RESEARCH CENTER FOR ACQUIRED IMMUNODEFICIENCY SYNDROME
LABORATORY OF VIRAL PATHOGENESIS

A long standing goal of our research group is to elucidate the molecular mechanisms of virus pathogenesis. We have been focusing on two human viruses, human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus type 1 (HSV-1).

1) Molecular analysis on interaction of hiv-1 and host factors: T. YOSHIDA, K. SATO, Y. SHINODA, T. KOBAYASHI, T. WATANABE, S. YAMAMOTO and Y. KOYANAGI

Changing cellular protein trafficking may represent a novel antiviral approach. We have discovered that an N-terminal deletion mutant of a membrane protein, CD63, (CD63ΔN) blocks entry of CXCR4-using, T-cell tropic human immunodeficiency virus type 1 (X4 HIV-1) by suppressing CXCR4 surface expression. This suppression was observed in CXCR4 but not in CD4, CCR5, CD25, CD71, or other tetraspanin proteins. From a series of extensive analyses including confocal and total internal reflection fluorescence microscopy examination, the suppression of CXCR4 expression on the plasma membrane appeared to be caused by mislocalization of CXCR4 and exclusive transportation of CXCR4 toward intracellular organelles mainly the late endosomes/lysosomes. Our data suggest that CXCR4 trafficking is able to be modified in terms of its recruitment to the plasma membrane without enhancing the degradation or arresting vesicular transport of CXCR4 (*Traffic*, 9: 540–558, 2008). Furthermore, we found that wild type CD63 molecule was eliminated from the plasma membrane of HIV-1-producing T cell after activation, followed by a decrease in the amount of virion-incorporated CD63, and oppositely, an increase in the infectivity of the released virions. On the other hand, we found that CD63 at the cell surface was preferentially embedded on the membrane of released virions in HIV-1 envelope protein (Env)-independent manner, and that virion-incorporated CD63 had a potential to inhibit HIV-1 Env-mediated infection in a strain-specific manner at the post-attachment entry step(s). In addition, these behaviors were commonly observed in the other tetraspanin proteins, such as CD9, CD81, CD82 and CD231. However, L6 protein, which has similar topology with tetraspanins but does not belong to tetraspanin superfamily, did not have the potential to prevent HIV-1 infection, even though its successful incorporation into the released particles (*J. Virol.*, 82:1021-1033, 2008). Taken together, these results suggest that tetraspanin proteins commonly have an unique potential to modulate HIV-1 infectivity through incorporation into released HIV-1 particles, and our findings may provide a clue to elucidate uncovered aspects of HIV-1 entry. We are also attempting to identify HIV-associated host factors using a variety of

genetics- and protein chemistry-based methods. Candidate of the HIV-associated host factors under investigation are interferon, cytokines, signal molecules, and membrane proteins.

2) HIV pathogenesis: K. SATO, N. MISAWA, J. CHUANYI NIE, H. KITAYAMA and Y. KOYANAGI

We developed a small animal model in which human cells (peripheral blood lymphocytes, PBL) are transplanted into a T, B, and NK cell-deficient mouse (NOD/SCID/IL-2Rγ^{null} mouse, NOG mouse) strain created in collaboration with the Central Institute for Experimental Animals in Kanagawa. In the human PBL-transplanted mice (hu-PBL-NOG-SCID mouse), abundant human CD4 (hCD4) cell killing was reproduced with CCR5-using (R5) HIV-1 infection and HIV-1 induced a neuropathology that resembled HIV encephalopathy observed in human patients. Using the model, we have found that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) but not Fas-ligand (FasL) has critical contribution in the HIV-1-induced $CD4^+$ T cell bystander killing as well as neuronal apoptosis in the central nervous system (CNS) (*J. Exp. Med.* 193, 651-659, 2001, *Proc. Natl. Acad. Sci. USA*. 100, 2777-2782, 2003). TRAIL could therefore be a potential target for the therapeutic intervention of HIV-induced bystander cell killing in HIV-1-infected patients. However, the chimeric mice transplanted with human PBL are not fully reconstituted with human blood cells. Thus, we recently developed a novel human-chimera mouse model, NOG-hCD34 mice by transplanting newborn NOG mice with $hCD34^+$ cells via hepatic injection. The longitudinal flow cytometric analyses showed that NOG-hCD34 mice were able to support human hematopoiesis and multilineage differentiation of human leukocytes for at least 44 weeks. When infected with CCR5-tropic or CXCR4-tropic HIV-1, NOG-hCD34 mice reproduced HIV-1-associated complications such as intrathymic infection, sequential decrease in peripheral as well as splenic hCD4/hCD8 ratio and depletion of splenic $hCD4^+hCD45RA^-hCCR5^+$ T lymphocytes. High plasma HIV-1 RNA load was observed in CCR5-tropic HIV-1 infected mice for prolonged period of time. The infected mice were depleted in peripheral $hCD45RA^-hCD4^+$ T lymphocytes, and there were marked peaks in the percentages of peripheral $hCD45RA^-hCD8^+$ T lymphocytes after HIV-1 infection. In the splenic T lymphocytes positive for HIV-1 p24, severe down-regulation of hCD4 surface expression was observed. These data suggests that NOG-hCD34 mouse can have variety of application in long-lasting and systemic studies of HIV-1 pathogenesis, as well as in the testing of anti-HIV-1 drug candidates.

3) Mechanism of Herpes virus and HIV-1 neuropathogenesis: H. KITAYAMA, A. ANDO and Y. KOYANAGI

Herpes simplex virus type 1 (HSV-1) causes fatal and sporadic encephalitis in human. The encephalitis-survivors frequently suffer from symptoms of memory deficits. It remains unclear how HSV-1 induces tissue damages in memory formation-associated brain tissues such as the hippocampus. In this study, we examined HSV-1 infection in the hippocampus using a rat HSV-1 infection model. We found profound pathological changes in the hippocampus and large numbers of HSV-1 antigen-positive cells in the dentate gyrus (DG) subfield of HSV-1-infected rats. To understand the precise mechanism of HSV-1-induced tissue damages in the hippocampus, we employed rat organotypic hippocampal slice cultures (OHC) as an in vitro HSV-1 infection model. In OHC, HSV-1 infection predominated in neuronal cells and the infected neuronal cells were severely damaged. Longitudinal analysis indicated that granule cells in DG subfield were extremely vulnerable to HSV-1 infection among neuronal cells in the hippocampus (Microbes Infect. 10:1514-1523, 2008). Since DG granule cells play a crucial role in memory formation, disruption of these cells may be a primary step leading to memory deficits.

HIV-1-infected macrophages damage mature neurons in the brain, though their effect on neuronal development has not been clarified. We show that HIV-1-infected macrophages produce factors that impair development of neuronal precursor cells, and that soluble viral protein R (Vpr) is one of the factors which have the ability to suppress axonal growth. Cell biological analysis revealed that extracellularly administered recombinant Vpr (rVpr) clearly accumulated in mitochondria where a Vpr-binding protein adenine nucleotide translocator (ANT) localizes, and decreased mitochondrial membrane potential ($\Delta\Psi m$), which led to ATP synthesis. The depletion of ATP synthesis reduced transportation of mitochondria within neurites. This mitochondrial dysfunction inhibited axonal growth even when the frequency of apoptosis was not significant. We also found that point mutations of arginine (R) residues to alanines (A) residues at positions 73, 77, and 80 rendered rVpr incapable of causing mitochondrial membrane depolarization and axonal growth inhibition. Moreover, the Vpr-induced inhibition was suppressed after the treatment with ubiquinone analogue (ubiquinone-10). Our results suggest that soluble Vpr is a major viral factor that causes disturbance of neuronal development through induction of mitochondrial dysfunction. Since ubiquinone-10 protects the neuronal plasticity *in vitro*, it may be a therapeutic agent that can offer defense against HIV-1-associated neurological disease (*J. Virol.* J. Virol. 82: 2528-2542, 2008). Our results suggest that HSV-1 itself and HIV-1 Vpr are the major factors causing disturbance of neuronal cell development.

LIST OF PUBLICATIONS
Research Center for Acquired Immunodeficiency Syndrome
Laboratory of Viral Pathogenesis

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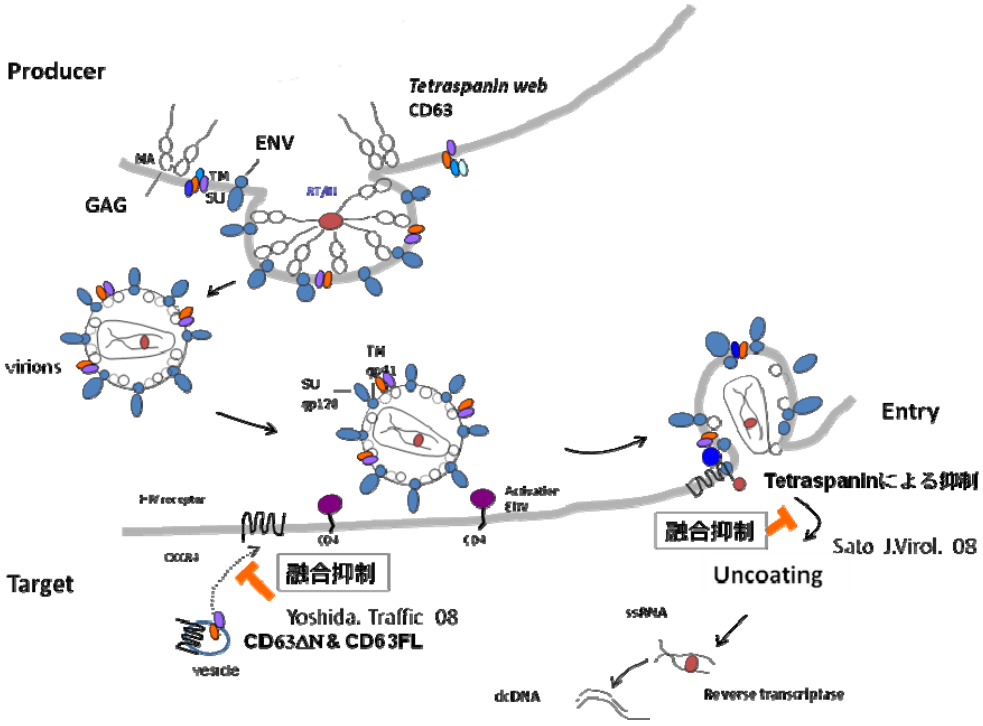
附属エイズ研究施設　　Research Center for Acquired Immunodeficiency Syndrome
ウイルス病態研究領域　　Laboratory of Viral Pathogenesis

北山裕子が平成 20 年 3 月に医学研究科博士課程を修了・学位を取得し、大鵬薬品に入社した。平成 20 年 4 月に生命科学研究科修士課程に横山貴章が入学した。8 月に Johnny Chuanyi Nie がカナダ British Columbia 大学に復学した。11 月 26 日から 28 日に第 22 回日本エイズ学会総会・学術総会を開催した。

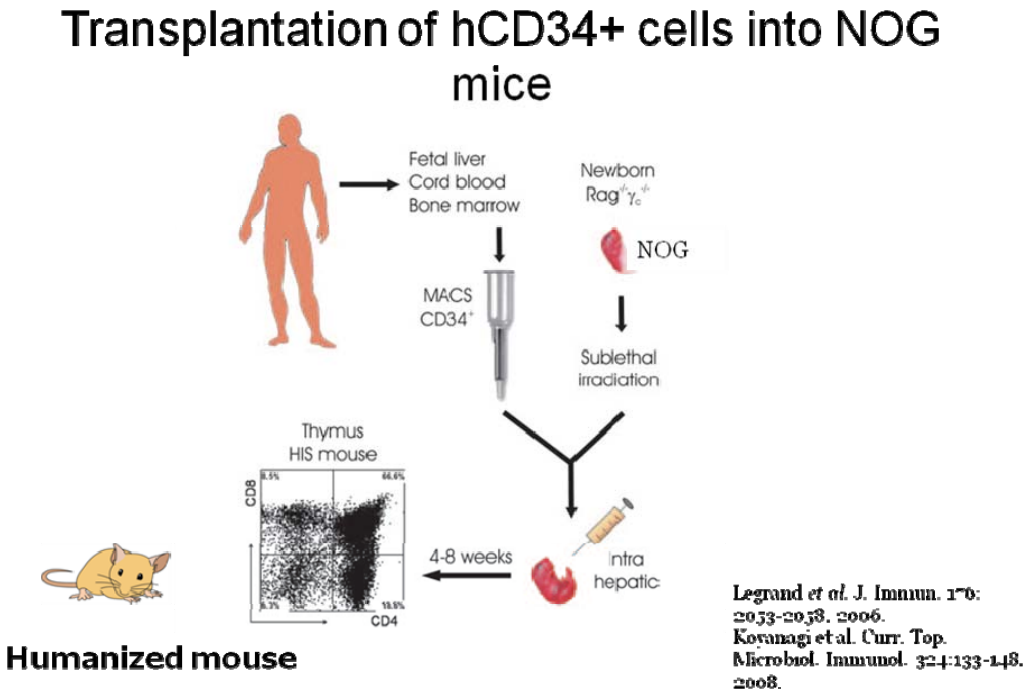
以下の 3 つのテーマについて研究を遂行している。

1) HIV 感染に関わる宿主因子の解析 : HIV を改良したレンチウイルスベクターを用いて HIV 感受性細胞に cDNA ライブラリを発現させ、HIV 感染に耐性を賦与する遺伝子探索実験系を確立した(*J. Virol.*78, 11352-11359, 2004)。これにより、CD63 の N 末端欠損　変異体(CD63ΔN)が、CXCR4 のゴルジ体から細胞質膜への移動を特異的に抑制することを見出した（芳田ら、*Traffic*, 9: 540–558, 2008）。さらに、CD63 と同じ分子群に分類される CD9, CD81, CD82, CD231 などのテトラスパニン分子は、ウイルス粒子のエンベロープに取り込まれ、このウイルス粒子の標的細胞との結合後の侵入過程を抑制すること、そして、この抑制作用はウイルス分離株特異的であることを見出した（佐藤ら、*J. Virol.*, 82:1021-1033, 2008）。これらの結果は、テトラスパニン分子がウイルス感染を抑制する活性を有することを示している（下図）。テトラスパニンに加え、遺伝学的ならびに蛋白質化学的手法により、新規 HIV 関連宿主因子の探索研究を行っている。現在、その候補分子として解析を行っているのは、インターフェロン、サイトカイン、シグナル分子、ユビキンチン関連分子、膜蛋白質などである。（芳田剛、佐藤佳、篠田康彦、山元誠司、小林朋子、渡部匡史、小柳義夫）



2) HIV 病原性解析：神奈川の実験動物中央研究所との共同研究により T, B, NK 細胞欠損マウス（NOG マウス）にヒト末梢血リンパ球を移植し、HIV 感受性マウスモデルを確立した。このモデルマウス（hu-PBL-NOG mouse）では、CCR5 を補受容体とする R5 HIV-1 の感染により CD4 細胞の破壊と中枢神経内の神経細胞の細胞死を引き起こすことができる。そして、これらの細胞死は Fas ligand でなく、TRAIL を介することがわかった(*J. Exp. Med.* 193, 651-659, 2001, *Proc. Natl. Acad. Sci. USA.* 100, 2777-2782, 2003)。すなわち、TRAIL に対する抑制薬は、エイズ患者に対する細胞障害治療薬となりうる可能性を示唆している。しかしながら、これらの末梢血移植マウスでは、すべての血液系細胞が構築されているのではない。そこで、新生児 NOG マウスの肝臓内にヒト CD34 陽性血液幹細胞を移植するヒト化マウスモデル(NOG-hCD34)を作製した（下図）。このマウスのなかでは、ヒト長期造血能、ならびに、リンパ球、マクロファージ、樹状細胞への分化増殖能が少なくとも 44 週間は維持された。そして、HIV 感染により胸腺細胞の破壊、末梢血ならびに脾臓における CD4/CD8 比の減少、そして、CD4 陽性 CD45 陰性 CCR5 陽性 T 細胞の欠乏が観察された。長期にわたる高ウイルス血症が R5 ウイルス感染により誘導され、CD45 陰性 CD4 陽性 T 細胞の減少と CD45 陰性 CD8 陽性 T 細胞の一過性の増加も観察された。さらに、脾臓内の HIV 抗原(p24)陽性細胞では、細胞表面上 CD4 分子の発現が極端に減少していた（投稿中）。これらの結果は、NOG-hCD34 マウスが、HIV 持続感染モデルならびに抗 HIV 候補薬の評価実験系となりうることを示唆している。

（佐藤佳、三沢尚子、Johnny Chuanyi Nie, 北山裕子、小柳義夫）



3）ヘルペスウイルスならびに HIV 脳炎の発症メカニズム：ラット海馬培養スライス片に GFP 発現 HSV（東京大学川口博士との共同研究により入手）を感染させると、このウイルスの感染播種の過程を観察できることがわかった。特に、神経幹細胞が局在し、それらの細胞分化が活発な海馬歯状回に対して HSV は強力な細胞障害を引き起こし、神経グリアの組織構成を破壊することがわかった(Microbes Infect. 10:1514-1523, 2008)。同じスライス培養法を用いて、HIV 感染マクロファージが神経細胞分化を抑制する因子を遊離していることを見出した（北山ら、*Microbiol. Immunol.* 52: 78-88, 2008, 下図左）。そして、その中で HIV がコードする Vpr 蛋白質が軸索伸長障害作用を有することがわかった。神経分化培養細胞に組換え Vpr 蛋白質を添加すると、ANT という Vpr 結合性蛋白質が局在するミトコンドリア分画にすばやく移行し、ミトコンドリアの膜電位低下、そして、ATP 産生を抑制する。さらにその ATP 産生低下が神経樹状突起のミトコンドリアへの移行を抑制する結果、軸索は伸長できないことがわかった (*J. Virol.* J. Virol. 82: 2528-2542, 2008, 下図右)。これらの結果は、HSV それ自身ならびに HIV Vpr 蛋白質は神経細胞分化障害の主な要因となっていることを示唆するものである。（北山裕子、安藤良徳、横山貴章、小柳義夫）