その相乗効果の有無を調べたところ，相乗指数（ The combination index ） は 0.9 以下となり，相乗効果を認めた（図 9）。

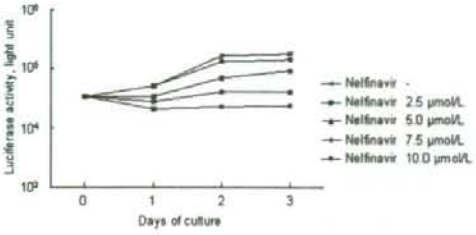
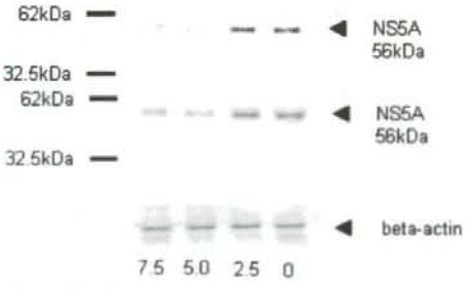


図 1.NFV の HCV-replicon に対する抑制作用



チン発現量

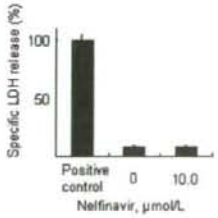


図 3.LDH 試験

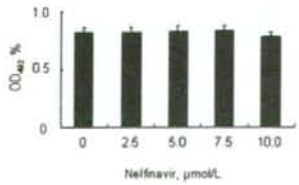


図 4.MTS 法

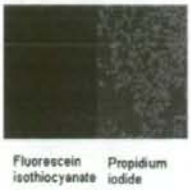


図 5.TUNNEL 染色

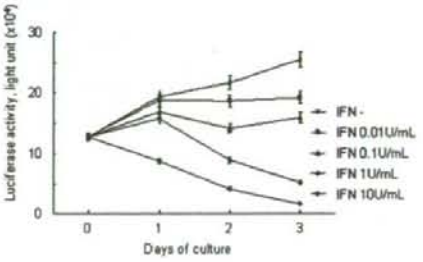


図 6.IFN の HCV-replicon に対する抑制作用

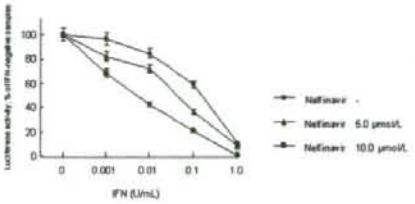


図 7.IFN と NFV の同時投与による HCV-replicon に対する抑制作用

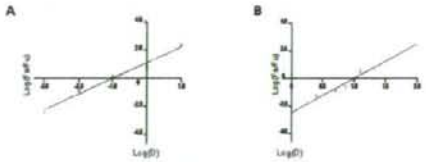


図 8. (A)NFV,線量効果曲線 y = 2.47x+0.43（B）IFN,線量効果曲線 y = 1.12x-0.14

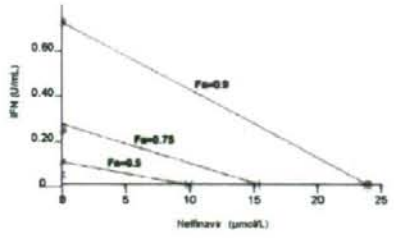


図 9. NFV と IFN の HCV-replicon 増殖に対するアイソボログラム解析

D. 考察

HCV, HIV 重複感染者に対する HAART 療法の PI 剤として NFV を選択することにより,IFN の効果を上げる可能性が示唆された.NFV の HCV に対する作用点を明らかにすることにより,細胞内における新たな HCV 複製制御機序の発見につながるものと考えられた

E. 結論

HCV、HIV 重複感染者に対する HAART 療法の PI 剤として NFV を選択することにより、IFN の効果を上げる可能性が示唆された。今後の課題は NFV の HCV に対する作用点を明らかにすることである。平成 21 年度以降の計画は、NFV 投与による肝細胞における遺伝子発現の変化についてマイクロアレイを用いて解析し、NFV の HCV 増殖抑制が既知の細胞内免疫などを介した作用であるのか、また別の新たな増殖調節機序の存在の可能性について評価を行うことである。

F. 健康危険情報

なし

G, 研究発表

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H. 知的財産権の出願・登録状況

なし

(共同研究者；山城剛　當間智)

厚生労働科学研究費補助金（創薬基盤推進研究事業）

AIDS 関連播種性 Mycobacterium avium 感染症の免疫学的機序の解明

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平成 20 年度研究分担報告書

研究要旨　Mycobacterium avium 感染症は、呼吸器疾患の一つである。HIV 感染、IL-12/IFN-γ 経路異常、移植等により全身感染を引き起こす。特に HIV 感染では独立した予後を決定する因子となり得る。しかし、その病態に関しては十分に明らかでない。我々は、その動物モデルとして、HIV 感染症に合併する播種性 M. avium 症と同様に経腸感染することが知られているブタ（276 例）を用いて基礎的検討を行い、さらに実際の AIDS 剖検例（5 例）を用いて感染病態に関する検討を行ってきた。ブタでの検討では、病巣は腸間膜リンパ節と肝臓に 98%以上の割合で感染病巣を認めた。その組織像は約半数の個体で非特異的な滲出性病巣を示した。肝病巣に着目すると、その病巣は 66%の個体で門脈域に限局し、組織像が浸潤性あるいは脾臓病変が存在する個体では有意に小葉内病巣を認めた。このことからブタは M. avium に経口感染し、腸粘膜を経てリンパ行性に全身に播種することが示唆された。このことを踏まえ AIDS 剖検症例で検討を行った。病変の組織像は、乾酪性肉芽腫が 1 例に認められた。4 例は炎症細胞の浸潤に乏しい泡沫状組織球の集簇からなっていた。また 3 例に消化管病巣を認め、4 例に腸間膜リンパ節に病巣を認めた。何れの症例においても肺病変は認められなかった。肺 M. avium 感染症においては、その組織像は類上皮細胞性肉芽腫の像を示すことが知られている。一方、AIDS 関連播種性 M. avium 感染症では泡沫状組織球の集簇が特徴的所見であり、今回の検討においてもそれに準ずるものであった。乾酪性肉芽腫を示した 1 症例は HAART 導入後であり、M. avium 診断時の CD 4 値は 162μ/ml と症例中最も高値であった。このことから宿主の免疫能が組織像に影響したと考えられる。また消化管および腸間膜リンパ節や大動脈周囲リンパ節に病巣を認めたことから、腸管が侵入門戸であり、経リンパ行性に病巣が拡大したことが推察される。しかし、血中や骨髄において菌が証明されていることから、後に血行性へと移行することが推測された。さらに免疫組織化学染色により感染病巣でのリンパ球の各分子の発現状況を検討した。その結果、腸粘膜およびリンパ節病巣では、CD4+細胞や CD56+細胞の減少、T-bet に比べ GATA-3 の優位な発現、CD3+細胞に比べ CD20+細胞の有為な増加を認めた。またリンパ節病巣では FoxP3、SOCS3 の発現増加が認められた。HIV 感染では腸粘膜での CD4+細胞の枯渇や NK 細胞の減少、機能不全が知られている。このことは菌の腸粘膜下での感染を容易にする。また SOCS3、FoxP3 の発現増加は IL-12/IFN-γ 経路の抑制に繋がる。このことから感染制御に重要な IFN-γ や TNF-α の産生低下により菌の増殖、肉芽腫の形成不全に至り、播種が成立すると考えられた。今後、他の臨床病型との比較からその病態を捉えていきたい。

A. 研究目的

Mycobacterium avium 感染症は、既往歴のない中高年女性を中心に環境中より経気道的に感染することで発症する呼吸器疾患の一つである。しかしながら HIV 感染症の流行とともに日和見的な全身感染の存在が明らかとなり、近年は、抗レトルウイルス薬による治療（HAART）の導入によりリンパ節炎などを主体とする免疫再構築に伴う M. avium 感染症が臨床的な重要性を増している。また AIDS 症例では経腸感染が主体であり、播種性であっても肺病変を形成する率は低いことが知られている。本研究の目的は、HIV 感染症に合併する M.avium 感染症の臨床病理学的解析を行うことを目的とする。

B. 研究方法

2002 年から 2004 年の間、沖縄県でと畜されたブタのうち、全身性に M. avium 感染による肉芽腫性病巣を認めた 276 個体、感染組織としては 3,312 検体（276 個体 x12 組織）を対象として用いた。全身感染の定義は、腸間膜リンパ節あるいは顎下リンパ節と肝臓あるいは脾臓や肺など実質臓器に感染病巣を認める個体とした。また AIDS 症例での検討に対しては、国立国際医療センターにて 2001

年から 2003 年までの間、剖検が行われた HIV 感染者のうち播種性 M. avium 感染が確認された 5 症例を対象とした。
得られた組織は、一般的な処理により組織標本を作製し、病理組織学的検討に用いた。
菌種の同定はアンプリコアマイコバクテリウムキット（日本ロッシュ社製）を用い、亜種の同定には遺伝子挿入配列 IS1245 の制限断片長多型配列を解析することにより行った。

（倫理面への配慮）
剖検材料の使用に関しては、研究機関および国際医療センターの倫理委員会の承認を受け、規則に従い実施した。実際には、剖検時の承諾書により研究目的に組織を用いる事の同意がなされている。またヒト組織材料を使用するため、下記の点を留意して実験を行った。

1）研究に用いる組織は、他の研究目的には使用しない。
2）カルテ等の個人情報に関しては、施設外に持ち出す際は、個人を特定を不可能とするための処置を行い、記号番号により管理した。
3）本研究により、直接提供者が医学上の利益・不利益を得ることはない。

C. 研究結果
ブタに関する研究については論文としてまとまったので PDF ファイルを添付する。ブタでの検討では、病巣は腸間膜リンパ節と肝臓に 98%以上の割合で感染病巣を認めた。その組織像は、約半数の個体で非特異的な滲出性病巣を示した。肝病巣に着目すると、その病巣は 66%の個体（n=112）で門脈域に限局し、組織像が浸潤性あるいは脾臓病変が存在する個体では有意に小葉内病巣を認めた。このことからブタは M.avium に経口感染し、腸粘膜を経てリンパ行性に全身に播種することが示唆された。このことを踏まえ AIDS 剖検症例で検討を行った。対象とした 5 例のうち多剤併用療法（HAART）導入前の症例は 2 例、導入後の症例は 3 例であった。全症例で抗結核薬の標準的な投与がなされていた。病変の組織像は、壊死を伴う被包化された肉芽腫が 1 例に認められた。4 例は炎症細胞の浸潤に乏しい泡沫状組織球の集簇からなっていた。また 3 例に消化管病巣を認め、4 例に腸間膜リンパ節に病巣を認めた。何れの症例においても肺病変は認められなかった。免疫応答宿主の肺 M. avium 感染症においては、その組織像は類上皮細胞性肉芽腫の像を示し、時に乾酪性であることが知られている。一方、AIDS 関連播種性 MAC 症では泡沫状組織球の集簇が特徴的所見であり、今回の検討においてもそれに準ずるものであった。壊死を伴う肉芽腫を示した 1 症例は HAART 導入後であり、M. avium 診断時の CD４値は 162μ/ml と症例中最も高値であった。このことから宿主の免疫能が組織像に影響したと考えられる。また消化管および腸間膜リンパ節や大動脈周囲リンパ節に病巣を認めたことから、腸管が侵入門戸であり、経リンパ行性に病巣が拡大したことが推察される。しかし、血中や骨髄において菌が証明されていることから、後に血行性へと移行することが推測された。

さらに免疫組織化学染色により感染病巣でのリンパ球の各分子の発現状況を検討した。腸粘膜およびリンパ節病巣では、CD4+細胞や CD56+細胞の減少、T-bet に比べ GATA-3 の優位な発現、CD3+細胞に比べ CD20+細胞の有為な存在を認めた。またリンパ節病巣では FoxP3、SOCS3 の発現増加が認められた。HIV 感染では腸粘膜での CD4+細胞の枯渇や NK 細胞の減少、機能不全が知られている。このことは菌の腸粘膜下での感染を容易にする。また SOCS3、FoxP3 の発現増加は IL-12/IFN-γ 経路の抑制に繋がる。このことから感染制御に重要な IFN-γ や TNF-α の産生低下により菌の増殖、肉芽腫の形成不全に至り、播種が成立すると考えられた。

D. 考察
HIV 感染では腸粘膜での CD4+リンパ球の枯渇や NK 細胞の減少、機能不全が知られている。このことは菌の腸粘膜下での感染を容易にする。また SOCS3、FoxP3 の発現増加は IL-12/IFN-γ 経路の抑制に繋がる。このことから感染制御に重要な IFN-γ や TNF-α の産生低下により菌の増殖、肉芽腫の形成不全に至り、播種が成立すると考えられた。

E. 結論
結核を中心とする基礎的な研究から、病原体の毒性や播種に関与する生体側の要因に関して提唱がなされてきた。しかし、こうした要因がヒトであるいは M. avium 感染症においても当てはまるのかは明らかではない。一方で CD8+あるいは γδ＋T 細胞ではなく CD4+T 細胞が M. avium 感染に対する免疫学的な要因として重要であることがマウスを用いた研究から明らかにされている。こうした背景から、我々はまず、これまでの検討で得られた進展機序に関する仮説を C57BL/6

beige マウスを用いて検討し、病理像との関係から免疫学的な検討を加えていく予定である。一方で播種を引き起こすもう一方の要因である菌側要因に関しては、in vitro でヒト腸上皮細胞（Caco-2 細胞）および肺胞上皮細胞（A549 細胞）を用いて現在、検討中である。

F. 健康危険情報
なし

G. 研究発表

1. 論文発表
(1) Hibiya K, Kazumi Y, Nishiuchi Y, Sugawara I, Miyagi K, Oda Y, Oda E, Fujita J Descriptive analysis of the prevalence and the molecular epidemiology of Mycobacterium avium complex-infected pigs that were slaughtered on the main island of Okinawa. Comp Immunol Microbiol Infect Dis. (in press)

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(2) 日比谷健司, 仲村秀太, 田里大輔, 知念寛, 比嘉太, 健山正男, 照屋勝治, 菊池嘉, 岡慎一, 望月眞, 遠藤久子, 藤田次郎 「AIDS 関連播種性 Mycobacterium avium 感染症の免疫学的機序」第 61 回 日本呼吸器学会 日本結核病学会 九州支部秋期学術講演会 宜野湾市

H. 知的財産権の出願・登録状況
なし

(共同研究者；日比谷健司、仲村秀太、田里大輔、 山城剛、 知念寛、比嘉太、健山正男、仲宗根勇、山根誠久、久場睦夫、鹿住祐子、菅原勇、稲垣考、市川和哉、小川賢二、西内由紀子、照屋勝治、菊池嘉、岡慎一、 望月眞、遠藤久子)

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レイアウト

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
Koyanagi Y, Tanaka Y, Ito M, and Yamamoto N.	Humanized mice for humanretrovirus infection	Nomura, T; Watanabe, T; Habu, S	Humanized Mice. Curr. Top. Microbiol. Immunol.	Springer	Berlin	2008	324: 133-48

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
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Yoshida T, Kawano Y, Sato K, Ando Y, Aoki J, Miura Y, Komano J, Tanaka Y, and Koyanagi Y.	A CD63 mutant inhibits T-cell tropic HIV-1 entry by disrupting CXCR4 trafficking to the plasma membrane.	Traffic	9	540–58	2008
Kondo K, Okuma K, Tanaka R, Matsuzaki G, Ansari AA, and Tanaka Y.	Rapid induction of OX40 ligand on primary T cells activated under DNA-damaging conditions.	Hum Immunol	69	533-42	2008

Zhang LF, Okuma K, Tanaka R, Kodama A, Kondo K, Ansari AA, and Tanaka Y.	Generation of mature dendritic cells with unique phenotype and function by in vitro short-term culture of human monocytes in the presence of interleukin-4 and interferon-beta.	Exp Biol Med (Maywood)	233	721-31	2008
Kamiyama H, Yoshii H, Tanaka Y, Sato H, Yamamoto N, and Kubo Y.	Raft localization of CXCR4 is primarily required for X4-tropic human immunodeficiency virus type 1 infection.	Virology	386	23-31	2009
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Ⅳ. 研究成果の刊行物・別刷

Humanized Mice for Human Retrovirus Infection

Y. Koyanagi(✉), Y. Tanaka, M. Ito, and N. Yamamoto

Abstract Inbred mice with specific genetic defects have greatly facilitated the analysis of complex biological events. Several humanized mouse models using the C.B.-17 *scid/scid* mouse (referred to as the SCID mouse) have been created from two transplantation protocols, and these mice have been utilized for the investigation of human immunodeficiency virus type 1 (HIV-1) and human T-lymphotropic virus type I (HTLV-I) pathogenesis and the evaluation of antiviral compounds. To generate a more prominent small animal model for human retrovirus infection, especially for examination of the pathological process and the immune reaction, a novel immunodeficient mouse strain derived from the NOD SCID mouse was created by backcrossing with a common γ chain (γ_c)-knockout mouse. The NOD-SCID γ_c^{null} (NOG) mouse has neither functional T and B cells nor NK cells and has been used as a recipient in humanized mouse models

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for transplantation of human immune cells particularly including hematopoietic stem cells (HSC). From recent advances in development of humanized mice, we are now able to provide a new version of the animal model for human retrovirus infection and human immunity.

Abbreviations AIDS: acquired immunodeficiency syndrome; APC: antigen-presenting cell; ATL: adult T-cell leukemia; AZT: azidothymidine; CCR5: cc-chemokine receptor 5; DC: dendritic cell; ddI: dideoxyinosine; DN: double negative; γ_c; common gamma chain; GVHD: graft-versus-host disease; HAM: HTLV-I-associated myelopathy; HIV-1: human immunodeficiency virus type 1; HSC: hematopoietic stem cell; HTLV-I: human T-lymphotropic virus type I; IL: interleukin; MHC: major histocompatibility complex; PBMC: peripheral blood mononuclear cell; SCID: severe combined immunodeficiency; SP: single positive; TRAIL: tumor necrosis factor (TNF)-related apoptosis-inducing ligand; TSP: tropical spastic paraparesis

1 Mouse/Human Chimeric Models for HIV-1 Infection Using SCID Mouse

The C.B.-17 *scid/scid* (SCID) mouse carries a spontaneously arising autosomal recessive mutation and was found to have severe combined immunodeficiency (SCID) by Bosma and colleagues (Bosma et al. 1983). This strain has a defect of DNA protein kinase and a lack of progenitors to T and B cells (Blunt et al. 1995; Boubnov and Weaver 1995; Kirchgessner et al. 1995; Miller et al. 1995; Peterson et al. 1995). Therefore, these mice are unable to reject the xenograft and they tolerate engraftment of human cells or tissues, followed by subsequent infection with human immunodeficiency virus type 1 (HIV-1). Two of these models are the SCID-hu thy/liv mouse developed by McCune and colleagues (McCune et al. 1988) and, the hu-PBL-SCID mouse developed by Mosier and colleagues (Mosier et al. 1988) (Fig. 1).

1.1 HIV-1 Infection in the SCID-hu thy/liv Mouse

The SCID-hu thy/liv mouse is generated by surgical coimplantation of a piece (1 mm) of human fetal thymus and liver under the murine kidney capsule. The implanted tissues produce a conjoint organ (thy/liv) that appears to reconstitute normal thymus for more than 1 year. The fetal liver provides hematopoietic precursors that are located in islets between the thymic lobes. Thymic epithelial cells are derived from the fetal thymus, whereas the hematopoietic cells including T cells and dendritic cells (DC) are derived from the liver (Vandekerckhove et al. 1992). The generated thymus is composed of more than 70% CD4CD8

Mouse strain	human tissue or cells	References
1) SCID-hu thy/liv mouse		
CB17 SCID mouse	human thy/liv tissue	McCune et al. 1988
2) Human-PBL-SCID mouse		
CB17 SCID mouse	human PBMC	Mosier et al.1991
NOD-SCID mouse	human PBMC	Koyanagi et al. 1997
NOG mouse	human PBMC	Nakata et al. 2005

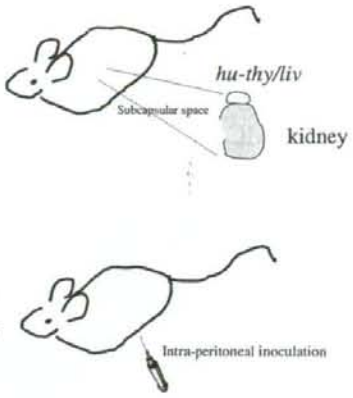

Fig. 1 Mouse/human chimeric models for HIV-1 infection. HIV-1-susceptible humanized mouse models have been reported from two transplantation protocols

double-positive (DP) cells, and the rest are CD4 or CD8 single-positive (SP) and double-negative (DN) T cells. Although these CD4 or CD8 SP cells migrate into the peripheral circulation and express a naive phenotype (>70% $CD45RA^+$), the numbers of these cells are very low (Jamieson and Zack 1999). In addition, no immune response to viral antigen has been found so far. Thus, the reconstitution of the human immune system is not complete in this mouse. Since some skill in the surgical operation to coimplant the thy/liv organ and systematic support on distribution of human fetal organs are required, the SCID-hu thy/liv mouse studies have been carried out in limited numbers of laboratories, mainly in the US.

The human thy/liv implant in SCID mice is highly susceptible to infection with HIV-1, including R5 and X4 HIV-1 (McCune et al. 1991; Namikawa et al. 1988). As the number of circulating human $CD4^+$ T cells is low as mentioned above, direct injection of virus into the implant is performed under anesthesia. The level of HIV-1 replication and potential with CD4 T cell depletion of X4 HIV-1 appears to be higher than that of R5 HIV-1 within a few weeks of infection by evaluation with PCR and flow cytometric analyses (Kaneshima et al. 1994). Immunohistological examination indicated that the infected cells initially appeared in the thymic cortical regions and subsequently spread through the entire organ after HIV-1 infection (Stanley et al. 1993). Flow cytometric analysis indicated that the CD4CD8 DP cells were almost completely eradicated and the ratio of $CD4^+$ and $CD8^+$ thymocytes was reversed after infection (Aldrovandi et al. 1993; Bonyhadi et al. 1993). Furthermore, SCID-hu thy/liv mouse have been also used to assess efficacy of several anti-HIV compounds administrated before infection, including azidothymidine (AZT) and 2′,3′-dideoxyinosine (ddI) (Kaneshima et al. 1991; McCune et al. 1990; Rabin et al. 1996).

1.2 HIV-1 Infection in the hu-PBL-SCID Mouse

The hu-PBL-SCID mouse is created by injection of peripheral blood mononuclear cells (PBMC) from healthy adults into the peritoneal cavity of SCID mice, and human $CD4^+$ and $CD8^+$ T cells circulate through the peritoneal cavity, peripheral blood, and organs such as spleen and liver for more than 1 month after PBMC injection (Mosier et al. 1988). The presence of human $CD4^+$ T cells makes this attractive as a model to study HIV-1 pathogenesis and evaluation of anti-HIV compounds (Mosier et al. 1991; Pastore et al. 2003; Hartley et al. 2004). Although it was initially reported as a model with little graft-versus-host disease (GVHD) in the hu-PBL-SCID mice, the high levels of T cell-reconstituted mice develop symptoms of GVHD within 2 months after injection of PBMC (Sandhu et al. 1995). Therefore, long-term observation may not be possible in this model. In addition, human $CD4^+$ and $CD8^+$ T cells in hu-PBL-SCID mice expressed the CD45RO antigen, a marker found in either activated or memory T cells (Tary-Lehmann and Saxon 1992). This is not similar to the ratio of normal adult PBMC, which contain approximately 50% $CD45RO^+$ and $CD45RA^+$ (naive) cells. Furthermore, human $CD4^+$ cells in the hu-PBL-SCID also express abundant levels of HIV-1 coreceptor CCR5, and accordingly, R5 but not X4 HIV-1 more actively replicates in this system (Mosier et al. 1993; Nakata et al. 2006). The relative ease with which this model can be generated and the high efficiency of R5 HIV-1 infection make this system very attractive to study HIV-1 pathogenesis for researchers who struggle in obtaining fetal organs for generating SCID-hu thy/liv mouse. Importantly, a significantly high level of HIV-1 replication correlates with severe depletion of human $CD4^+$ T cells within 2 weeks after infection, and the replication is dependent on Nef protein (Kawano et al. 1997). Thus, this model appears to be adequate for short-term investigation of HIV-1 replication and pathogenesis. As mentioned above, the reconstituted $CD4^+$ and $CD8^+$ T cells are strongly activated and have memory phenotypes, indicating that these cells are xenoreactive proliferated but anergic T cells (Tary-Lehmann and Saxon 1992; Tary-Lehmann at al. 1995). In this model, neither thymopoiesis nor hematopoiesis is generated (Koyanagi, unpublished observations). Thus, it is assumed that the pathological events of HIV-1 infection in this model include that in mature T cells in humans.

1.3 Human Acquired Immune Responses in the hu-PBL-SCID Mouse

Of interest, some immune responses are induced, including humoral and cellular immune responses in the hu-PBL-SCID mouse with administration of various antigens (Gorantla et al. 2005; Ifversen P and Borrebaeck 1996; Nonoyama et al. 1993; Sandhu et al. 1994). However, there are two major limitations to development of strong human immune responses in the hu-PBL-SCID mice. The first is the lack of

appropriate human antigen-presenting cells (APC) including DC. The second is the lack of a suitable microenvironment such as the presence of normal lymphoid organs and architecture that may facilitate induction and maintenance of immune effector cells (Tary-Lehmann et al. 1995). To overcome the lack of APC, Delhem et al. used autologous skin that contains tissue DC as a source of APC and succeeded in demonstrating the induction of primary MHC-restricted human T cell responses against HIV-1 envelope in the hu-PBL-SCID mice (Delhem et al. 1998). Santini et al. have reported that inactivated HIV-1-pulsed, monocyte-derived, and matured human DC can stimulate human anti-HIV-1 antibody production by B cells from HIV-1-negative donors in the SCID mouse system, and that this immune response is partially protective (Santini et al. 2000). However, there had been no attempts to overcome the lack of a suitable microenvironment in this hu-PBL-SCID mouse until our report (Yoshida et al. 2003).

To overcome the deficiency of a suitable microenvironment in the hu-PBL-SCID mouse system, we attempted to transfer PBMC together with inactivated HIV-1-pulsed autologous monocyte-derived DC directly into the mouse spleen (Yoshida et al. 2003). The intrasplenic inoculation of PBMC was found to reduce excessive GVHD compared to the intraperitoneal transfer method (Tanaka et al., unpublished observations). In addition, with this procedure we could obtain larger yields of human T cells than with the conventional intraperitoneal transfer. Therefore, we reasoned that the microenvironment in the mouse spleen should provide human T cells with optimum conditions for activation (Fig. 2). With this new immunizing protocol, we have succeeded in eliciting a protective $CD4^+$ T cell immunity against R5 HIV-1 infection. We were surprised to see that the immunized mice were totally resistant against challenge with live R5 HIV-1 (Yoshida et al. 2003). The protective immunity was induced equally with either R5 or X4 HIV-1 as an antigen. The sera from the immunized mice contained a soluble R5 HIV-1 suppressive factor that was mainly synthesized by human $CD4^+$ T cells in response to HIV-1 antigen, specific peptides of HIV-1 according to MHC class II haplotypes (Yoshida et al. 2005), but

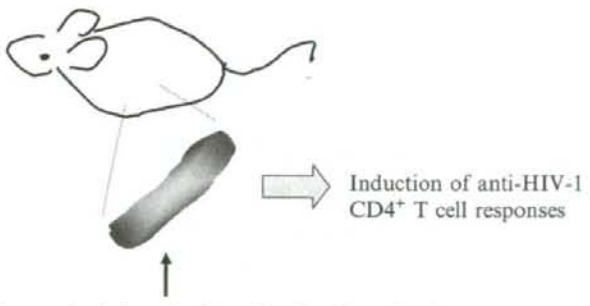

Fig. 2 Human acquired immune responses in the hu-PBL-SCID mouse. Protective $CD4^+$ T cell immunity against R5 HIV-1 infection is induced by PBMC transfer together with inactivated HIV-1-pulsed autologous DC directly into the mouse spleen

not ovalbumin as a control. The factor appears to be unrelated to the currently identified R5 HIV-1 suppressive cytokines examined by neutralization assay using antibodies against CCR5-binding β-chemokines, IL-4, IL-10, IL-12, IL-13, IL-16, MCP-1, MCP-3, IFN-α, IFN-β, TNF-α, and TNF-β.

Our study indicates that a DC-based HIV-1 vaccination can induce HIV-1-reactive human $CD4^+$ T cells producing a yet-undefined R5 HIV-1 suppressor factor. The demonstration made by Lu et al. (Lu et al. 2003) that DC pulsed with AT-2 inactivated simian immunodeficiency virus (SIV) can also stimulate protective anti-SIV specific T cell and antibody responses in rhesus monkeys suggests a rational basis for the DC-based immunization against HIV-1 infection. This idea is further supported by the recent findings by Lu et al. (Lu et al. 2004), who showed the efficacy of a therapeutic DC-based whole HIV-1 virion vaccination for HIV-1 infection.

In order to achieve successful DC-based immunization against HIV-1, a large number of monocyte-derived mature DC with immuno-stimulating activity is expected. A conventional method for generating DC from monocytes has been established, and there are commercially available kits for monocyte purification. In addition, we recently found a simple method to isolate monocytes from bulk PBMC by using antichemokine receptor monoclonal antibody-coated plates (Nimura et al. 2006). These monocytes could be differentiated to Th1-inducing DC and expressed low levels of cell surface CD4 and CCR5. When sensitized with inactivated HIV-1, these DC could induce the R5 HIV-1-suppressing factor in the hu-PBL-SCID mouse (Nimura et al. 2006). Because human monocytes and immature DC are still susceptible to R5 HIV-1 infection, this method, which can induce HIV-1-resistant DC, will be helpful for HIV-1-infected individuals in generation of therapeutic DC against acquired immunodeficiency syndrome (AIDS).

2 Development of Immunodeficient Mouse Strains for Improvement of Human Cell Reconstitution and HIV-1 Infection

2.1 Generation of Novel Immunodeficient Mouse Strains

By introduction of the *scid/scid* mutation in various strains of inbred mice possessing defects in innate immunity, we surveyed congenic mouse strains to find an adequate immunodeficient mouse for transplantation of human cells and HIV-1 infection. From extensive transplantation experiments with human PBMC in immunodeficient mouse strains, the NOD-SCID mouse, which contains some defects in the function of complements and macrophages, was found to be a most suitable strain as a recipient for human cell reconstitution as well as HIV-1 infection (Koyanagi et al. 1997a). The number of human cells in the human PBMC-transplanted NOD-SCID (hu-PBL-NOD-SCID) mice was more than

three- to fivefold higher than that in the conventional hu-PBL-SCID mice. More importantly, the levels of HIV-1 viremia were significantly high in the HIV-1 infected hu-PBL-NOD-SCID mice. Some HIV-1-infected mice exhibited more than 1 ng of p24 antigen per milliliter in plasma, which is a much higher concentration than that in HIV-1-infected patients, and showed severe CD4 depletion after intraperitoneal inoculation with R5 HIV-1. Using this mouse model, we found that tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) but not Fas ligand induced bystander killing of $CD4^+$ T cells in the mouse spleen (Miura et al. 2001). In addition, when these HIV-1-infected mice were treated with a sublethal dose of lipopolysaccharide, HIV-1-infected cells migrated into mouse brain tissue and induced apoptosis in neuronal cells via TRAIL molecules expressed on the HIV-1-infected cells, probably macrophages (Miura et al. 2003). These data suggested that TRAIL should have a pathological role of disease progression for AIDS.

Recently, further genetic manipulation has been performed. A more profoundly severe immunodeficient mouse strain, defective in common γ (γ_c) chain, which is a component of receptors for IL-2, IL-7, and other cytokines and critical for generation of NK and T cells, was generated from the NOD-SCID mouse (Ito et al. 2002) and the recombination activating gene-2 $(RAG\text{-}2)^{null}$ mouse. The $RAG\text{-}2^{null}$ mouse is also a genetically manipulated immunodeficient strain, which has a defect in the differentiation of T and B cells. NOD-SCID γ_c^{null} (NOG) and $RAG\text{-}2/\gamma_c^{null}$ mouse strains were then generated, and these mice have been confirmed to have neither functional T and B cell nor NK cells and have been used as humanized mouse models by transplantation of human immune cells. The level of human cell reconstitution was greatly improved in NOG as well as $RAG\text{-}2/\gamma_c^{null}$ mice transplanted with human PBMC (hu-PBL-NOG and hu-PBL-$RAG\text{-}2/\gamma_c^{null}$ mice) (Nakata et al. 2005; Koyanagi et al. 1997b). Moreover, a technical improvement should be noted in that it is not necessary for preceding treatment with antibody, an anti-IL-2 receptor β chain antibody or an anti-asialo GM-1 antibody, to protect mouse NK cell differentiation in recipient mice.

2.2 *HIV-1 Infection in the hu-PBL-NOG Mouse*

Using NOG mice, we indicated the usefulness of the mouse strain for evaluation of an anti-HIV-1 compound (Nakata et al. 2006). HIV-1 suppressive efficacy of a small molecule of CCR5 antagonist was confirmed in the mice 1 day after HIV-1 inoculation. The level of viral loads and HIV-1-induced CD4 depletion was dramatically suppressed after treatment of a CCR5 antagonist, suggesting that the NOG mouse should serve as a small animal model for evaluation of anti-HIV-1 compounds (Nakata at al. 2006). Furthermore, it is noteworthy that this hu-PBL-NOG model provides a greater reproducibility of high viremia levels than the conventional HIV-1-infected SCID mouse models and that the high level of viremia achieved in this mouse model made it possible to monitor the changes in the