rev		構造およびアクセサリータンパク質（Nef を除く）の発現増強
アクセサリータンパク質		
Vif		抗ウイルス細胞因子 APOBEC3G の不活化
Vpr		ウイルス DNA の核移行
Vpu		ウイルス粒子の放出促進
Nef		細胞表面における MHC-Ⅰ発現の抑制

図 1　HIV-1 ゲノムとウイルスタンパク質

LTR：long terminal repeat（長末端反復配列）

HIV-1 のゲノム構造の模式図を上に，ウイルスタンパク質の機能を下に示した。ゲノムはプロウイルス（図 3）の構造である。機能は数多くある報告[1, 3]のうち，主要なものだけを記載した。

子，調節遺伝子，アクセサリー遺伝子に分類され，その産物も同様に 3 群に分類されている[3]。構造タンパク質はウイルス粒子の主要構成成分であり，Tat，Rev および Vpu を除く他のウイルスタンパク質も粒子内に存在する（図 2）。感染細胞内にのみ存在するこれら 3 種類のタンパク質は，ウイルス遺伝子やタンパク質の発現制御あるいはウイルス粒子の産生効率に関与している。構造タンパク質はすべてのレトロウイルスに共通に存在するが，調節タンパク質は霊長

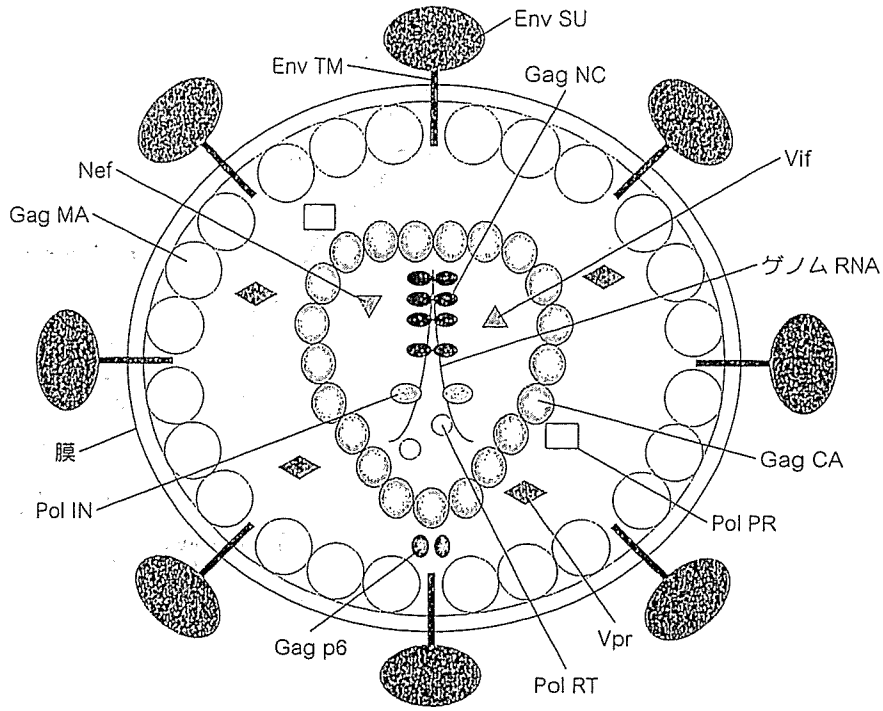

図2　HIV-1 粒子の構造

SU：surface protein（外膜タンパク質）gp120, TM：transmembrane protein（膜貫通タンパク質）gp41

HIV-1 粒子を構成するウイルスタンパク質を模式的に示した。Vif および Nef は粒子中に微量しか存在しないため，その生物学的意義については議論がある。ウイルスタンパク質の略称については図1参照のこと。

類レトロウイルス（白血病ウイルス，免疫不全ウイルス，スプーマウイルス）に特徴的で，アクセサリータンパク質はそれぞれの霊長類レトロウイルスに特異的である。

2）ウイルス複製機構

HIV-1 もレトロウイルスの一員であるから，基本的にはこのウイルス群特有の複製様式に従って増殖するが，構造遺伝子のみからなる単純なレトロウイルスに比べてはるかに複雑な複製制御機構がある。図3に感染細胞における HIV-1 複製の主な過程とそれに関与するウイルスタンパク質をまとめた。ウイルス粒子の細胞への吸着・侵入に始まり，プロウイルスの形成，プロウイルスからの遺伝子発現を経て，ウイルス粒子の放出，感染性粒子への成熟に至るのがレトロウイルス共通の生活環である。HIV-1 が特徴的であるのは，上記調節タンパク質とアクセサリータンパク質によって制御される複製過程である（図1および図3）。また，Nef の活性に代表されるように，細胞レベルでは解析困難な，個体内ウイルス複製や病原性に重要な機能もある。

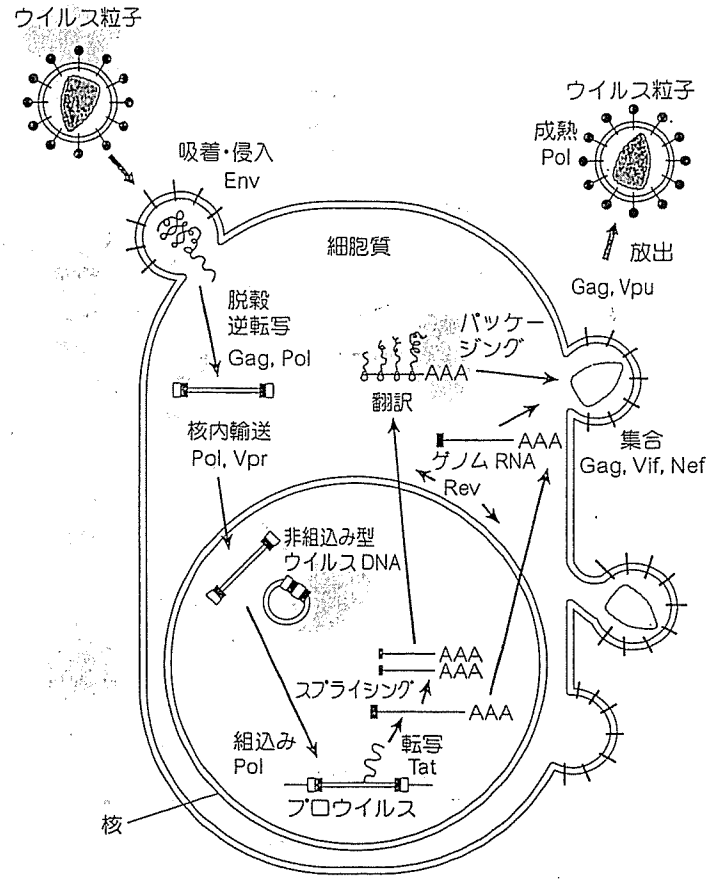

図 3　HIV-1 の複製サイクル（文献 1 より一部引用改変）
HIV-1 の一複製サイクル（吸着・侵入からウイルス粒子の成熟まで）を模式的に示した。複製過程とそれに関与するウイルスタンパク質をまとめた。ウイルスタンパク質の略称および主要機能については図 1 参照のこと。

2．シクロフィリンと HIV-1 複製

シクロフィリン A は HIV-1 Gag（group specific antigen：群特異抗原）の CA（capsid：カプシド）領域（図 1 および図 2）に特異的に結合する（HIV-2 など他の霊長類免疫不全ウイルスの Gag には結合しない。また，他のイムノフィリン FKBP〔FK-binding protein：FK 結合タンパク質〕は HIV-1 Gag に結合しない）ので，シクロスポリンは効率的にこれを阻害する[4, 5]。シクロスポリンの持つ免疫抑制作用[6]はこの阻害効果に影響しない[5]。単に結合するだけでなく，このシクロフィリン A/Gag CA 相互作用はウイルス学的に極めて重要で，HIV-1 複製の初

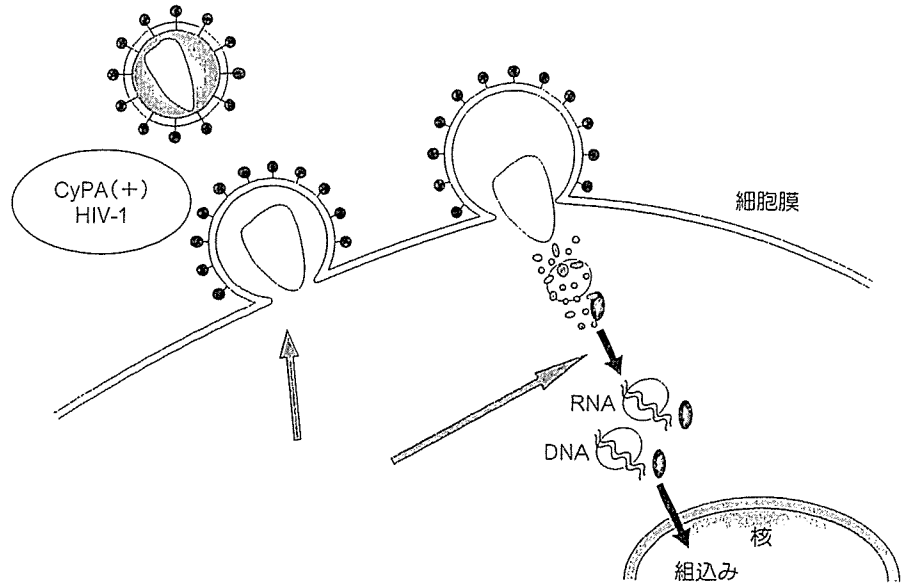

図4　シクロフィリンとHIV-1の複製

シクロフィリンAによるHIV-1複製の増強のメカニズムの概略を示した。シクロフィリンA(CyPA)存在下で産生されたHIV-1粒子は感染直後あるいは脱殻過程(灰色の矢印)が効率良く進行する。

期過程の効率を格段に増強させる（図4）。

現在，抗HIV-1治療の主体は多剤併用療法（HAART：highly active anti-retroviral therapy）である[7]。ウイルスの逆転写酵素やプロテアーゼに対する阻害剤を種々組合わせて用いることで顕著な治療効果が認められている。しかし，HAART法も，ウイルス増殖を完全には抑え込めないこと，抵抗性ウイルスの出現，さらには副作用など，克服すべき課題も多い。一方，シクロスポリンの感染個体における抗HIV-1効果は，免疫抑制作用を欠損させたアナログを含めて未だ明確になっていない。他の薬剤との併用など，今後抗HIV-1戦略の一つとして考慮する必要があると思われる。

3．HIV-1 DT

HIV-1の大きなウイルス学的特徴の一つにその宿主域の狭さがある。HIV-1はチンパンジーとヒトにしか感染せず，また，感染してもチンパンジーはエイズを発症しない。このため，ウイルスと感染細胞の分子生物学的解析が急速に進展しても，その成果がエイズ発症機構の解明や治療法の確立などの究極の目標達成に直接結びついていない。エイズの動物モデルを樹立するため様々なアイデアが試されたが，マウスなどの小動物を用いた実験系は目標にほど遠いも

のであった。筆者らは，HIV-1とサル免疫不全ウイルス（SIV：simian immunodeficiency virus）との間でキメラウイルス（SHIV：simian and human immunodeficiency）を作製し，カニクイザルやアカゲザルを用いた感染実験系の構築に成功した[8]。図5に示したように，実験用のサルで感染・増殖するSHIVを使用することにより，HIV-1遺伝子をゲノムに持つウイルスの動物実験が可能となった。しかし，このSHIVやその改変ウイルスはSIVの遺伝子やシークエンスを多く持つので，HIV-1とはかなり異なるウイルスであるといえる。

最近，サル細胞でのHIV-1複製阻害に関与する2つの細胞因子，TRIM5αおよびAPOBEC3G，が同定された（図6）。いずれのタンパク質もヒトとサル細胞に共通して存在するが，サルのものがより強力にHIV-1複製を阻害すると考えられている。TRIM5αによる複製阻害にはウイルスGag CA領域（図1および図2）が関与し，一方，APOBEC3Gを不活化するのはウイルスのVifである（図1）。筆者らもSHIV研究から，HIV-1のGag CA領域をSIVの対応する領域に置換することでサル細胞でのウイルス複製能を獲得するのではないかと考

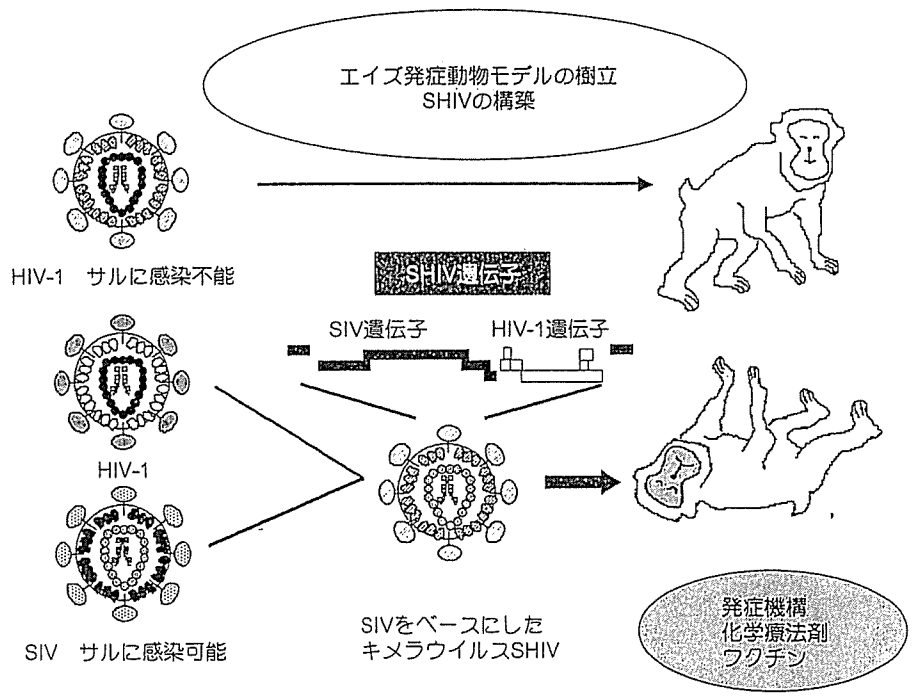

図5　SHIVに基づくHIV-1動物モデル

SIVとHIV-1との間で作製したキメラウイルスSHIVを用いたサル（アカゲザルあるいはカニクイザル）感染実験について，その概略および目標を示した。SHIVはSIVと同様にサルに感染・増殖してエイズを発症させる。

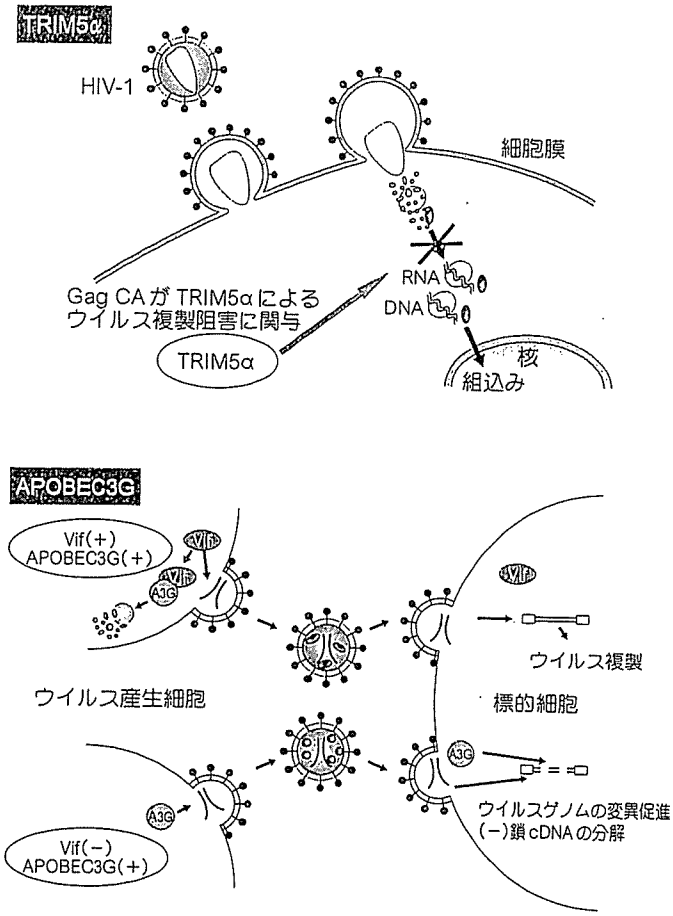

図 6　サル細胞における HIV-1 複製阻害因子
A3G：APOBEC3G
現在までに同定されているウイルス複製阻害因子である TRIM5α および APOBEC3G について，その作用機構の概略を示した。

え，キメラ部分がごく僅かの新しいキメラウイルス（図 7）を作製した[9]。このウイルスはヒト細胞で効率良く増殖し，かつ，シクロスポリンに抵抗性となる点では SIV と同じであったが，サル細胞では増殖できなかった。可能性として考えられたのは，HIV-1 Vif がサル APOBEC3G を十分不活化できないことであった。筆者らは *gag* 遺伝子の一部だけでなく *vif* 遺伝子も SIV 由来のウイルスクローンを構築した（図 7）。HIV-1 dual-tropic（DT）と名付けたこのウイルスは，ヒトおよびサル細胞で良く増殖した。ゲノム長の約 95%が HIV-1 由来であり，今までに報告のあるサル細胞で感染・増殖可能なキメラウイルスのうち最も HIV-1 に近い。今後，この

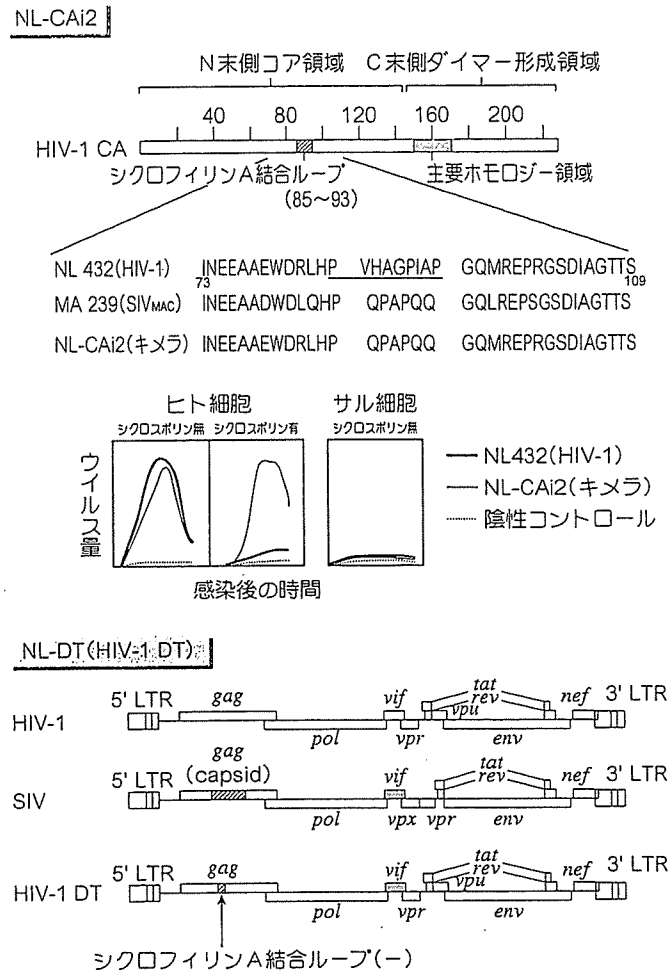

図7　新しいキメラウイルスの構築

HIV-1 をベースにした新しいキメラウイルス（NL-CAi2 および NL-DT）の構造を示した。NL-CAi2 は HIV-1 のシクロフィリン結合ループのみがアカゲザル由来 SIV（SIV_{MAC}）の配列に置換されており，シクロスポリン存在下で SIV_{MAC} と同様に増殖する。ただし，サル細胞には感染性がない。数字は NL432 CA のアミノ酸番号。NL-DT は NL-CAi2 の置換に加え *vif* 遺伝子も SIV_{MAC} のものと置換してあり，サル細胞およびヒト細胞で増殖する。

ウイルスを様々に改変したクローンを用いてサル感染実験を行うことで，ウイルスタンパク質と個体内ウイルス複製やウイルス病原性との関係解明，さらには，新しい化学療法剤やワクチンの開発に向けた研究を展開させていく予定である。

おわりに

シクロフィリンAとHIV-1 Gag CAの結合は，このウイルスの複製や種間トロピズムに大きな生物学的意義を持つ。霊長類免疫不全ウイルスの中でHIV-1のみがシクロフィリンA依存性に増殖するが，この現象のウイルス側決定領域CAがHIV-1の狭い宿主域の一因ともなっている。このテーマに関する研究は，エイズという世界レベルの感染症[10]に対抗する基礎戦略および個体モデルの確立に極めて重要である。日本を含むアジア・アフリカ地域でHIV-1感染者やエイズ患者が激増している現在，このような基礎研究に基づく基礎/臨床の有機的協力体制の構築が求められている。

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Chapter II

The Role of HTLV-I-Infected CD4$^+$ T Cells as Activated Th1 Cells in the Immunopathogenesis of HTLV-I-Associated Myelopathy /Tropical Spastic Paraparesis

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Abstract

Chronic myelitis, characterized by perivascular cuffing and parenchymal infiltration of lymphocytes, is the primary neuropathological feature of human T lymphotropic virus type 1 (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the exact cellular and molecular events underlying the induction of chronic inflammation in the spinal cord by HTLV-I are still unclear, long-standing bystander mechanisms, such as the destruction of surrounding nervous tissue by the interaction between HTLV-I-infected CD4$^+$ T cells and HTLV-I-specific cytotoxic T cells in the spinal cord, are believed to play an important role in the immunopathogenesis of HAM/TSP. As the first step in this scenario, exaggerated transmigration activity of HTLV-I-infected CD4$^+$ T cells to the spinal cord is a most important prerequisite. In this regard, we demonstrated that HTLV-I-infected CD4$^+$ T cells of HAM/TSP patients,

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compared to anti-HTLV-I-seropositive carriers, have heightened transmigrating activity to tissues based on the up-regulation of matrix metalloproteinase expression. Is Th1 or Th2 activation involved in increased transmigrating activity of HTLV-I-infected $CD4^+$ T cells in HAM/TSP patients? The facts, such as increased levels of Th1 cytokines expression with Th1 cytokine-related signaling molecules in the peripheral blood T cells of HAM/TSP patients, indicated that the immunological status of HAM/TSP is undoubtedly based on Th1 activation. Most importantly, HTLV-I-infected $CD4^+$ T cells are concentrated in the cell population having transmigration potential to the tissues with Th1 characters. In studying the signaling pathways in the regulation of Th1 activation, we found that p38 mitogen-activated protein kinase (p38 MAPK) was involved. In addition, our data indicated that activation of this signaling might be involved in the high HTLV-I proviral load in HAM/TSP patients. However, the transmigrated HTLV-I-infected $CD4^+$ Th1 cells must settle locally over a long period for the establishment of long-standing perpetuation of a chronic inflammatory state. Our study revealed that the peripheral blood $CD4^+$ T cells of HAM/TSP patients are resistant to apoptosis triggered through mitochondrial death pathway by up-regulation of Bcl-xL expression. Collectively, these findings strongly suggest that HTLV-I-infected $CD4^+$ T cells of HAM/TSP patients, having the characteristics of activated Th1 based on activation of p38 MAPK signaling, concomitant with up-regulated Bcl-xL expression, have the potential to trigger the neuropathological process in the spinal cord.

Introduction

Human T lymphotropic virus type I (HTLV-I), a member of the Oncovirinae subfamily in the family Retroviridae, is the causative agent of two clinically disparate diseases, namely adult T-cell leukemia (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [1, 2]. With the discovery of HAM/TSP, it has become evident that HTLV-I has the remarkable capacity for aggressive lymphoproliferation and profound chronic inflammation. However, the precise pathophysiologic mechanisms underlying these entirely different clinical conditions caused by HTLV-I are poorly understood. Although high HTLV-I proviral load in the peripheral blood is an important factor in the development of HAM/TSP [3, 4], it is unclear how such a situation is induced. In addition, it is not clear why only a very small proportion of HTLV-I-infected individuals develop either of these HTLV-I-associated diseases.

As the name indicates, the principal clinical manifestation of HAM/TSP is spastic paraplegia or paraparesis, characterized by a slowly progressive course of prominent upper motor neuron involvement and mild sensory deficit with sphincteric disturbance [5-7]. Not unexpectedly, the primary neuropathological feature of HAM/TSP is chronic inflammation of the spinal cord, mainly the lower thoracic cord, characterized by perivascular cuffing and parenchymal infiltration of mononuclear cells [8, 9]. Immunohistochemical studies have revealed that $CD4^+$ and $CD8^+$ T cells and macrophages infiltrate active, chronically inflammed lesions in the spinal cords of HAM/TSP patients with short-lived illness. Also, apoptosis was found in $CD45RO^+$ T cells, the main target of HTLV-I infection, with distribution of $TIA\text{-}1^+$ $CD8^+$ T cells in the lesions [10, 11]. These findings suggest that interactions between HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific $CD8^+$ cytotoxic T cells in the spinal cord are operative in the immunopathological process leading to HAM/TSP. Moreover, bystander mechanisms, such as destruction of the surrounding nervous tissue induced by the interactions

between both T-cell populations, are probably critical [12]. Finally, the fact that anti-apoptotic proteins are also expressed in both $CD4^+$ and $CD8^+$ T-cell populations [11] might explain the long-standing perpetuation of a chronic inflammatory state in the spinal cord lesions of HAM/TSP patients.

HTLV-I-infected $CD4^+$ T cells are the first responders in the immunopathogenesis of HAM/TSP. That is, exaggerated transmigration activity of HTLV-I-infected $CD4^+$ T cells to the spinal cord is a most important prerequisite for the establishment of chronic myelitis. In this review, we will analyze the immunological abnormalities in the peripheral blood $CD4^+$ T cells of HAM/TSP patients, such as transmigration, cytokine expression, anti-apoptotic protein expression, in comparison with anti-HTLV-I-seropositive carriers, and discuss the importance of HTLV-I-infected $CD4^+$ T cells as activated Th1 cells in the development of HAM/TSP.

1. Exaggerated Transmigrating Activity of Peripheral Blood HTLV-I-Infected $CD4^+$ T Cells in HAM/TSP Patients

We previously reported significantly increased adherence of peripheral blood T cells to human endothelial cells (EC) in HAM/TSP patients, compared to T cells from anti-HTLV-I-seropositive carriers and anti-HTLV-I-seronegative controls [13], as evidenced by the adherence of activated $CD4^+$ T cells (rather than $CD8^+$ T cells) to EC with heightened lymphocyte function antigen-1 (LFA-1) expression [14]. Subsequently, we identified the cells transmigrating through EC in HAM/TSP patients as activated $CD4^+$ T cells [15]. Although these findings indicate that the peripheral blood $CD4^+$ T cells of HAM/TSP patients have a prominent potential to transmigrate through EC, theses cells must penetrate and transverse the subendothelial basement membrane after trasmigrating through the EC for the invasion of tissues. Therefore, we investigated the transmigrating activity of peripheral blood $CD4^+$ T cells of HAM/TSP patients through a reconstituted basement membrane (RBM) [16], using Transwell cell-culture chambers [17]. Either peripheral blood $CD4^+$ or $CD8^+$ T cells were applied to the upper compartment of the Transwell inserts, which were polyvinylpyrrolidone-free polycarbonate filters of 8-μm pore size pre-coated with laminin on the lower surface and RBM (Matrigel) on the upper surface, respectively. After a 6-hour incubation period, the transmigrating cells in the lower chamber were counted by trypan blue staining. As shown in figure 1a, the percentage of transmigrating $CD4^+$ T cells was significantly higher than that of $CD8^+$ T cells in patients with HAM/TSP (n = 18). By contrast, no significant difference was observed in the percentage of transmigrating $CD4^+$ and $CD8^+$ T cells in controls (n = 16, including 8 anti-HTLV-I-seropositive carriers and 8 anti-HTLV-I-seronegative individuals) (figure 1b). When only transmigrated $CD4^+$ T cells were compared, the percentage of transmigrating cells in HAM/TSP patients (range, 3.0% - 40.1%; mean $\pm$ SD, 16.5% $\pm$ 12.0%) was significantly higher than that in either anti-HTLV-I-seropositive carriers (range, 0.5% - 5.0%; mean $\pm$ SD, 2.8% $\pm$ 1.8%) or anti-HTLV-I-seronegative controls (range, 0.3% - 6.9%; mean $\pm$ SD, 2.8% $\pm$ 2.6%), respectively (figure 2). Since the lower surface of the polycarbonate filters of the Transwell inserts was coated with a high concentration of laminin, the ligand of very late activating antigen-6 (VLA-6) [18], there was some possibility that the

increased transmigration activity of $CD4^+$ T cells in HAM/TSP patients might be related to the increased numbers of VLA-6^+ $CD4^+$ T cells. However, no significant differences were noted in the percentage of VLA-6^+ $CD4^+$ T cells between HAM/TSP patients and controls (data not shown).

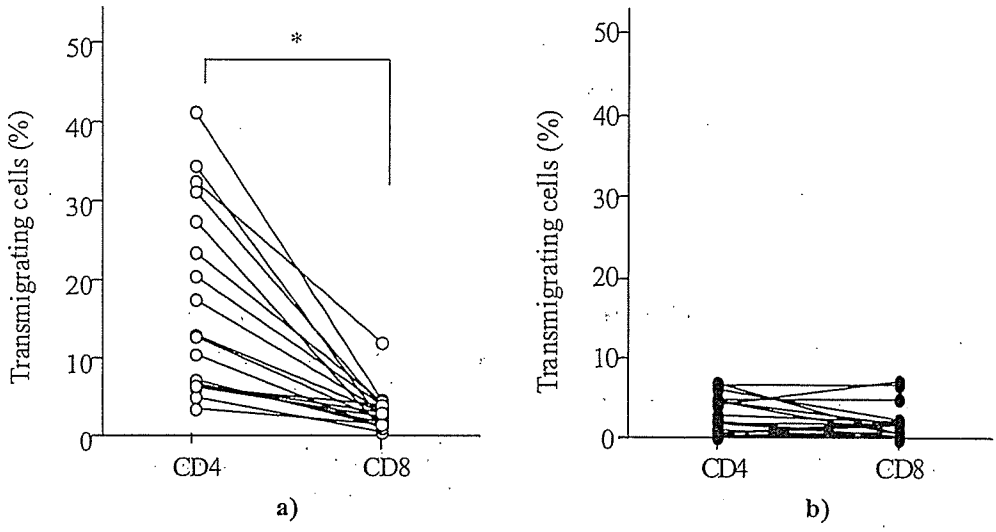

Figure 1. Percentage of transmigrating $CD4^+$ and $CD8^+$ T cells. The percentage of transmigrating cells was calculated using the formula, transmigrating cells/applied cells x 100 (%). a) The percentage of transmigrating $CD4^+$ T cells, compared to transmigrating $CD8^+$ T cells, was significantly higher in 18 HAM/TSP patients. b) In 16 controls (including 8 anti-HTLV-I-seropositive carriers), there was no significant difference between the percentage of transmigrating $CD4^+$ and $CD8^+$ T cells. $*p < 0.01$. Wilcoxon single-rank test was used for statistical analysis. Quotation from Ref. 16.

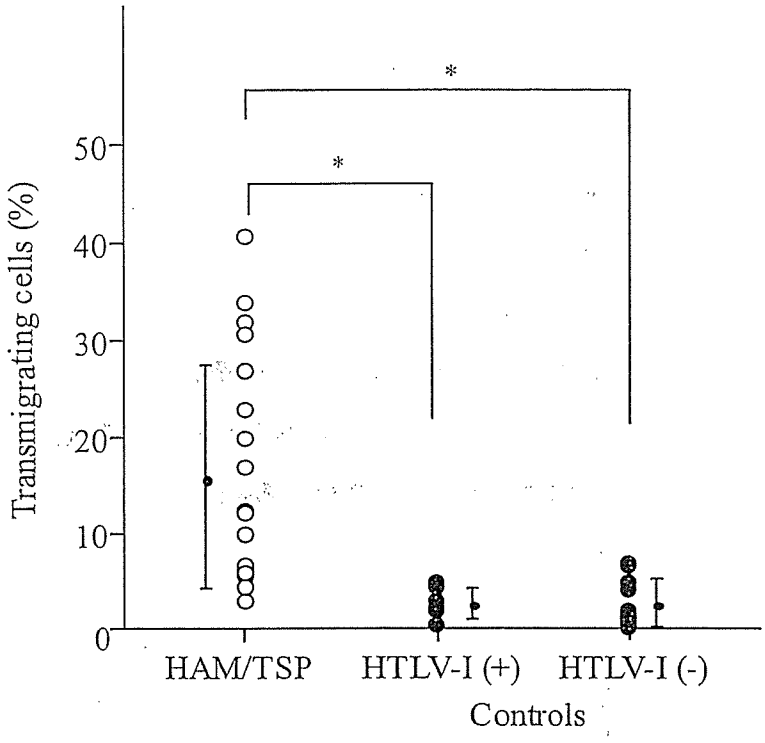

Figure 2. Comparison of the percentage of transmigrating CD4$^+$ T cells between HAM/TSP patients and controls. CD4$^+$ T cells of HAM/TSP patients (open circles) were significantly increased in comparison with either anti-HTLV-I-seropositive carriers (HTLV-I (+)) or anti-HTLV-I-seronegative controls (HTLV-I (-)) (closed circles). *$p < 0.01$; closed circle and bar, mean ± SD. Mann-Whitney U-test was used for statistical analysis. Quotation from Ref. 16.

Next, in analyzing the HTLV-I proviral load in transmigrated and non-transmigrated CD4$^+$ T cells by competitive PCR, using oligonucleotide primers of the HTLV-I pX gene, in 9 HAM/TSP patients and in 5 anti-HTLV-I-seropositive carriers, we found that the HTLV-I proviral load in transmigrated CD4$^+$ T cells from HAM/TSP patients was two- to eight-fold higher than in non-transmigrated CD4$^+$ T cells (figure 3). By contrast, no significant difference was found in HTLV-I proviral load in transmigrated and non-transmigrated CD4$^+$ T cells from anti-HTLV-I-seropositive carriers, although the copy numbers of HTLV-I provirus were very low (figure 3).

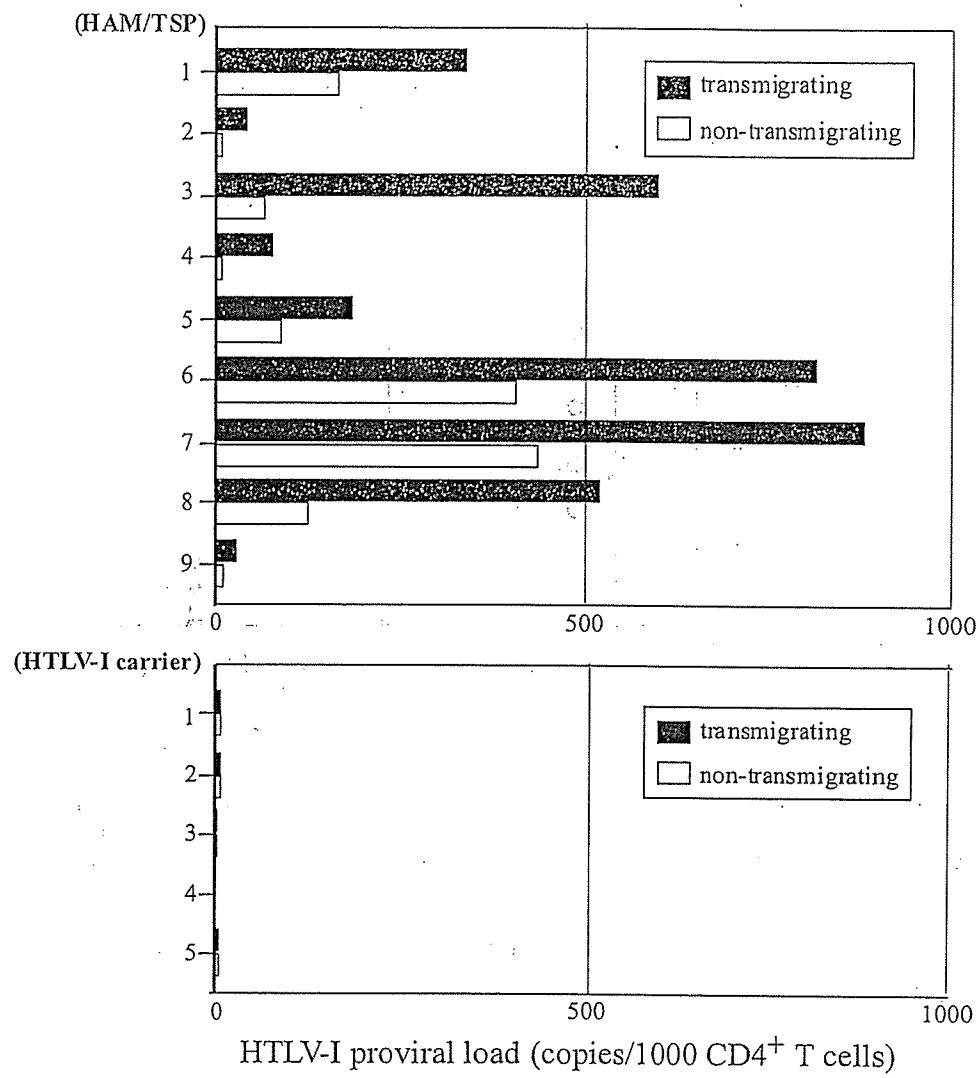

Figure 3. Quantitative analysis of HTLV-I proviral DNA in $CD4^+$ T cells. HTLV-I proviral load was compared between transmigrating and non-transmigrating $CD4^+$ T cells. HTLV-I proviral DNA, quantitated in the transmigrating $CD4^+$ T cells from the peripheral blood of 9 HAM/TSP patients, was higher than in non-transmigrating $CD4^+$ T cells. No significant difference in HTLV-I proviral load was found among 5 anti-HTLV-I-seropositive carriers (HTLV-I carrier). Quotation from Ref. 16.

Our data clearly demonstrated that $CD4^+$ T cells of HAM/TSP patients have significantly increased transmigrating activity through RBM, compared to that of anti-HTLV-I-seropositive carriers and HTLV-I-seronegative individuals. Moreover, the HTLV-I proviral load in transmigrated $CD4^+$ T cells of HAM/TSP patients was increased significantly as compared to that in non-transmigrated $CD4^+$ T cells. These findings strongly suggest that HTLV-I-infected $CD4^+$ T cells in HAM/TSP patients have exaggerated transmigration activity to extravasate from the blood to central nervous system tissues.

The findings mentioned above, such as the heightened adherence to EC and exaggerated transmigrating activity through EC and RBM of peripheral blood $CD4^+$ T cells, particularly

HTLV-I-infected CD4$^+$ T cells, from HAM/TSP patients giving rise to the phenomenon derive from *in vitro* studies. In this regard, although the rolling phenomenon is necessary *in vivo* for the initial attachment of T cells to EC before the adhesion of T cells to EC [19], the selectin family of adhesion molecules, including E-selectin and L-selectin, plays an important role [20]. We previously reported that serum levels of both soluble forms of E- and L-selectin were significantly elevated in HAM/TSP patients [21]. The degree of lymphocyte adherence to EC and the activated state of EC *in vivo* are reflected in a soluble form of both selectins in sera because soluble forms of E- and L-selectin are derived from activated EC membrane and cell surface of lymphocytes following attachment to EC, respectively [22, 23]. Therefore, elevated levels of soluble forms of selectins in HAM/TSP patients suggest that the peripheral blood CD4$^+$ T cells actively adhere to EC and transmigrate through EC and BM to the tissues *in vivo* in HAM/TSP patients. In addition, the possibility of active transmigration of peripheral blood CD4$^+$ T cells of HAM/TSP patients to the tissues *in vivo* might be also supported by the existence of other HTLV-I-associated diseases including Sjögren's syndrome [24], alveolitis [25] and uveitis [26], each of which occasionally occurs in conjunction with HAM/TSP. Indeed, we previously reported T- lymphocytic infiltration in labial salivary glands of all HAM/TSP patients examined and a very high frequency of Sjögren's syndrome in HAM/TSP patients [27]. We also reported a HAM/TSP patient with multi-organ inflammatory diseases, including Sjögren's syndrome, uveitis and interstitial cystitis, who had a high HTLV-I proviral load in the peripheral blood, suggesting that increased numbers of HTLV-I-infected cells might potentially induce systemic inflammation in several organs [28].

Overall, our findings indicate that peripheral blood HTLV-I-infected CD4$^+$ T cells from HAM/TSP patients exhibit highly invasive activities to extravasate from the blood to tissues. These activities presumably serve to trigger the first steps of the pathogenetic process in the spinal cords of HAM/TSP patients.

2. Mediators that Induce the Exaggerated Transmigrating Activities of Peripheral Blood HTLV-I-Infected CD4$^+$ T Cells in HAM/TSP Patients

Matrix metalloproteinases (MMPs) are important mediators, which play a critical role in the transmigration of T cells to tissues and the degradation of the extracellular matrix [29]. Among these MMPs, gelatinase, such as MMP-2 and MMP-9, cleave native collagen types IV and V, gelatine, fibronectin and laminin [30], which are the main components of the vascular endothelium and basement membrane. In the first step of T-cell transmigration into tissues, the interaction between each adhesion molecule, such as the integrin family, and the vascular EC or the vascular BM, subsequently followed by the induction of MMPs, is a very important process. To clarify the role of mediators in the induction of activated transmigrating activity of HTLV-I-infected CD4$^+$ T cells of HAM/TSP patients, we investigated MMP-2 in the peripheral blood T cells of HAM/TSP patients after their contact to vascular cell adhesion molucule-1 (VCAM-1), which is a ligand for the $\alpha 4\beta 1$ integrin known as very late activating antigen-4 (VLA-4) [31]. After the peripheral blood T cells of HAM/TSP patients were co-

cultured with or without VCAM-1 (+) cells for 12 hrs, only T cells were collected. After that, the collected T cells were cultured in serum-free conditions for 6 hrs and the culture supernatant was collected and designated as T sup. As shown in gelatin zymography (figure 4a), the gelatinolytic activity of 72-kDa gelatinase (MMP-2) in T sup was higher when T cells were co-cultured with VCAM-1 (+) cells compared to T sup of cultured T cells alone in 5 HAM/TSP patients than 5 control patients. As shown in figure 4a, 67-kDa gelatinase, which is the active form of 72-kDa gelatinase (MMP-2), was detected in only T sup derived from T cells of 4 of 5 HAM/TSP patients co-cultured with VCAM-1 (+) cells but not in T sup derived from T cells of all 5 control patients. In addition, anti-MMP-2 immunoblot analysis of samples from 3 HAM/TSP patients showed that the high gelatinolytic activity of MMP-2 was due to increased production of MMP-2 protein (figure 4b). For analysis of gelatinolytic activity of MMP-2, gels were photographed and scanned, then imported into Adobe Photoshop software and densitometric analysis was performed using the NIH Image Analysis software. The incremental ratio (IR) of gelatinolytic activity of MMP-2 was determined as follows: IR = densitometric counts in each T sup when co-cultured with VCAM-1 (+) cells / densitometric counts in each T sup when T cells were cultured alone. When we compared the IR among HAM/TSP patients, anti-HTLV-I-seropositive carriers and anti-HTLV-I-negative controls, the IR in HAM/TSP patients was significantly higher than that in anti-HTLV-I-seropositive carriers and in anti-HTLV-I-negative controls (figure 5). Next, in order to investigate whether the mechanisms of the induction of MMP-2 in T cells are really based on VCAM-1/VLA-4 interaction, we pretreated VCAM-1 (+) cells with anti-VCAM-1 blocking monoclonal antibody before co-culture with T cells in 5 HAM/TSP patients. In this case, the IR was determined as follows: IR = densitometric counts in each T sup when cultured with VCAM-1 (+) cells pretreated with anti-VCAM-1 blocking monoclonal antibody or control (irrelevant) monoclonal antibody / densitometric counts in each T sup when T cells were cultured alone. As shown in figure 6, although the IR of T sup of T cells co-cultured with VCAM-1 (+) cells pretreated by control monoclonal antibody were similar to the IR of T sup derived from T cells co-cultured with non-pretreated VCAM-1 (+) cells, the IR of T sup from T cells co-cultured with VCAM-1 (+) cells pretreated by anti-VCAM-1 blocking monoclonal antibody were significantly reduced, indicating that VCAM-1/VLA-4 interaction plays an important role in up-regulation of gelatinolytic activity of MMP-2 in T cells of HAM/TSP patients. As far as we compared the percentage of VLA-4^+ cells in cultured T cells from HAM/TSP patients with those of control patients, no significant differences were found between both groups.

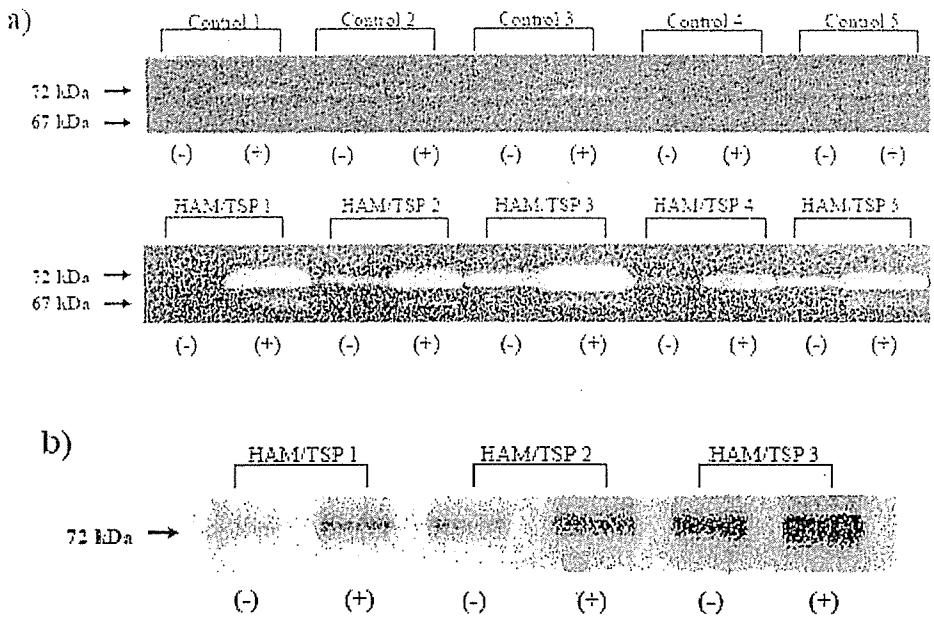

Figure 4. Representative gelatin zymography of gelatinolytic activity of MMP-2 (a) and immunoblot of MMP-2 protein (b) in T sup after co-culture with VCAM-1 (+) or after culture of T cells alone (-). (a) Gelatinolytic activity of MMP-2 (72-kDa gelatinase) in T sup was higher when T cells were co-cultured with VCAM-1 (+) cells compared to T sup from cultures of T cells alone in five HAM/TSP patients than in five controls. Gelatinolytic activity of 67-kDa gelatinase, which is the active form of 72-kDa gelatinase (MMP-2), was detected in only T sup derived from T cells of four of five HAM/TSP patients co-cultured with VCAM-1 (+) cells. (b) Immunoblot analysis of immunoprecipitates of T sup of three HAM/TSP patients indicating an up-regulated state of MMP-2 protein production. Arrow indicates MMP-2 (72-kDa gelatinase). Quotation from Ref. 31.

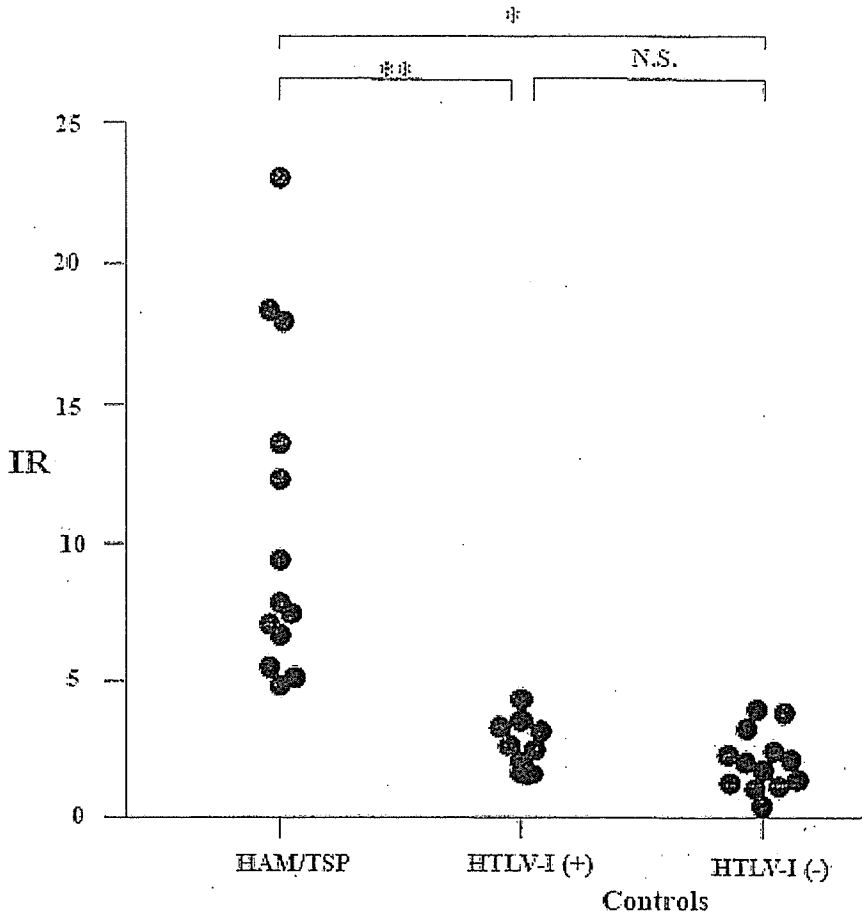

Figure 5. Comparison of IR of gelationolytic activity of MMP-2 (72-kDa gelatinase) in T sup of co-cultured T cells with VCAM-1 (+) cells or T sup derived from cultures of T cells alone between HAM/TSP and controls. IR of gelatinolytic activity of MMP-2 was determined as follows: IR = densitometric counts in each T sup when cultured withVCAM-1 (+) cells / densitometric counts in each T sup when cultured T cell alone. IR is significantly higher in HAM/TSP patients than in controls including anti-HTLV-I-seropositive carriers. HTLV-I (+): anti-HTLV-I-seropositive carriers; HTLV-I (-): anti-HTLV-I-seronegative controls. $*p < 0.0001$, $**p = 0.0002$, N.S.; not significant. Mann-Whitney's U-test was used for statistical analysis. Quotation from Ref. 31.

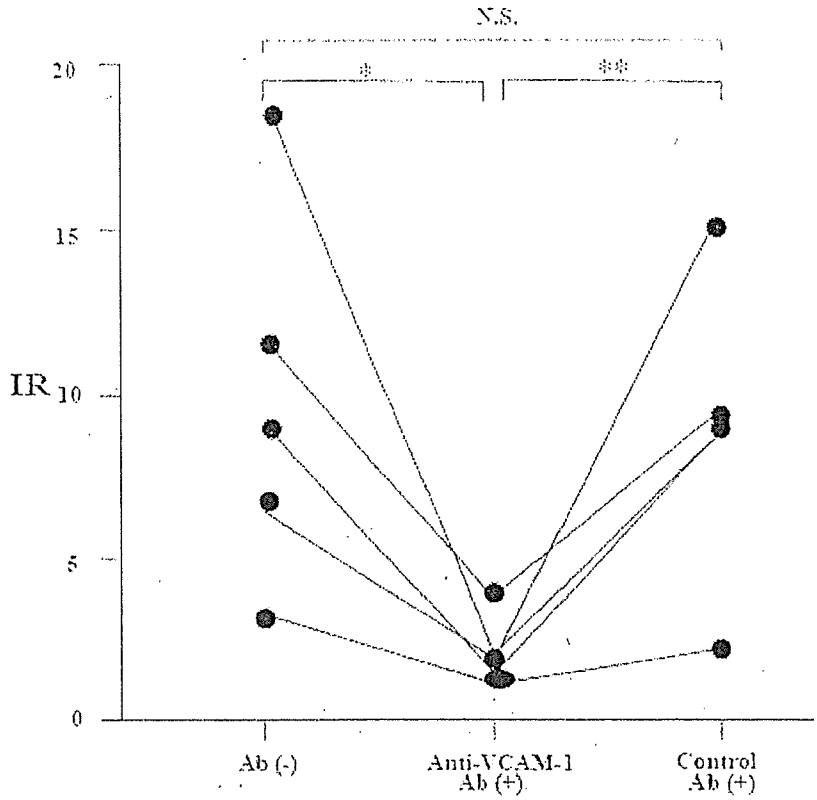

Figure 6. Effect of pretreatment of VCAM-1 (+) cells with anti-VCAM-1 monoclonal antibody on gelatinolytic activity of MMP-2 (72-kDa gelatinase) in T sup. IR of gelatinolytic activity of MMP-2 was determined as follows: IR = densitometric counts in each T sup of T cells co-cultured with VCAM-1 (+) cells or VCAM-1 (+) cells pretreated with anti-VCAM-1 monoclonal antibody or control monoclonal antibody / densitometric counts in each T sup of cultured T cell alone. IR in T sup of T cells co-cultured with VCAM-1 (+) cells pretreated with anti-VCAM-1 monoclonal antibody was significantly reduced, compared to IR of T sup derived from T cells co-cultured with VCAM-1 (+) cells pretreated with control antibody or non-pretreated VCAM-1 (+) cells. Ab (-): no treatment; Anti-VCAM-1 Ab (+): pretreatment with anti-VCAM-1 antibody; Control Ab (+): pretreatment with control antibody. *p = 0.0324, **p = 0.0237, N.S.; not significant. Wilcoxon single-rank test was used for statistical analysis. Quotation from Ref. 31.

In this study, we demonstrated that VCAM-1/VLA-4 interaction can induce up-regulation of MMP-2 activity in the peripheral blood T cells of HAM/TSP patients based on up-regulation of its expression. We previously reported increased adherence of peripheral blood CD4$^+$ T cells to human EC in HAM/TSP patients, as described in section 1 [14]. Therefore, it is suggested that the adherence of the peripheral blood CD4$^+$ T cells to EC itself triggers MMPs activation in these cells for the transmigration to the tissues in HAM/TSP patients. Although our data presented in this section are based on adhesion molecule/ligand interactions, we previously showed that the gelatinolytic activity of MMP-9 in the peripheral blood CD4$^+$ T cell of HAM/TSP patients, treated with phorbol myristate acetate (PMA), was more up-regulated than that of control patients despite no significant difference in the condition without the treatment with PMA between both groups (unpublished data). Thus, the peripheral blood CD4$^+$ T cells of

HAM/TSP patients might have the potential to be able to easily induce MMPs activation by triggering lymphocyte activation.

The immunopathological analysis of spinal cord lesions in HAM/TSP patients revealed that infiltrating mononuclear cells expressed VLA-4 concomitantly with highly expressed VCAM-1 on vascular endothelium, and MMP-2 and MMP-9 are also expressed on infiltrating mononuclear cells with disruption of vascular endothelium in chronic active lesions [32, 33]. Therefore, our *in vitro* data are supported by these histopathological findings in spinal cords of HAM/TSP patients. In addition, the importance of MMP in the transmigration of T cells, by the degradation of the extracellular matrix, is also supported by the finding that the transmigration of the peripheral blood $CD4^+$ T cells of HAM/TSP patients was significantly inhibited by the selective MMP inhibitor, *N*-biphenyl sulfonyl-phenylalanine hydroxamic acid (BPHA), in an *in vitro* transmigration assay system [34].

The exact mechanisms by which such an activated status of peripheral blood $CD4^+$ T cells of HAM/TSP patients [35, 36], induces an up-regulated state of MMP expression after activation, such as the cell adherence by adhesion molecule/ligand interaction and T-cell stimulator, remain unknown. At least the MMP-2 gene promoter seems not to be influenced by HTLV-I tax gene, since it contains neither TPA-responsive elements or NF-κB elements nor other tax-responsive elements [37]. Therefore, up-regulation of MMP-2 expression by VCAM-1/VLA-4 interaction might not be directly based on HTLV-I infection itself in HAM/TSP patients but on the altered intracellular signal transduction or cytokine expression in the peripheral blood $CD4^+$ T cells, particularly HTLV-I-infected T cells, of HAM/TSP patients. On the other hand, the MMP-9 gene promoter contains NF-kB, SP-1 and AP-1 responsive elements [38]. Therefore, HTLV-I tax can activate the MMP-9 promoter and induce MMP-9 expression in HTLV-I-infected T cells through the action of these transcription factors [39]. In this regard, it has been reported recently that the longer d(CA) repeat alleles in MMP-9 promoter polymorphism were more frequently observed in HAM/TSP patients than in HTLV-I seropositve asymptomatic carriers, suggesting that the longer alleles are involved in the up-regulated MMP-9 expression observed in HAM/TSP patients [40].

Aminopeptidase-N (AP-N), which is a widely distributed transmembrane ectoenzyme in mammalian cells [41, 42], is another proteases that degradates extracellular matrix. We previously reported that AP-N activity in peripheral blood T cells of HAM/TSP patients was increased significantly, as compared to that of controls [16]. AP-N, like MMP, has the ability to degrade type IV collagen, which is one of the main components of the basement membrane. As such, both proteinases might be involved in not only the transmigration to the tissues but also the tissue destruction, such as the damage of the blood-brain barrier [43, 44].

Our findings indicate that VCAM-1/VLA-4 interaction can induce up-regulation of MMP-2 in peripheral blood T cells of HAM/TSP patients, compared to control patients including anti-HTLV-I-seropositive carriers. However, in considering the increased transmigrating activity of peripheral blood $CD4^+$ T cells of HAM/TSP patients, HTLV-I-infected $CD4^+$ T cells of HAM/TSP patients seem to easily transmigrate to the spinal cords by using increased activity of MMP-2, MMP-9 and AP-N as the mediators