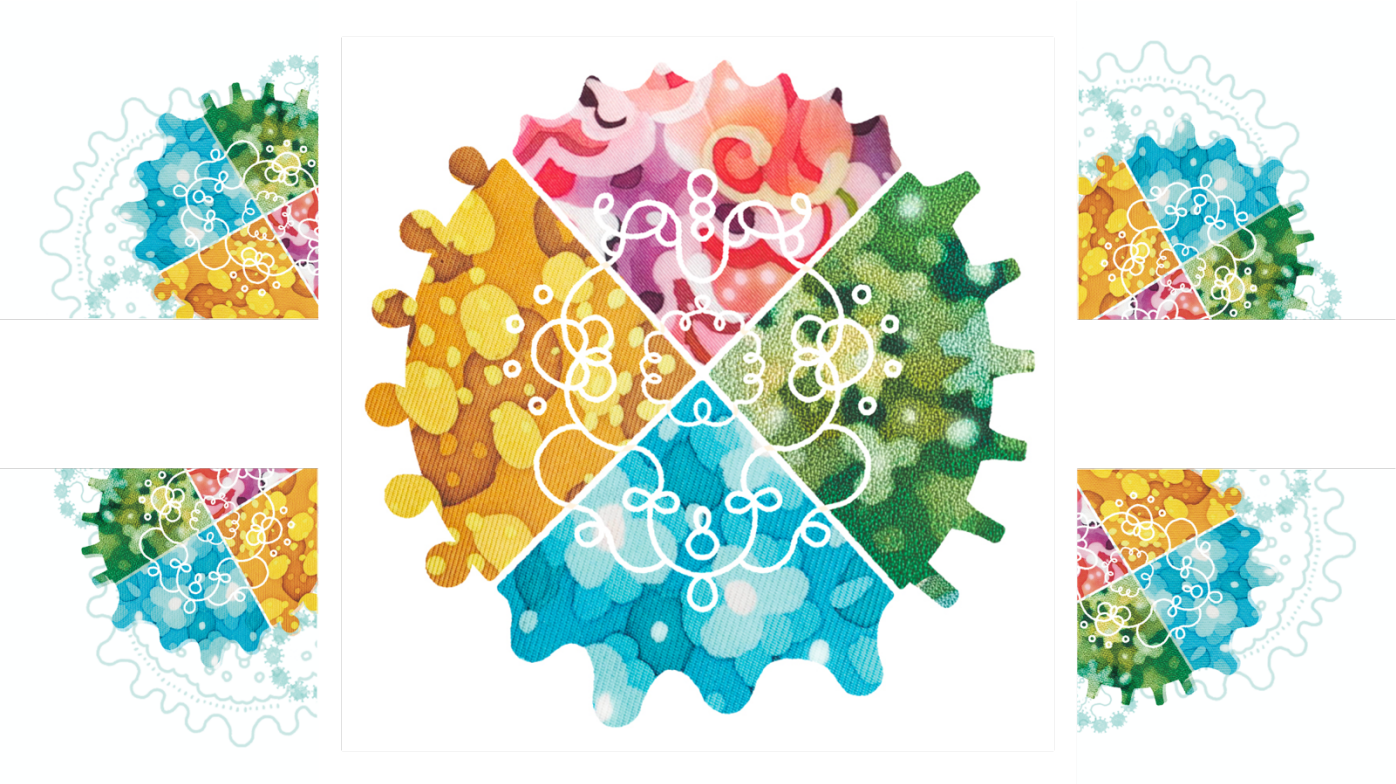
24th Kumamoto AIDS Seminar

6th November - 7th November, 2023

Kumamoto Kenmin Koryukan-Parea

(Parea Kumamoto Prefectural Community Center)

Kumamoto City, Japan



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Kumamoto University

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Naofumi Takahashi, Assistant professor, Joint Research Center for Human Retrovirus Infection, Kumamoto University

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Yuka Matsuoka & Atsuko Murakawa
Joint Research Center for Human Retrovirus Infection, Kumamoto University
1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan
TEL : +81 96-373-6830
Email : satolab-sec@kumamoto-u.ac.jp/satolab-sec4@kumamoto-u.ac.jp

The 24th Kumamoto AIDS Seminar Time Table

	DAY 1 November 6th (Monday)	DAY 2 November 7th (Tuesday)
▼9:00		**Session III** 9:00-9:50
		Coffee Break 9:50-10:05
▼10:00	Registration Poster hanging up Collaboration Meeting	**Session IV** 10:05-11:50
▼11:00		
▼12:00	Opening Remarks 12:50-13:00	Lunch 11:50-13:00
▼13:00	**Session I** 13:00-13:50	**Session V** 13:00-14:20
	Coffee Break 13:50-14:00	
▼14:00	**Session II** 14:00-15:30	Closing Remarks 14:20-14:30
▼15:00	Coffee Break 15:30-15:45	Collaboration meeting, One-on one meeting, and Lab tour at Kumamoto University
▼16:00	**Poster Presentation** 9F Meeting Room 1 15:45-17:05	
▼17:00	Coffee Break/voting for the best poster award 17:05-17:15	
	Keynote Lecture 17:15-18:15	
▼18:00		
▼19:00	**Reception** Kumamoto Hotel Castle 19:00-21:00	
▼20:00		

Day1 November 6th, Monday

10:00 - 12:50	**Registration, Poster hanging up & Collaboration Meeting**		

Kumamoto Kenmin Koryukan Parea 10F Parea Hall 12:50-18:15

12:50 Opening Remarks **Hisao Ogawa (President of Kumamoto University)**

Session I	**Application of Tocky system for virus research**		**13:00-13:50 〔50〕**
		Chairs: Seiji Okada (Kumamoto University, Japan), Yorifumi Satou (Kumamoto University, Japan)	
13:00 - 13:25 【25】	**O1 - 1**	Takushi Nomura Kumamoto University, Japan	Analysis of T-cell responses associated with age-dependent severity in a SARS-CoV-2 infected mouse model
13:25 - 13:50 【25】	**O1 - 2**	Omnia Reda/Wajihah Sakhor Kumamoto University, Japan	HIV-Tocky system to visualize proviral expression dynamics
13:50-14:00 〔10〕	**Coffee Break**		
Session II	**Selected talk from poster presentation**		**14:00-15:30 〔90〕**
		Chairs: Naofumi Takahashi (Kumamoto University, Japan), Kenji Sugata (Kumamoto University, Japan)	
14:00-14:15 【15】	**SP - 1**	Mst Monira Begum Kumamoto University, Japan	Correlation of some virological characteristics of SARS-CoV-2 variants of concern and variants of interest with spike protein-mediated fusogenicity
14:15-14:30 【15】	**SP - 2**	Randa Abdelnaser Kumamoto University, Japan	A new function of M-Sec, the host factor for an efficient cell-to-cell transmission of HIV-1 and HTLV-1
14:30-14:45 【15】	**SP - 3**	Yoshihiko Goto Kumamoto University, Japan	HLA-C-restricted CTLs specific for SARS-CoV-2 nucleocapsid display potent antiviral activity across viral variants and are maintained as a long-lived memory cells
14:45-15:00 【15】	**SP - 4**	Alitzel Anzurez NIID, Japan	Pro-inflammatory cytokine production after SARS-CoV-2 infection in people living with HIV
15:00-15:15 【15】	**SP - 5**	Perpetual Nyame Kumamoto University, Japan	A derivative compound, HT-7, inhibits HIV-1 release by inducing BST2/tetherin on the cell surface
15:15-15:30 【15】	**SP - 6**	Devon Weterings Imperial College London, UK	Characterisation of the HTLV-1-specific cytotoxic T-lymphocyte response and viral gene expression in HTLV-1 carriers at high risk of developing Adult T-cell Leukaemia/Lymphoma
15:30-15:45 〔15〕	**Coffee Break**		
Poster presentation (9F Meeting Room 1)			15:45-17:05 〔80〕
15:45-16:25 〔40〕	**Discussion Group A** ***Chairs: Hesham Nasser, Kaho Matsumoto (Kumamoto University, Japan)***		
16:25-17:05 〔40〕	**Discussion Group B** ***Chairs: Monde Kazuaki, Godfrey Barabona (Kumamoto University, Japan)***		
17:05-17:15 〔10〕	***Coffee Break/Voting for Best poster award***		
Keynote Lecture			**17:15-18:15 〔60〕**
		Chairs: Masafumi Takiguchi (Kumamoto University, Japan), Masao Matsuoka (Kumamoto University, Japan)	
17:15-17:45 【30】	**O1 - 3**	Yosuke Maeda Kumamoto University, Japan	Coreceptors and HIV-1 pathogenesis
17:45-18:15 【30】	**O1 - 4**	Charles Bangham Imperial College London, UK	HTLV-1: transcriptional burst and selective clonal survival
19:00-21:00 〔120〕	**Reception at Kumamoto Hotel Castle (Best poster award)**		

Day2 November 7th, Tuesday

Kumamoto Kenmin Koryukan Parea 10F Parea Hall 9:00-14:30

Session III Immunology			**9:00-9:50【50】** ***Chairs: Tetsuro Matano (NIID, Japan), Ai Tachikawa (NIID, Japan)***
9:00-9:25【25】	**O2 - 5**	Chihiro Motozono Kumamoto University, Japan	Molecular mechanism of SARS-CoV-2 spike escape from HLA-A*24:02-restricted T cell response
9:25-9:50【25】	**O2 - 6**	Hiroyuki Yamamoto NIID, Japan	SIVmac239-specific neutralizing antibody responses: Targeting specificity, predispositions
9:50-10:05〔15〕	**Coffee Break**		
Session IV Virology			**10:05-11:50【105】** ***Chairs: Kenji Maeda (Kagoshima University, Japan), Terumasa Ikeda (Kumamoto University, Japan)***
10:05-10:30【25】	**O2 - 7**	Manabu Aoki Kumamoto Health Science University, Japan	GRL-142 Binds to and Impairs HIV-1-Integrase-Nuclear Localization Signal and Exerts Potent Activity against INSTI-Resistant HIV-1
10:30-10:55【25】	**O2 - 8**	Yasumasa Iwatani Nagoya Medical Center, Japan	Host APOBEC3A cytidine deaminase drives mutations in the SARS-CoV-2 genome
10:55-11:20【25】	**O2 - 9**	Akatsuki Saito Miyazaki University, Japan	An inhaled ACE2 decoy confers protection against SARS-CoV-2 infection in preclinical models
11:20-11:50【30】	**O2 - 10**	Jiri Zahradnik Charles University in Prague, Czech Republic	Probing Host-Pathogen Protein Interactions by Yeast display
11:50-13:00〔70〕	**Lunch Time**		
Session V Clinical infectious diseases			**13:00-14:20【80】** ***Chairs: Hiyoyuki Gatanaga (ACC, Japan), Shinya Suzu (Kumamoto University, Japan)***
13:00-13:25【25】	**O2 - 11**	Kouki Matsuda Kagoshima University, Japan	Identification of HIV blips and associated immunological factors as indicators of HIV reservoir
13:25-13:50【25】	**O2 - 12**	Koji Watanabe ACC, Japan	Epidemiological strategy towards sexually transmitted amebiasis
13:50-14:20【30】	**O2 - 13**	Aileen Rowan Imperial College London, UK	Evolution of HTLV-1-induced cancer
14:20-14:30〔10〕	**Closing Remarks**		**Takamasa Ueno [Campus Head, Huretro Inst., Kumamoto Univ.]**

15:00-18:00 **Collaboration meeting, One-on one meeting, and Lab tour at Kumamoto University**

Poster Session

■ Poster Discussion: November 6th, Monday

Group A : 15:45-16:25 [40 min.]

Meeting Room 1, Parea Kumamoto Prefectural Community Center 9F

Group A Chairs: Hesham Nasser, Kaho Matsumoto (Kumamoto University)		
Poster No	**Presenter**	**Title**
P-01	Youssef Eltalkhawy (Kumamoto University)	iPS cell–derived model to study the interaction between Tissue Macrophage and HIV-1
P-02	Akira Kawashima (Kumamoto University)	Advancing Entamoeba histolytica Diagnostics: Validation of qPCR-based assays
P-03	Naofumi Takahashi (Kumamoto University)	HIV-1 infection in $CD34^+$ monocytes
P-04	Md Samiul Alam Rajib (Kumamoto University)	Characterization of host genome in HIV-1 infected CD4+ T-cells from the patients under long-term combined Anti-Retroviral Therapy (cART)
P-05	Zavuga Zuberi (Kumamoto University)	Longitudinal characterization of HIV-1 provirus landscape in ART-treated individuals
P-06	Sharmin Nahar Sithi (Kumamoto University)	Establishment of recombinant SIV to characterize viral reservoirs *in vivo*
P-07	Mitsuyoshi Takatori (Kumamoto University)	Tissue-specific pathogenesis and tumor heterogeneity in lymphoma-type ATLL
P-08	Akhinur Rahman (Kumamoto University)	Exploring the role of CTCF in HIV-1 provirus silencing using multiple in-vitro latent clones
P-09	Kaho Matsumoto (Kumamoto University)	Isolation of monoclonal antibodies with neutralization and ADCC activities against SARS-CoV-2 variants
P-10	Omnia Reda (Kumamoto University)	HIV-Tocky system to visualize proviral expression dynamics
P-11	Hossain Md Belal (Kumamoto University)	Intelli-OVI: A new-generation clinical tool for monitoring emerging viral infections
P-12	Wajihah Sakhor (Kumamoto University)	Recombinant HIV model system joined with parallel integration site, transcriptomic and epigenomic analysis to discover factor regulating the fate of HIV activation and latency
P-13	Theodore Asigbee (Kumamoto University)	HTLV-1 specific immune responses in latently HTLV-1 infected individuals
P-14 (SP-1)	Mst Monira Begum (Kumamoto University)	Correlation of some virological characteristics of SARS-CoV-2 variants of concern and variants of interest with spike protein-mediated fusogenicity
P-15	Bago Mussa (Kumamoto University)	The role of dysregulated extracellular vesicles' miRNAs in the modulation of chronic inflammation during HIV infection
P-16	Sara Habash (Kumamoto University)	Characterization of self-renewing murine macrophages with different origins
P-17 (SP-2)	Randa Abdelnaser (Kumamoto University)	A new function of M-Sec, the host factor for an efficient cell-to-cell transmission of HIV-1 and HTLV-1
P-18 (SP-3)	Yoshihiko Goto (Kumamoto University)	HLA-C-restricted CTLs specific for SARS-CoV-2 nucleocapsid display potent antiviral activity across viral variants and are maintained as a long-lived memory cells
P-19	Takeshi Nakama (Kumamoto University)	Cross-reactivity of HLA-A*24:02-restricted T-cell receptors towards SARS-CoV-2 mutations
P-20	Ryo Shimizu (Kumamoto University)	APOBEC3 degradation is the primary function of HIV-1 Vif determining virion infectivity in the myeloid cell line THP-1
P-21	Hesham Nasser (Kumamoto University)	Spike protein-mediated membrane fusion assay enables tracing the evolution of SARS-CoV-2 and variants

■ Poster Discussion: November 6th, Monday
Group B : 16:25-17:05 [40 min.]

Meeting Room 1, Parea Kumamoto Prefectural Community Center 9F

Group B Chairs: Monde Kazuaki, Godfrey Barabona (Kumamoto University)		
Poster No	**Presenter**	**Title**
P-22	Aritsu Yoshiki (Kumamoto University)	Development of altered peptide ligands containing non-natural amino acids that efficiently induce antiviral T cell responses
P-23	Chatherine Silas Mtali (Kumamoto University)	Establishment of SARS-CoV-2 infected Nr4a3-Tocky mice model with different severity
P-24	Mako Toyoda (Kumamoto University)	Analysis of virological and immunological characteristics of SARS-CoV-2 spike variants with mutations at position L452
P-25	Wright Amesimeku (Kumamoto University)	Isolation of resistant mutants against Lenacapavir using the Capsid Library System
P-26	Mark Mutethia Ndubi (Kumamoto University)	Characterization of antigen-specific T cell responses in third-dose SARS-CoV-2 vaccinated Japanese with HIV-1 infection
P-27	Dechuan Kong (Kumamoto University)	HIV-1 minigene system to establish an in vitro latency model
P-28	Issac Ngare (Kumamoto University)	HIV-1 broadly Neutralizing antibodies in Newly diagnosed Tanzanians
P-29	Ishrat Jahan (Kumamoto University)	The establishment of transgenic mouse system to characterize HTLV-1-driven $CD4^+$ T cell immortalization mechanism
P-30 (SP-4)	Alitzel Greet Anzurez Reyes (National Institute of Infectious Disease)	Pro-inflammatory cytokine production after SARS-CoV-2 infection in people living with HIV
P-31	Mayu Okumura (Kumamoto University)	Effect of CXCR4 oligomeric states on HIV-1 infectivity
P-32	Khaled Elgeshy (Kumamoto University)	Development of Biomarker for predicting hepatocarcinogenesis after Hepatitis C virus elimination
P-33	Rise Kurokawa (Kumamoto University)	Analysis of immune responses associated with age-dependent severity in SARS-CoV-2-infected Nr4a3-Tocky mice
P-34	Itnarin Mongkon (Kumamoto University)	The anti-tumor effect of Andrographolide against Primary effusion lymphoma
P-35	Huanyu Li (Kumamoto University)	In vitro priming of HLA-A*24:02-restricted variants-specific CD8+ T cells in SARS-COV-2 infection
P-36	Jakir Hossain (Kumamoto University)	Human endogenous retrovirus-K (HERV-K) antisense RNA is induced by SOX4 in the teratocarcinoma cells
P-37	Joyce Appiah-Kubi (Kumamoto University)	RESEARCH ON THE MECHANISM OF ACCELERATION OF HIGHLY REPLICATION-COMPETENT HIV-1 MATRIX (MA) MUTANTS
P-38 (SP-5)	Perpetual Nyame (Kumamoto University)	A derivative compound, HT-7 inhibit HIV-1 release by inducing BST2/tetherin on the cell surface
P-39	Daniel Enriquez-Vera (Kagoshima University)	Implementation of an artificial antigen presenting cell system for HTLV-1 Tax-specific peptides to evaluate HLA-restricted CD8+ T cell exhaustion
P-40 (SP-6)	Devon Weterings (Imperial College London)	Characterisation of the HTLV-1-specific cytotoxic T-lymphocyte response and viral gene expression in HTLV-1 carriers at high risk of developing Adult T-cell Leukaemia/Lymphoma
P-41	Patricia Watber (Imperial College London)	Understanding ATL oncogenesis by detecting driver mutations in HTLV-1 carriers

Session I
O1-1

Analysis of T-cell responses associated with age-dependent severity in a SARS-CoV-2 infected mouse model

Rise Kurokawa[1], Chatherine Silas Mtali[1], Reda Omnia[2], Sakhor Wajihah Binti[2], Yorifumi Satou[2], Masahiro Ono[3,4] and ***Takushi Nomura***[1,5]

[1]*Division of Virology and Pathology, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[2]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[3]*Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, London, United Kingdom*
[4]*Collaboration Unit for Infection, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[5]*AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan*

COVID-19 pneumonia is prevalent in older infected humans, while the age-dependent mechanisms of the pathogenesis remain unclear. The lungs in the early phase of SARS-CoV-2 infection present coexist environments of tissue aggression by viral replication, exogenous and endogenous antigens production, and tissue inflammation; thus, the influence of immunopathology on the containing of severe pneumonia could be significant. Nr4a3-Timer mouse is a knock-in mouse of the fluorescent timer protein as a reporter of the Nr4a3 receptor downstream of the TCR signal, enabling in vivo analysis of the temporal dynamics of activation of antigen-specific T cells. In the present study, we established the SARS-CoV-2 infected Nr4a3-Tocky mice model, which was suitable for analyzing dynamics and responses of antigen-specific T-cell activation in the lung and identified different disease phenotypes depending on the age of the mice. Old infected mice developed severe pneumonia, whereas middle-aged infected mice exhibited mild pneumonia with transient weight loss followed by recovery. The middle-aged infected mice induced significantly higher frequencies of activated antigen-specific T cells in the lungs than the old infected mice, implying an inverse correlation between the induction of T cell responses in the lung and the severity of pneumonia. Since the infiltration of inflammatory monocyte macrophages into the lungs was observed at the same level in both middle-aged and old infected mice, induced antigen-specific T-cell fractions in middle-aged mice could contribute to the control of viral replication and the suppression of lung inflammation. This study suggests that T-cell dysfunction due to immune aging would be associated with the severity of SARS-CoV-2 infection.

Session I
O1-2

HIV-Tocky system to visualize proviral expression dynamics

***Omnia Reda*[1,2]*, Wajihah Sakhor*[1]*, Masahiro Ono*[3]*, Yorifumi Satou*[1]

[1]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*

[2]*Microbiology Department, High Institute of Public Health, Alexandria University, Egypt*

[3]*Department of Life Sciences, Imperial College London, London, UK,*

The stably integrated pool of HIV-1 proviruses in the host genome stands against curative strategies. This reservoir is extremely heterogeneous with respect to host cell type, anatomical location, integration site, and replication fitness. During the initial phase of infection, only a few infected cells can resist host immune clearance or cytopathic effect and establish this resistant pool. The mechanisms underlying HIV latency initiation are not fully resolved yet. We, here, propose and validate a new reporter model for monitoring HIV-1 provirus silencing and reactivation using a Timer of cell kinetics and activity (Tocky). The HIV-Tocky system uses fluorescent Timer which is a mCherry-derived monomeric fluorescent protein whose emission spectrum spontaneously shifts from blue (t ½ ~ 4h) to red fluorescence (t ½ ~ 120h) allowing the observation of progressions of infected cells from early productive (B+), persistence (B+R+), recently silenced (R+) to latent infection (TN) with time. To our knowledge, this is the first report to distinguish two latent subsets: a directly non-expressing population and a recently silenced after brief expression. In-depth integration site analysis from Jurkat T cells infection suggested that the distribution of proviruses in directly latent cells was similar to that in actively transcribing cell population, whereas recently silenced cells tended to harbor proviruses integrated into heterochromatin. Furthermore, we established a library of various single integration clones which we utilized to demonstrate the efficiency of the block-and-lock strategy by capturing the fast dynamics of silencing that were overlooked in previous models. We, next, extended infection into primary CD4+T cells where we employed single-cell multiomic (scRNA-seq and scATAC-seq; Assay for Transposase-Accessible Chromatin) to obtain parallel information on the transcriptome as well as the epigenetic profile of the same cell at a single cell level. scRNA-seq transcriptomic profile together with RNA velocity analysis predicts two major routes after acute infection which is going into apoptosis or latency. Pseudo bulk motif activity analysis of scATAC-seq data uncovers transcription factors enrichment trend in which early productive (B+) and persistence (B+R+) populations are enriched with AP-1 family, latent infection (TN) is enriched with CTCF while the recently silenced population (R+) are enriched with RFX family. In summary, we propose HIV-Tocky system to serve as a time-sensitive model that can capture the kinetics of provirus expression. Moreover, we are currently looking at the dynamics and interplay of integration site, epigenomics and transcriptomics to uncover potential factor regulating the fate of HIV activation and latency.

Session II
SP-1(P-14)

Correlation of some virological characteristics of SARS-CoV-2 variants of concern and variants of interest with spike protein-mediated fusogenicity

***Mst Monira Begum*[1,2]*, Kimiko Ichihara*[1]*, Otowa Takahashi*[1]*, Nasser Hesham*[1,3]*, Michael Jonathan*[1,2]*, The Genotype to Phenotype Japan (G2P-Japan) Consortium, Kei Sato*[4]*, Terumasa Ikeda*[1]

[1]*Division of Molecular Virology and Genetics, Joint research center for Human Retrovirus infection, Kumamoto University, Kumamoto, Japan*
[2]*Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan*
[3]*Department of Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*
[4]*Division of Systems Virology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the global pandemic of the coronavirus disease 2019 (COVID-19). The spike (S) protein of SARS-CoV-2 plays an essential role in mediating membrane fusion of the virus with the target cells, triggering viral entry into target cells. Several reports demonstrated that the fusogenicity of the S protein in SARS-CoV-2 variants is closely associated with the intrinsic pathogenicity of the virus determined by a hamster model. However, the association of S fusogenicity with other virological parameters is unclear. Therefore, we investigate the correlation of the virological parameters of eleven previous variants of concern (VOCs) and variants of interest (VOIs) with S protein-mediated fusogenicity. The fusion activity mediated by S protein is strongly correlated with the S1/S2 cleavage of S protein in the transfected HEK293 cells and plaque size formed by clinical isolates in VeroE6/TMPRSS2 cells. However, the fusogenicity of S protein is not associated with pseudovirus infectivity measured in HOS-ACE2/TMPRSS2 cells, S protein-mediated entry efficiency into HOS-ACE2/TMPRSS2 cells, and viral replication kinetics in VeroE6/TMPRSS2 cells. Altogether, our data suggest that similar to fusion activity obtained by S protein-mediated membrane fusion assay, S1/S2 cleavage efficiency and plaque size may be a potential indicator to predict the intrinsic pathogenicity of newly emerged SARS-CoV-2 variants.

Session II
SP-2(P-17)

A new function of M-Sec, the host factor for an efficient cell-to-cell transmission of HIV-1 and HTLV-1

***Randa Abd-ElNasser**, Youssef M. Eltalkhawy, Naofumi Takahashi, Shinya Suzu*

Division of Infection & Hematopoiesis, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto

We have demonstrated that a cellular protein M-Sec (also known as tnfaip2) contributes to an efficient cell-to-cell transmission of both HIV-1 and HTLV-1. M-Sec is originally identified as a critical regulator of the F-actin^{+} long membrane extensions connecting distant cells referred to as tunneling nanotubes (TNTs), which is beneficial for the cell-to-cell transmission of those viruses. In addition, our recent study revealed that M-Sec facilitates the assembly of the viral structural protein Gag of HTLV-1, which is requisite for the formation of viral particles. Although this function of M-Sec more directly contributes to the cell-to-cell viral transmission, the underlying molecular basis remains unclear. In this study, we show that M-Sec regulates not only viral Gag protein but also the cell surface receptor for M-CSF, the most important cytokine for monocytes/macrophages. M-Sec knockdown in macrophages reduced the number and size of TNTs, as expected. Meanwhile, we found that M-Sec knockdown or pharmacological M-Sec inhibition also reduced the cellular responses to M-CSF, such as M-CSF-promoted migration, phagocytosis, nitric oxide production, and osteoclast differentiation. This inhibition appeared to be specific to M-CSF since the M-Sec knockdown did not affect the response to another cytokine GM-CSF or bacterial lipopolysaccharide (LPS). Consistent with the poor response to M-CSF, M-Sec knockdown/inhibition reduced the activation of M-CSF receptor and downstream cascades. Of interest, M-Sec knockdown did not affect the basal expression level of M-CSF receptor, but significantly reduced M-CSF-triggered internalization of M-CSF receptor and their re-expression on cell surface, the process of which is required for maximal signaling by receptor tyrosine kinases including M-CSF receptor. Exogenous expression of M-Sec in HEK 293 cells accelerates not only downregulation but also re-expression of M-CSF receptor on cell surface. These results suggest that M-Sec regulates the stability or intracellular trafficking M-CSF receptor, and thereby maximizes the response of macrophages to M-CSF. Given that M-Sec regulates plasma membrane structure exemplified by TNTs, our findings imply that M-Sec also regulates intracellular structures involved in endocytic trafficking of proteins, including M-CSF receptor and viral Gag protein.

Session II
SP-3(P-18)

HLA-C-restricted CTLs specific for SARS-CoV-2 nucleocapsid display potent antiviral activity across viral variants and are maintained as a long-lived memory cells

***Yoshihiko Goto*[1,3]*, Mako Toyoda*[1]*, Toong Seng Tan*[1]*, Hiroshi Hamana*[2]*, Takeshi Nakama*[1]*, Hanyu Li*[1]*, Yoshiki Aritsu*[1]*, Mizuki Kitamatsu*[4]*, Hiroyuki Kishi*[2]*, Yusuke Tomita*[3]*, Takuro Sakagami*[3]*, Takamasa Ueno*[1] *Chihiro Motozono*[1]

[1]*Division of Infection and immunity, Joint Research Center for Human Retrovirus infection, Kumamoto University*

[2]*Department of Immunology, Faculty of Medicine, Academic Assembly, University of Toyama*

[3]*Department of Respiratory Medicine, Faculty of Life Sciences, Kumamoto University*

[4]*Department of Applied Chemistry, Faculty of Science and Engineering, Kindai University*

Regardless of the progressive emergence of Variant of Concerns (VOCs), SARS-CoV-2 virus remains susceptible to CTL recognition. However, we previously reported that a spike mutation in several VOCs can confer escape from immunodominant CTL responses. Therefore, it is becoming important to unveil potent CTL responses targeting conserved epitopes across SARS-CoV-2 VOCs. Here, we focused on T cell responses to SARS-CoV-2 nucleocapsid (N) antigen due to the most abundant and highly conserved protein. IFN-γ ELISpot assay using N overlapping peptide pools revealed several immunodominant regions in convalescents with a haplotype *HLA-A24-B52-C12* which is the most prevalent in Japan. The N-specific T cell lines were responded in the context of HLA-C*12:02, but not other alleles, indicating N protein preferentially induce HLA-C-restricted T cells. Based on epitope prediction database, we identified the KF9 as an immunodominant epitope restricted by HLA-C*12:02. In fact, ex vivo KF9/HLA-C12 tetramer assay showed the higher frequency of KF9/C12-specific T cells in convalescents with HLA-C*12:02 and in vitro-expanded T cell lines were found to produce IFN-γ, TNFα and IL-2 and upregulate CD107a, suggesting multifunctional nature and cytotoxic potential. Moreover, we demonstrated that the T cell lines comparably suppressed viral replication of prototype and Omicron variants when the target cells expressed HLA-C*12:02 KF9-specific memory $CD8^+$ T cells were maintained as effector memory and TEMRA phenotype 6 months and 1 year after infection and were proliferated as effector memory subsets in *in vitro* stimulation, suggesting a potent cytotoxic potential upon reinfection. Taken together, we identified SARS-CoV-2 N-specific CTLs showing the potent antiviral activity across VOCs and being maintained as a high qualitative memory with the rapid proliferative capacity in the context of HLA-C*12:02, highlighting the importance of HLA-C restriction molecules to control newly emerging SARS-CoV-2 VOCs, in addition of HLA-A and HLA-B and providing the great insights into the future vaccine development including N antigen.

Session II
SP-4(P-30)

Pro-inflammatory cytokine production after SARS-CoV-2 infection in people living with HIV.

***Alitzel Anzurez*[1,2]*, Lucky Runtuwene*[1]*, Thao Thi Thu Dang*[1,2]*, Kaori Hosoya-Nakayama*[1]*, Aki Tanabe*[1]*, Michiko Koga*[4]*, Yukihiro Yoshimura*[5]*, Natsuo Tachikawa*[5]*, Tetsuro Matano*[1,2,3]*, Ai Kawana-Tachikawa*[1,2,3]

[1]*AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan*
[2]*Joint Research Center for Human Retrovirus Infection, Kumamoto, Japan*
[3]*Department of AIDS Vaccine Development, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[4]*Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[5]*Department of Infectious Diseases, Yokohama Municipal Citizens' Hospital, Kanagawa, Japan*

Introduction. Dysregulated inflammatory responses after SARS-CoV-2 infection cause severe COVID-19. Although HIV infection is considered to be one of the risk factors for severity, inflammatory responses during acute SARS-CoV-2 infection have yet to be evaluated in people living with HIV (PLWH). In this study, levels of multiple cytokines were measured in PLWH during the acute and recovery phases of COVID-19.

Materials and Methods: Nineteen PLWH and 18 HIV-seronegative individuals with SARS-CoV-2 infection were enrolled in this study. Plasma samples were collected at 1-14 days (acute phase) and 1-2 months (recovery phase) post-symptom onset. Levels of 20 pro-inflammatory cytokines were measured using multiplex Luminex assay system (Invitrogen): GM-CSF, IFNα, IFNγ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, TNFα, IP-10, MCP-1, MIP-1α, MIP-1β, ICAM-1, CD62E, and CD62P. Plasma samples at 6-12 months after SARS-CoV-2 infection were also measured to define the basal levels of these cytokines.

Results. The levels of most measured cytokines in the acute phase were significantly elevated compared to the basal levels and declined towards the recovery phase. The levels of IL-1α, IL-8, and IP-10 in the acute phase were significantly higher in severe cases than in mild cases. Although the basal levels of CD62E, ICAM-1, and IP-10 were significantly higher in PLWH, there was no significant difference in the level of each cytokine between the groups in the acute phase.

Conclusion. Inflammatory responses are induced in PLWH at a similar level to HIV-seronegative individuals after SARS-CoV-2 infection, despite their chronic pro-inflammatory status.

Session II
SP-5(P-38)

A derivative compound, HT-7, inhibits HIV-1 release by inducing BST2/tetherin on the cell surface

***Perpetual Nyame*[1]*, Akihiro Togami*[2]*, Tomofumi Yoshida*[1]*, Takuya Masunaga*[2]*, Hiromi Terasawa*[1]*, Nami Monde*[1]*, Yurika Tahara*[2]*, Tomohiro Sawa*[1]*, Yorifumi Satou*[3]*, Mikako Fujita*[2]*, Yosuke Maeda*[1]*, Hiroshi Tateishi*[2]*, Kazuaki Monde*[1]

[1]*Department of Microbiology, Faculty of Life Sciences, Kumamoto University, Kumamoto 860-8556, Japan*
[2]*Medical and Biological Chemistry Science Farm Joint Research Laboratory, Faculty of Life Sciences, Kumamoto University 862-0973, Kumamoto, Japan*
[3]*Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto 860-8556, Japan*

The zeal to end the HIV-1 epidemic has led to the discovery of potent antiviral therapy that hinders different processes in HIV-1 replication. Although the overwhelming approval of novel ART is promising, discovering a new antiviral drug that has a repressive effect on the late stages of HIV-1 replication. In this respect, the HIV-1 Gag protein, which drives the HIV-1 assembly has lately been of great interest. In this study, a new high-performance screening system using T cell lines, the Vpr-HiBiT technique, was established to indirectly quantify the amount of HIV-1 release. Candidate compounds from the Ono Pharmaceutical Compound Library were tested for their efficacy in obstructing HIV-1 release by infecting Jurkat T cells expressing Vpr-HiBiT with a single round VSV-G pseudo typed NL4-3. The HT-7 derivative compound was designed with EC50 and CC50 of 95.8μM and 418.9μM respectively. In our search to investigate the process/processes targeted by HT-7 that impair viral release, the expression of BST2/tetherin and Gag punctate formation were evaluated by flow cytometry and confocal laser imaging. In addition to increasing GagVenus signal intensity in the infected HT-7 treated cells, partial disruption of Gag accumulation on the plasma membrane was also visible. In particular, cell surface expression of the host factor (BST2/tetherin), which impedes HIV-1 release, increased significantly with HT-7 treatment. The absence of Vpu that counteracts the BST2/tetherin prevented HT-7 from suppressing the release of HIV-1. The implications are that the upregulation of BST2/tetherin by HT-7 led to HIV-1 release inhibition. The HT-7, which was discovered by the Vpr-HiBiT technology, is a potential antiviral agent that suppresses the release of HIV-1 by inducing BST2/tetherin expression on the cell surface. The affordable, short turnaround time and high performance of the Vpr-HiBiT technique highlight it as a test tool for viral research.

Session II
SP-6(P-40)

Characterisation of the HTLV-1-specific cytotoxic T-lymphocyte response and viral gene expression in HTLV-1 carriers at high risk of developing Adult T-cell Leukaemia/Lymphoma

***Devon Weterings*[1]*, Lisa Lam Chiou Yee*[1]*, Graham P. Taylor*[1,2]*, Lucy B. Cook*[1,3]*, Aileen Rowan*[1]

[1]*Section of Virology, Department of Infectious Disease, Imperial College London, London, UK,*
[2]*National Centre for Human Retrovirology, Imperial College Healthcare NHS Trust, London, UK,*
[3]*Department of Haematology, Imperial College Healthcare NHS Trust, London, UK*

Adult T-cell leukaemia/lymphoma (ATL) is caused by chronic infection with the retrovirus human T-cell leukaemia virus type 1 (HTLV-1). Despite the constitutive expression of viral genes by malignant cells, little is known about how the antiviral immune response contributes to oncogenesis. We recently reported that clonally expanded ATL-like HTLV-1 infected cells circulate in the blood up to 10 years before the onset of ATL symptoms, and that HTLV-1-carriers with circulating ATL-like clones have a high risk of developing ATL. Here, we characterized antiviral cytotoxic T-lymphocyte (CTL) function and viral gene expression in HTLV-1 carriers who have suspected premalignant lesions, ATL-like clones, circulating in their blood.

We studied peripheral blood mononuclear cells from three groups of patients: HTLV-1-carriers who had circulating ATL-like clones but no clinical symptoms of malignancy (high-risk carriers, n=12), HTLV-1-carriers with matched proviral loads but without detectable ATL-like clones (controls, n=12) and patients with ATL (n=9). CTL killing and viral gene expression was assayed by co-culture of *ex vivo* infected $CD4^+$ T-cells with autologous $CD8^+$ T-cells, followed by flow cytometric analysis of cell survival. ATL-like clones were identified by staining for T-cell receptor Vβ subunits, and HTLV-1-infected cells were identified by staining extracellularly for CADM1 and intracellularly for viral protein Tax. IFN-γ production by CD8+ T cells in response to overlapping 15mer peptides corresponding to Tax and HBZ was assayed by ELISpot.

CD8+ T-cells from high-risk HTLV-1-carriers and patients with ATL were significantly less efficient at killing Tax-expressing HTLV-1-infected cells and produced significantly lower levels of IFN-γ in response to Tax peptides than CD8+ T-cells from the control group. Furthermore, in 50% of high-risk HTLV-1-carriers the ATL-like clones did not express immunodominant viral protein Tax. Overall, these findings show early indications of both inefficient HTLV-1-specific CTLs and immune evasion in HTLV-1 carriers with circulating ATL-like clones.

Keynote Lecture
O1-3

Coreceptors and HIV-1 pathogenesis

Yosuke Maeda

Department of Microbiology, Faculty of Life Sciences, Kumamoto University

The chemokine receptors CCR5 and CXCR4 function as coreceptors for HIV-1 entry into CD4+ T cells. In general, R5 viruses that exclusively utilize CCR5 are predominant throughout the course of infection, and associated with transmission between humans. These findings were confirmed by evidence that individuals having homozygous CCR5 delta 32 mutation are resistant to HIV-1 infection, and the heterozygous form is associated with slower progression to AIDS. On the other hand, viruses that utilize CXCR4 emerge at the late stage of infection in approximately half of HIV-1-infected individuals, and are associated with an accelerated loss of CD4+ T cells and faster disease progression. Among them, viruses exclusively using CXCR4 are called X4 viruses, while viruses using both CCR5 and CXCR4 are called R5X4 viruses, respectively. Thus, X4 viruses have been suggested to be evolved from pre-existing R5 viruses by a stepwise transition via intermediate R5X4 viruses. However, underlying mechanism of coreceptor switch from CCR5 to CXCR4 is not fully elucidated. Furthermore, these findings were obtained from subtype B HIV-1 predominantly circulating in North America and Western Europe. In contrast, little is known about the frequency of CXCR4-using viruses and switching mechanism from R5 to X4 in subtype AE HIV-1 predominantly circulating in Southeast Asia. I will present the data how HIV-1 switches coreceptor from CCR5 to CXCR4 especially in subtype AE HIV-1.

Keynote Lecture
O1-4

HTLV-1: transcriptional burst and selective clonal survival

Helen Kiik[1], Saumya Ramanayake[1], Aris Aristodemou[1], Hiroko Yaguchi[1], Anat Melamed[1], Dale A. Moulding[2], Abhyudai Singh[3] and ***Charles R M Bangham***[1]

[1]Department of Infectious Diseases, Faculty of Medicine, Imperial College London, London, UK;
[2]University College London, UK;
[3]Computational Biology Department, School of Computer Science, Carnegie Mellon University, Pittsburgh 15213, PA, USA

The plus-sense strand of the HTLV-1 provirus is expressed in rare, intermittent bursts. We studied the dynamics and consequences of the plus-strand burst in two naturally infected, non-malignant T cell clones transduced with a short-lived green fluorescent protein (d2EGFP) Tax reporter system, and in polyclonal HTLV-1-infected peripheral blood mononuclear cells. We used RNA-seq, single-molecule RNA-FISH, time-lapse live-cell imaging, single-cell tracking and mathematical modelling. The duration of the burst in the two T-cell clones was ~100 h and ~400 h respectively – substantially longer than previous estimates. We show that the burst exerts strong effects on the host cell: it immobilizes the cell, increases the risk of apoptosis, transiently slows cell-cycle progression and proliferation, enhances activation of certain T-cell genes and pathways including NF-κB and the DNA damage response. After termination of the burst, there is a compensatory surge in proliferation of the infected cell, which must contribute to survival of the clone in vivo.

We previously reported that the HTLV-1 provirus binds the chromatin architectural protein CTCF and disrupts the structure and transcription of the neighbouring host genome, forming chromatin loops between the provirus and host chromatin. However, it was not known whether these effects were caused simply by the presence of the provirus or by its transcription. We now show that the plus-strand burst leads to loss of the abnormal chromatin loops downstream (3′) of the provirus; the loops are re-formed following termination of the burst.

The putative benefit to HTLV-1 of binding CTCF is not known. We reported in 2022 that HTLV-1-infected (and HIV-1-infected) T cell clones are more likely to persist long-term in vivo if the provirus lies near the nuclear lamina or the nucleolar periphery, which are associated with (reversible) transcriptional repression. There is evidence that CTCF tethers chromatin to these nuclear sites. We therefore hypothesize that CTCF benefits HTLV-1 by promoting temporary transcriptional silencing, reducing the chance of detection by the strong immune response.

Session III
O2-5

Molecular mechanism of SARS-CoV-2 spike escape from HLA-A*24:02-restricted T cell response

Chihiro Motozono

Division of infection and immunity, Joint research center for Human Retrovirus infection, Kumamoto University, Kumamoto, Japan

Although the variant of the SARS-CoV-2 virus shows resistance to neutralizing antibody, molecular basis of immune escape from the cellular immunity remains unclear. Here, we demonstrate that the SARS-CoV-2 spike-derived NF9 ($S_{448-456}$: NYNYLYRLF) is an immunodominant epitope presented by HLA-A*24:02 in convalescents and vaccinated-donors. Single cell transcriptomic analysis reveals that public TCR clonotypes comprised of TRAV12 and TRBV6 with unique CDR3β motifs are identified in both of convalescents and vaccinated-donors. A naturally-occurring L452R mutation in Delta/Omicron BA.5 variant is located at 5th position within the NF9 epitope (NF9-5R) and these public TCRs do not retain the recognition towards the NF9-5R peptide. The crystal structure of the public TCR-NF9/A24 complex reveals that the CDR1α/CDR3α loop in TRAV12 and the CDR3β loop in TRBV6 play a major role in recognition of peptide, contributing to the selection of the public TCR clonotype. Moreover, introduction of L452R mutation disturbs the interaction of CDR3α loop with the main contact residue Y453, results in loss of TCR recognition. Intriguingly, variant (NF9-5R)-specific T cells are not induced in convalescents infected with Delta/Omicron BA.5 variant. Taken together, we highlight the molecular basis of the SARS-CoV-2 L452R escape from HLA-A24 restricted T cell immunity.

Session III
O2-6

SIV_{mac239}-specific neutralizing antibody responses: Targeting specificity, predispositions

***Hiroyuki Yamamoto*[1,2,3]**

[1]*AIDS Research Center, National Institute of Infectious Diseases, Musashi-Murayama City, Tokyo*
[2]*Department of Biomedicine, University Hospital Basel, Basel, Switzerland*
[3]*Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*

Impaired neutralizing antibody (NAb) induction contributes to viral persistence of HIV and simian immunodeficiency virus (SIV), and characterizing NAb induction patterns is important for antibody-based HIV control strategies. We have identified via comprehensive cohort analysis that a subgroup of rhesus macaques infected with the highly difficult-to-neutralize SIV_{mac239} strain can nevertheless induce virus-specific NAbs, particularly under enhanced Env-specific B-cell maturation following selection of a *nef* mutant attenuated in B-cell inhibitory signaling. This poses another fundamental question on the virus-targeting specificity of such NAb responses. Comparison of eight SIV_{mac239}-specific NAb inducers (including six with the *nef* mutant selection) versus seven NAb non-inducers for plasma viral Envelope (Env) polymorphisms found that NAb inducers exerted higher Env gp120 selective pressure signatures. A panel neutralizing assay utilizing mutant SIVs identified Env mutations A138S in variable region 1 (V1), G201D in V2, A417T and 420-426 deletion in V4, and N476D in V5 as NAb escape mutations. Quadruple Env V1+V2+V4+V5 mutation impaired neutralizing titers to a level comparable to NAb non-inducers. Results collectively suggest that *in vivo* SIV_{mac239}-specific NAb responses globally targets multiple regions, cohering pan-cognate B-cell enhancement, at a functional level.

Session IV
O2-7

GRL-142 Binds to and Impairs HIV-1-Integrase-Nuclear Localization Signal and Exerts Potent Activity against INSTI-Resistant HIV-1

Manabu Aoki[1,2,3], *Hiromi Aoki-Ogata*[1,3], *Haydar Bulut*[1], *Hironori Hayashi*[4], *Nobutoki Takamune*[5], *Naoki Kishimoto*[5], *Hiroki Tanaka*[3], *Kazuya Hasegawa*[6], *Arun K. Ghosh*[7], *Shinichi Machida*[3], *Shogo Misumi*[5], *and Hiroaki Mitsuya*[1,3,8]

[1]*NCI, NIH, Bethesda, MD, USA*
[2]*Kumamoto Health Sci Univ, Kumamoto, Japan*
[3]*Natl Cent for Glob Health & Med Res Inst, Tokyo, Japan*
[4]*Tohoku Univ Grad Sch of Med, Miyagi, Japan*
[5]*Dept of Envir and Mol Health Sci, Kumamoto Univ, Kumamoto, Japan*
[6]*JPN Synchrotron Radiation Res Inst, Hyogo, Japan*
[7]*Purdue University, West Lafayette, IN, USA*
[8]*Kumamoto Univ Hosp, Kumamoto, Japan*

We established a multi-class-drug-resistant HIV-1 variant (HIV_{KGD}) by consecutively exposing an HIV-1 variant to various antiretroviral agents including IN-strand-transfer-inhibitors (INSTIs). However, HIV_{KGD} was found extremely susceptible to a previously reported HIV-1 protease inhibitor, GRL-142, with IC_{50} of 130 femtomolar. Amino acid substitutions in HIV_{KGD}'s IN-gene were associated with HIV_{KGD}'s extreme susceptibility to GRL-142. When cells were infected with HIV_{KGD}'s IN-gene-containing recombinant-HIV and cultured in the presence of GRL-142, a significant decrease of unintegrated 2-LTR-circular DNA was observed, suggesting that nuclear import of pre-integration complex (PIC) was severely compromised. X-ray crystallographic analyses revealed that GRL-142 binds to and covers the critical sequence (DQAEHLK) of HIV-1-integrase-nuclear localization signal (NLS) and was assumed to sterically block the transport of GRL-142-bound HIV_{KGD}'s PIC into the nucleus. INSTI-resistant variants isolated from patients with AIDS, who were heavily INSTI-experienced and had developed resistance to RAL and/or DTG, were susceptible to GRL-142, suggesting that NLS-targeting agents could serve as agents for salvage therapy for highly INSTI-resistant-variant-harboring individuals. GRL-142's extremely potent activity against multi-drug-resistant HIV-1 variant (HIV_{KGD} and other variants with RAL- and/or DTG-resistance) was associated three inhibition mechanisms (i) proteases' enzyme inhibition, (ii) protease's dimerization inhibition, and (iii) impairment of HIV-1-NLS. Further design and optimization based on GRL-142 to potently block both IN and PR might lead to the development of promising treatment regimens with highly effective antiretroviral agents.

Session IV
O2-8

Host APOBEC3A cytidine deaminase drives mutations in the SARS-CoV-2 genome

Yoshihiro Nakata[1], *Hirotaka Ode*[1], and ***Yasumasa Iwatani***[1,2]

[1]*Department of Infectious Diseases and Immunology, Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Aichi, Japan*
[2]*Department of AIDS Research, Division of Basic Medicine, Nagoya University Graduate School oof Medicine, Nagoya, Aichi, Japan*

The number of rapid genetic variations in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA genome has been increasing during viral transmission in human populations since the end of 2019. Previous analysis of the SARS-CoV-2 genome database has suggested the involvement of a host APOBEC-like mutation signature, i.e., a high prevalence of cytidine (C)-to-uridine (U) mutations. However, the substantive host factors driving SARS-CoV-2 genome mutations have not yet been identified. In this talk, I show several lines of evidences that human APOBEC3A (A3A) cytidine deaminase plays a critical role in the induction of C-to-U substitutions in the SARS-CoV-2 genome. The APOBEC3 family comprises seven members (A, B, C, D, F, G and H) in primates and exhibits potent antiviral activity against retroviruses and retroelements.

First, our bioinformatics analysis of the chronological genetic changes in the sequence database indicated that the largest UC-to-UU mutation signature, consistent with APOBEC-recognizing nucleotide motifs, was predominant in single-stranded RNA (ssRNA) regions of the viral genome. In SARS-CoV-2-infected cells, exogenous A3A expression, but not expression of other human APOBEC proteins, induced UC-to-UU mutations in viral RNA (vRNA). Additionally, mutated C bases were often situated at the tip of a bulge or loop region in the vRNA secondary structure. Interestingly, A3A mRNA expression was drastically increased by interferons (IFNs) and tumour necrosis factor-α (TNF-α) in epithelial cells derived from the respiratory system, a site of efficient SARS-CoV-2 replication. Moreover, an elevated UC-to-UU mutation rate was detected in SARS-CoV-2 produced from lung epithelial cells treated with IFN-ß and TNF-α, but not from CRISPR/Cas9-based A3A knockout cells. Taken together, these findings demonstrate that A3A is a primary host factor that drives mutations in the SARS-CoV-2 RNA genome by RNA editing.

Session IV
O2-9

An inhaled ACE2 decoy confers protection against SARS-CoV-2 infection in preclinical models

Akatsuki Saito

Department of Veterinary Medicine, Faculty of Agriculture, University of Miyazaki

The Omicron variant continuously evolves under the humoral immune pressure exerted by vaccination and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and the resulting Omicron subvariants display further immune evasion and antibody escape. An engineered angiotensin-converting enzyme 2 (ACE2) decoy composed of high-affinity ACE2 and an IgG1 Fc domain could offer an alternative modality to neutralize SARS-CoV-2. We previously reported its broad spectrum and therapeutic potential in rodent models. Here, we demonstrate that the engineered ACE2 decoy retains neutralization activity against Omicron subvariants, including the currently emerging XBB and BQ.1 strains, which completely evade antibodies currently in clinical use. SARS-CoV-2, under the suboptimal concentration of neutralizing drugs, generated SARS-CoV-2 mutants escaping wild-type ACE2 decoy and monoclonal antibodies, whereas no escape mutant emerged against the engineered ACE2 decoy. Furthermore, inhalation of aerosolized decoys improved the outcomes of rodents infected with SARS-CoV-2 at a 20-fold lower dose than that of intravenous administration. Last, the engineered ACE2 decoy exhibited therapeutic efficacy for cynomolgus macaques infected with SARS-CoV-2. These results indicate that this engineered ACE2 decoy represents a promising therapeutic strategy to overcome immune-evading SARS-CoV-2 variants and that liquid aerosol inhalation could be considered as a noninvasive approach to enhance the efficacy of COVID-19 treatments.

Session IV
O2-10

Probing Host-Pathogen Protein Interactions by Yeast display

Miguel Padilla Blanco, ***Jiri Zahradník***

First Faculty of Medicine at Biocev, Charles University, Vestec-Prague, Czechia

In this presentation, we will dive into the methodology of yeast display and its multifaceted applications, including deep mutational scanning, affinity maturation, and long-term in vitro neutral evolution. Our focus will be on probing host-pathogen protein interactions, with a particular emphasis on SARS-CoV-2 and other coronaviruses. These techniques, traditionally employed in protein engineering, have recently risen to prominence as indispensable tools for dissecting the intricate interfaces between hosts and pathogens.,

Beyond structural similarities, we will explore the distinctive amino acid compositions characterizing the interfaces between SARS-CoV-2, ACE2, and other ACE2-binding coronaviruses. While these receptor-binding domains (RBDs) share the same ACE2 binding location, they diverge significantly in 11 interface residues, a divergence further compounded by emerging variants such as EG2 and BA.2.86. The existence of parallel solutions for the same interaction suggests a remarkable interface plasticity, even under the strong selective pressure driving tight binding.

Could current in vitro evolution techniques explain observed plasticity and help us to draw the future trends? Can an integration of protein engineering approaches enhance our understanding of host-pathogen dynamics and improve development of the next generation of vaccines and antibodies?

Session V
O2-11

Identification of HIV blips and associated immunological factors as indicators of HIV reservoir size

Kouki Matsuda[1,2]*, Kenji Maeda*[1]

[1]*Division of Antiviral Therapy, Joint Research Center for Human Retrovirus Infection, Kagoshima University, Kagoshima, Japan*
[2]*AIDS Clinical Center (ACC), National Center for Global Health and Medicine (NCGM), Tokyo, Japan*

The difficulty in eliminating the provirus incorporated into HIV-infected cells with current cART alone is a barrier to cure. In addition, it is extremely difficult to collect and analyze tissues such as lymph nodes where HIV reservoir cells are thought to be localized. In this study, we identified clinical biomarkers reflecting the size of residual HIV reservoirs in individuals with HIV infection. Twenty-five participants infected with HIV were enrolled in this study. All participants had been receiving cART for at least 5 years and maintained a low viral load (< 20 copies/mL) during therapy. First, we analyzed HIV proviruses using an intact provirus DNA assay (IPDA). The proportion of intact proviruses was significantly lower than that of defective proviruses, and most proviruses became defective after cART. $CD4^+$ T cells isolated from peripheral blood mononuclear cells were stimulated with PMA/Ionomycin *ex vivo*. An elevated level of HIV mRNA expression in cells was defined as the number of transcription-competent peripheral viral reservoirs. Then, we divided the 25 participants into two groups: 15 reactivation-positive and 10 reactivation-negative, and analyzed the difference, and identified some factors to differentiate these two groups, that can be biomarkers reflecting the amount of residual HIV reservoir cells of the individuals with HIV. The reactivation-positive group had a higher frequency of Blips, a phenomenon in which the viral load observed during cART temporarily increased to detectable levels. We identified blips as a clinical manifestation correlating with the residual HIV peripheral reservoir size. We also found that some blood factors, including anti-HIV antibody titers, associated with blips, can also be useful as biomarkers that reflect the number of residual HIV reservoirs. These indicators may be used to predict the therapeutic effects when HIV curative therapy is established.

Session V
O2-12

Epidemiological strategy towards sexually transmitted amebiasis.

Koji Watanabe [1,2], *Akira Kawashima* [1,2], *Yasuaki Yanagawa* [1,3]

[1]*AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo,*
[2]*Center for AIDS Research, Kumamoto University, Kumamoto, Japan.*
[3]*Department of Medicine, Stanford University, CA, USA.*

Amebiasis, caused by *Entamoeba histolytica*, is currently spreading not only in developing countries but also in some developed countries, including Taiwan and Japan. Unlike an amebiasis in the developing countries, where contaminated food/water are the source of transmission, amebiasis is transmitted as sexually transmitted infections (STIs) in developed countries. Asymptomatic carriers are the main source in such situation. Furthermore, it is the key for the epidemiological control to effectively identify & treat asymptomatic carriers in the high-risk population. For making it come true, we should know two things; 1) To determine who is at high risk for amebiasis (the target for the screening test), and 2) To establish the screening method for asymptomatic carriers.
Seroprevalence survey is known as an effective method for epidemiological assessment of *E. histolytica*. We previously reported that seropositivity of HIV-1 positive and negative MSM (Men who have sex with men) are 23.1% and 6.7%, respectively. It is already well-known that MSM is at high risk for *E. histolytica*, and good candidate for the screening test. Interestingly, our recent study showed that seropositivity of the female clients at STI clinic in Tokyo was 8.7 %, which was the same level as the male clients at the same clinic. These data strongly suggest that *E. histolytica* is currently spreading also among sexually active female in Japan. It is probably because most female clients at STI clinic are engaged in oral-anal sexual contact as a commercial sex worker, however, more detailed data should be collected in future epidemiological studies.
On the other hand, our clinical studies showed that peptic ulcer in the large intestine are surely found even among asymptomatic *E. histolytica* carriers. Moreover, seropositivity of *E. histolytica* among asymptomatic carriers are 90% or more. In the recent cross-sectional study, we performed serological screening test for *E. histolytica* for asymptomatic 312 HIV-negative MSM, and successfully identified 8 asymptomatic carriers by stool PCR from 20 seropositive individuals. Taken together, serological screening approach followed by the stool PCR constitutes a potentially practical (non-invasive & cost-effective) epidemiological strategy.
In conclusion, active epidemiological surveys, in combination with an “effective” serological screening for asymptomatically infected individuals, is one of the practical strategies for reducing sexually transmitted *E. histolytica* infections.

Session V
O2-13

Evolution of HTLV-1-induced cancer

*Sonia Wolf[1], Patricia Watber[1], Devon Weterings[1], Anat Melamed[1], Graham Taylor[1,2], Lucy Cook[1,2,3] and **Aileen Rowan**[1].*

[1]Section of Virology, Department of Infectious Disease, Faculty of Medicine, Imperial College London, United Kingdom
[2]National Centre for Human Retrovirology, Imperial College Healthcare NHS Trust, St Mary's Hospital, London, United Kingdom
[3]National Centre for Human Retrovirology, Imperial College Healthcare NHS Trust, St Mary's Hospital, London, United Kingdom

Human T cell Leukaemia virus type-1 (HTLV-1) chronically infects 10-20 million people worldwide and causes Adult T cell leukaemia/lymphoma (ATL) in 5% of virus carriers. The virus establishes tens of thousands of long-lived infected T-cell clones in each carrier, each of which contains a DNA copy of the viral genome integrated into the host cell DNA. In >90% of ATL cases, the malignant cells are descended from a single T-cell clone which has accumulated thousands of mutations within the host and viral genes. Our research aims to identify the key events that drive one of the thousands of infected T-cell clones to become malignant in a minority of virus carriers.
We previously showed that premalignant cells are detectable circulating in the blood at frequencies of >1% of peripheral blood mononuclear cells (PBMC) years before the onset of ATL symptoms. Here, we evaluated the rate at which otherwise healthy HTLV-1 carriers who have circulating ATL-like clones develop ATL. To evaluate the somatic mutational landscape and viral and host gene expression in ATL-like clones, we flow-sorted clones using anti-T-cell receptor (TCR) V-beta subunit antibodies combined with cell-surface markers which identify HTLV-1-infected cells (CCR4+CD26- cells). As immune escape mutations are common in ATL, we also characterised antiviral cytotoxic T-cell responses in carriers with and without circulating ATL-like clones.
Together, our data reveals that there is evidence of pre-malignant disease and immune dysregulation in HTLV-1 carriers before any symptoms of ATL begin, offering a rare opportunity to study the host-pathogen relationship during the development of virus-induced cancer. Furthermore, the ability to predict the onset of malignancy will enable clinicians to better identify at-risk carriers and raises the possibility of testing interventions to prevent or delay the onset of ATL.

Poster presentation
P-01

iPS cell–derived model to study the interaction between Tissue Macrophage and HIV-1

Youssef M. Eltalkhawy[1], *Naofumi Takahashi*[1], *Yasuo Ariumi*[1], *Jun Shimizu*[2], *Kazuo Miyazaki*[2], *Satoru Senju*[3], *Shinya Suzu*[1]

[1]*Joint Research Center for Human Retrovirus Infection, Kumamoto University, Honjo 2-2-1, Kumamoto-city, Kumamoto 860-0811, Japan*
[2]*MiCAN Technologies Inc., Goryo-ohara 1-36, Kyoto 615-8245, Japan*
[3]*Department of Immunogenetics, Graduate School of Medical Sciences, Kumamoto University, Honjo 2-2-1, Kumamoto-city, Kumamoto 860-0811, Japan*

Despite effective antiretroviral therapy (ART), HIV-1 persists in cells including macrophages, which is a critical obstacle to cure of HIV-1. However, the precise role of macrophages in HIV-1 infection remains unclear because they reside in tissues that are not easily accessible. Monocyte-derived macrophages (MDMs) are widely used as a model. However, another model is needed, as recent studies revealed that most tissue- resident macrophages originate from the yolk sac and fetal liver precursors rather than monocytes, and the embryonic macrophages possess a proliferating capability that MDMs lack. In this study, we therefore tested whether human induced pluripotent stem (iPS) cellderived immortalized macrophage-like cells (iPS-ML) are a useful self-renewing macrophage model. iPS-ML from five different donors were used in this study. The single round HIV-1 infection system was used which comprised of GFP-expression NL-43 strain coupled with VSV-G envelope protein. Gag was assessed by p24 ELISA in the supernatant as well as flowcytometric intracellular staining. Fate of the proviral copy was assessed using near-full length proviral PCR and viral transcription was assessed using qRT-PCR.

iPS-ML cells proliferate in a cytokine-dependent manner, retain macrophage functions, support HIV1 replication, and exhibit infected MDMs-like phenotypes, such as enhanced tunneling nanotube formation and cell motility, and resistance to viral cytopathic effect. However, several differences are also observed between MDMs and iPS-ML, most of which can be explained by the proliferation of iPS-ML. For instance, proviruses with large internal deletions are enriched more rapidly in iPS-ML. Interestingly, inhibition of viral transcription by HIV-1-suppressing agents is more obvious in iPS-ML. Our present data proposes that iPS-ML model is suitable for mimicking the interplay between HIV-1 and self-renewing tissue macrophages that cannot be fully modeled by MDMs alone.

Poster presentation
P-02

Advancing Entamoeba histolytica Diagnostics: Validation of qPCR-based assays

Akira Kawashima [1, 2, 3], *Koji Watanabe* [1, 2, 3], *Hiroyuki Gatanaga* [1, 2]

[1]*The Joint Research Center for Human Retrovirus Infection Kumamoto University Campus, Kumamoto City, Kumamoto, Japan*
[2]*AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan*
[3]*Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan*

Amebiasis is a protozoan infection that spreads through oral transmission and causes diseases such as colitis and liver abscess. Particularly in developed countries, it is known as a sexually transmitted infection (STI) that frequently occurs among men who have sex with men (MSM) and people engaging in oral-anal sex. Current diagnostic tests for *Entamoeba histolytica* include direct fecal microscopic examination, rapid fecal antigen test, serum anti-amoebic antibody test, and pathological examination. Still, a more sensitive and specific polymerase chain reaction (PCR) test is available. However, the PCR test requires complicated sample processing and nucleic acid extraction and is only performed in a few research institutions, not on a commercial basis.

Our primary objective, in collaboration with the Department of Parasitology at the National Institute of Infectious Diseases, was to optimize and validate Taqman-probed quantitative PCR (Taq-qPCR) assays for more accurate diagnosis of amebiasis. Despite our initial Taq-qPCR efforts, we observed a significant number of low-titer positives (Ct values > 35) that required further validation to distinguish true positives.

In order to evaluate the quality of the Taq-qPCR method, we introduced droplet digital PCR (ddPCR). ddPCR assay is a third-generation PCR method that provides absolute quantification, and it is known to be highly accurate even at low concentration ranges that are difficult to detect with qPCR. First, we successfully detected low burden of HM1:IMSS laboratory strain using our primer-probe sets. Furthermore, it was confirmed that amplitude of positive droplets at 35 PCR cycles is high enough to differentiate from negative droplets. Next, we will perform ddPCR using different primer-probe sets, and compare the amplitude between positive and negative droplets. Finally, we will identify the best primer-probe sets using clinical samples in the future experiments.

In summary, by using advanced techniques such as ddPCR and exploring novel primer-probe sets, we aim to increase the diagnostic accuracy for *Entamoeba histolytica*. This research has the potential to fill a diagnostic gap by improving current methods.

Poster presentation
P-03

HIV-1 infection in CD34$^+$ monocytes

Naofumi Takahashi, *Osamu Noyori, Youssef M. Eltalkhawy, Shinya Suzu*

Division of Infection & Hematopoiesis, Joint Research Center for Human Retrovirus Infection, Kumamoto University

HIV-1 persists in cellular reservoirs despite effective antiretroviral therapy (ART). Although CD4$^+$ T cells are the well-known reservoir, increasing evidence suggests that myeloid cells, including circulating monocytes, are also an important viral reservoir. However, it is not fully understood what are preferentially infected monocyte subsets in vivo. We previously reported that CD34$^+$ monocytes carried HIV-1 proviruses more frequently than remaining and majority fraction of monocytes. In this study, we initially found that this fraction harbors the proviruses even in the virologically suppressed patients on ART. Therefore, we asked how CD34$^+$ monocytes are infected and persist. We identified cells similar to CD34$^+$ monocytes (CD45$^+$CD14$^+$) in human lymph nodes. They expressed higher levels of HIV-1 receptors CD4 and CCR5 compared to the rest of monocytes, which suggested that they are counterpart of those in peripheral blood. Of note, when compared to CD34$^-$ monocytes, both peripheral and lymph node CD34$^+$ monocytes highly expressed CCR7 and sphingosine-1-phosphate receptor 1 (S1PR1), which regulate migration and egress of monocytes into and from the lymph nodes, respectively. In addition, analysis of lymph nodes from SIV-infected rhesus macaques revealed persistence of proviruses in CD34$^+$ monocytes as well as T cells. Taken together, these results raise a new possibility that lymph node CD34$^+$ monocytes, which originate from the circulation, are infected with HIV-1 owing to their high susceptibility to HIV-1, and return to circulation, which explains the detection of proviral DNA in peripheral CD34$^+$ monocytes even after long-term ART.

Poster presentation
P-04

Characterization of host genome in HIV-1 infected CD4+ T-cells from the patients under long-term combined Anti-Retroviral Therapy (cART)

***Samiul Alam Rajib*[1]*, Kouki Matsuda*[2]*, Wajihah Binti Sakhor*[1]*, Sharmin Nahar Sithi*[1]*, Akhinur Rahman*[1]*, Omnia Reda*[1]*, Benjy Jek Yang Tan*[1]*, Md Belal Hossain*[1]*, Shuzo Matsushita*[3]*, Kenji Maeda*[2]*, Hiroyuki Gatanaga*[4]*, Kiyoto Tsuchiya*[4]*, Yorifumi Satou*[1]

[1]*Division of Genomics and Transcriptomics, The Joint Research Center for Human Retrovirus Infection, Kumamoto University*
[2]*TheJoint Research Center for Human Retrovirus Infection, Kagoshima University*
[3]*Division of Clinical Retrovirology, The Joint Research Center for Human Retrovirus Infection, Kumamoto University*
[4]*AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo*

Human Immunodeficiency Virus type 1 (HIV-1) infiltrates host cells, and integrates its genetic material into host genome known as provirus. This provirus, whether intact or impaired, poses a challenge to complete viral elimination. The persistence of intact proviruses contributes to viral reservoir establishment by integrating into genes associated with cellular growth. This phenomenon, reminiscent of the retrovirus HTLV-1, has been linked to the gradual accumulation of cancer-related gene mutations in Adult T-cell Leukemia/Lymphoma (ATL). This raises the prospect of similar occurrences in people living with HIV (PLWH). Long-term cART induces viral latency, prompting investigation into potential cancer-related gene mutations within expanded infected cells, which could contribute to persistent health issues like non-AIDS-defining cancers in PLWH. To address this question, we are selectively isolating clonally expanded CD4+ T-cells at a single-cell resolution from PLWH who have sustained antiretroviral therapy for over two decades, maintaining undetectable viral levels for six months, and sustaining CD4+ T-cell counts exceeding 350 cells/mL. Peripheral blood mononuclear cells (PBMC) were acquired from eight patients attending the Dr. Matsushita's Clinic between 1997 and 2023. DNA and RNA were isolated from PBMCs subjected to stimulation with Phorbol ester Myristate Acetate (PMA) and Ionomycin for 24 hours. Proviral loads (PVL) were assessed across different time points, revealing a progressive increase in PVL for four patients despite prolonged cART. Utilizing Ligation-Mediated PCR, we identified expanded clones within three patient samples. Collaborating with NCGM, Tokyo, we expanded our study cohort by incorporating 30 additional patient samples collected throughout the first study year. In our ongoing project, we plan to isolate HIV-1-infected cells, followed by amplification of their genomic DNA via Multiple Displacement Amplification (MDA). A methanol-fixing-based sorting technique has been developed to selectively isolate HIV-1-infected CD4+ T-cells from long-term antiretroviral-treated patient samples. These isolated cells will undergo exome sequencing for mutation analysis within a cancer-related gene panel, further elucidating the intricate relationship between HIV-1 infection and chronic health conditions.

Poster presentation
P-05

Longitudinal characterization of HIV-1 provirus landscape in ART-treated individuals

Zavuga R Zuberi[1], *Samiul Alam Rajib*[1], *Kouki Matsuda*[2], *Kenji Maeda*[2], *Hiroyuki Gatanaga*[3], *Kiyoto Tsuchiya*[3] *and Yorifumi Satou*[1]

[1]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan*
[2]*Division of Antiviral Therapy, Joint Research Center for Human Retrovirus Infection, Kagoshima University, Kagoshima, Japan*
[3]*AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan*

Prolonged antiretroviral therapy (ART) results in plasma viral suppression in HIV-1-infected individuals. The persistence of latently infected HIV-1 reservoirs, such as CD4+ T cells, with the potential to drive viral rebound is a key obstacle in achieving an HIV-1 cure. However, long-acting ART can potentially improve HIV care by reducing the adherence burden, impacting the provirus landscape, and preventing drug resistance. We have hypothesized the viral sequences may contribute to the HIV-1 provirus landscape changes between treatment-naïve and ART-treated individuals. Therefore, this study aims to characterize the provirus landscape in the peripheral blood among HIV-infected individuals before and after ART. A longitudinal study of paired serum and peripheral blood mononuclear cells (PBMCs) samples (n=38) from treatment-naïve and ART-treated individuals was adopted. In treatment-naïve individuals, serum HIV-1 RNA samples were amplified by a two-amplicons approach and sequenced to identify multiple conserved regions of the replication-competent HIV-1 virus populations. Then, the designed primers targeting multiple regions of HIV-1 will amplify the HIV-1 proviral DNA from PBMCs of treatment-naïve and ART-treated individuals, followed by proviral sequences analysis. Concurrently, the HIV-1 RNA transcription between treatment-naïve and ART-treated individuals will be captured by the double-R assay based on π-code end-point PCR assay using the IntelliPlex™ system that simultaneously targets highly conserved ‘R’ region of the HIV-1 5’-long terminal repeats (LTR) and 3’-LTR regions. In addition, the IntelliPlex™ system will be used to detect the drug resistance mutations during ART by wild-type and drug-resistance-specific probes for the HIV-1 RNA during ART. We’ve currently achieved a 97.3% (n=37) success rate of amplifying both 5’- and 3’-half-length genome PCR (-hgPCR) amplicons, which decreased with samples having lower plasma viral loads. Moreover, 6 out of 13 (46.2%) samples with both 5’- and 3’-hgPCR amplicons were sequenced by short-reads sequencing using Illumina MiSeq. Considering the variability of HIV-1 sequence subpopulations observed among the studied participants (n=6), we plan to use nanopore long-reads sequencing to efficiently estimate the consensus sequences of HIV-1 RNA from serum samples.

Poster presentation
P-06

Establishment of recombinant SIV to characterize viral reservoirs *in vivo*

***Sharmin Nahar Sithi*[1], *Kazuaki Monde*[2], *Kenji Sugata*[1], *Wajihah Sakhor*[1], *Samiul Alam Rajib*[1], *Omnia Reda*[1], *Takuya Yamamoto*[3], *Yorifumi Satou*[1].**

[1]*Division of Genomics & Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[2]*Department of Microbiology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan*
[3]*Center for Vaccine & Adjuvant Research, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan*

The integration of viral DNA into the host genome is a critical event in the cycle of replication and pathogenesis of HIV. Although most HIV-infected cells are rapidly eliminated *in vivo*, integrated replication-competent but transcriptionally silent HIV provirus can form a viral reservoir and may remain latent despite combination anti-retroviral therapy (cART). The latency mechanism of HIV *in vivo* is yet not fully understood. On the other hand, SIV is a primate lentivirus and SIV infection of macaques closely mimics the pathogenesis, virology, immunology, and pathology of HIV infection in human. So, SIV-infected macaques are found as useful animal model for people living with HIV.
In this study, we have aimed to establish recombinant SIV with Timer, a dual fluorescent protein to visualize provirus expression dynamics and latent reservoirs *in vivo*. Timer fluorescent protein forms a short-lived chromophore that emits blue fluorescence ($t_{1/2}$~4h) during active transcription which matures into red fluorescence ($t_{1/2}$~120h) upon silencing. Utilizing this unique feature of Timer fluorescent protein, we constructed replication competent recombinant SIV-Timer-IRES-Nef where Timer coding sequences were inserted in the *nef* region of SIVmac239 genome and the expression of Timer fluorescent protein is driven by 5′ LTR. First, we observed Timer fluorescent protein expression in transfected 293T cells. To establish the system *in vitro*, we infected PM1-CCR5 cell line by SIV-Timer and monitored Timer expression for 5 days in flowcytometry. After 1 day of infection, $Blue^+$ cells were found which shifted to Red^+ on 3 days post infection. Moreover, to know the replication capacity, Gag p27 was observed in the infected cells. At present, we are validating the construct *in vitro* before infecting macaques. Our aim is to infect macaques with SIV-Timer and obtain PBMC, lymph nodes, spleens of the infected macaques at different time points and analyze them by single cell analysis to identify genetic and epigenetic regulators contributing establishment of latent reservoirs.

Poster presentation
P-07

Tissue-specific pathogenesis and tumor heterogeneity in lymphoma-type ATLL

Mitsuyoshi Takatori[1], *Kenji Sugata*[1], *Benjy Tan Jek Yang*[1], *Shugo Sakihama*[2], *Hiromu Yano*[3], *Yoshihiro Komohara*[3], *Yuetsu Tanaka*[2], *Atae Utsunomiya*[4], *Takuya Fukushima*[2], *Kennosuke Karube*[5], *Yorifumi Satou*[1]

[1]*Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto*
[2]*Laboratory of Hemato-Immunology, Graduate School of Health Sciences, University of the Ryukyus, Okinawa*
[3]*Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto*
[4]*Hematology, Imamura General Hospital, Kagoshima*
[5]*Department of Pathology and Laboratory Medicine, Graduate School of Medicine, Nagoya University*

Adult T-cell leukemia/lymphoma (ATLL) is a CD4 T-cell malignancy encompassing various subtypes, including lymphoma-type, cutaneous-type, and other variants characterized by tissue-specific tumor growth. Despite previous reports highlighting genomic aberrations in tumor cells from peripheral blood and tissues, the mechanisms underlying this abnormality and their relationship with the tumor microenvironment remain unclear. Our research focused on elucidating these differences by examining lymph nodes containing ATLL cells. We aim to clarify the differences in the characteristics of tumor cells based on the location of ATLL cells and further explore the tumor microenvironment in lymph nodes. To achieve this, we conducted high-resolution gene expression analysis using single-cell RNA sequencing (scRNA-seq) on peripheral blood samples and lymph node lesions from ATLL patients. Furthermore, we performed spatial transcriptomics to identify the tissue-specific localization of tumor cells and investigate the tumor immune microenvironment in lymph node lesions. We subsequently integrated and analyzed data obtained from scRNA-seq and spatial transcriptome analysis to uncover ATLL pathogenesis specific to lymph nodes. As a result, distinct patterns of gene expression and cellular dynamics were identified in peripheral blood and lymph node lesions, suggesting their involvement in the progression of the disease and treatment responses within the tissue. Additionally, we present spatial analyses elucidating ATLL cell localization and intratumoral heterogeneity.

Poster presentation
P-08

Exploring the role of CTCF in HIV-1 provirus silencing using multiple in-vitro latent clones

Akhinur Rahman[1,2], *Omnia Reda*[1,2,3], *Wajihah Sakhor,*[1,2] *Misaki Matsuo*[1], *Hiroaki Takeuchi*[4], *Kenji Sugata*[1], *Yorifumi Satou*[1]

[1]*Division of Genomics & Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[2]*Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japa*
[3]*Microbiology Department, High Institute of Public Health, Alexandria University, Alexandria, Egypt*
[4]*Department of Molecular Virology, Tokyo Medical and Dental University, Tokyo, Japan*

The persistence of HIV-1 provirus in latent reservoirs including CD4+ T cells, myeloid-linage cells during antiretroviral therapy is a major obstacle to cure HIV-1. Experimental tools like single cell, next generation sequencing and efforts from numerous researches have started shedding lights to the factors that contribute to HIV-1 silencing and maintenance of reservoir. Recent reports show that CTCF (CCCTC-binding factor) is upregulated in latent HIV-1 population and depletion of CTCF can inhibit the latent phenotype. Though the role of CTCF in retroviruses like HTLV, BLV is well recognized, how CTCF influences HIV-1 latency is not yet elucidated.

To investigate the role of CTCF in HIV-1 silencing, we used several HIV-1 clones like J1.1, J-Lat 9.2, J-Lat 10.6, Jurkat-timer C10, ACH2 and THP1#2-16. The integration site and the provirus sequence from these clones were determined by using DNA capture-seq method. To understand the epigenetic environment of provirus, we performed ChIP-seq (H3K4me3, H3K36me3 & H3K27me3) and RNA-seq. Interestingly, most of the clones have integration site inside actively transcribing gene. Then, we checked the probability of CTCF binding throughout the HIV-1 sequence using PWMTools and compared the in-silico CTCF binding score of HTLV-1, BLV and HIV-1. The probability score of HIV-1 was similar to HTLV-1 and BLV. We also performed CTCF Chromatin immunoprecipitation (ChIP) of all these clones and quantified the ChIP DNA for the provirus regions that showed top 3 highest score of in-silico binding. The ChIP-qPCR result suggests that CTCF binding sites inside provirus are different in these clones.

In summary, we are exploring whether the CTCF binding to provirus or nearby host regions influences HIV-1 silencing. If yes, we also want to explore how it influences provirus latency.

Poster presentation
P-09

Isolation of monoclonal antibodies with neutralization and ADCC activities against SARS-CoV-2 variants

***Kaho Matsumoto*[1]**, *Takeo Kuwata[1], Yu Kaku[2], Masayuki Amano[1], Mikiko Shimizu[1], Biswas Shashwata[1], Shuzo Matsushita[1]*

[1]Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan
[2]Division of Systems Virology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Neutralizing monoclonal antibodies were potent therapeutic drugs against SARS-COV-2 in the early phase of COVID-19 pandemic, but Omicron variants, which emerged in November 2021, are not neutralized by the antibodies clinically approved. To develop monoclonal antibodies effective against various variants, we isolated monoclonal antibodies from a breakthrough infection (BT) case during Omicron wave in October 2022. B cells were separated by magnetic beads, and IgG+IgM- memory B cells were single-cell sorted using receptor-binding domain (RBD) or spike (S) from prototype SARS-COV-2 as a probe. Recombinant IgG antibodies were produced in cells transfected by plasmids expressing heavy and light chains, which were cloned from the sorted B cells.

Total of 181 monoclonal antibodies were isolated from the Omicron BT case. Among them, 135 antibodies (75%) bound to the S protein from prototype SARS-COV-2, and 29 antibodies (16%) neutralized prototype SARS-COV-2. In addition to neutralizing activity, we examined antibody-dependent cellular cytotoxicity (ADCC) of antibodies by measurement of FcγRIIIa-mediated luciferase reporter activation. Twenty eight antibodies (15%) showed ADCC activity against the target cells expressing S proteins from prototype SARS-COV-2, but only 15 of them showed ADCC activity against Omicron BA.4/5 variant. Interestingly, 13 of these cross-reactive antibodies were isolated by the S probe, suggesting the ADCC epitope outside RBD, which is conserved among variants. Neutralizing antibodies were isolated by the RBD probe (59%) more than the S probe, because RBD is the main epitope for neutralization.

We are now analyzing neutralization and ADCC activities of newly isolated monoclonal antibodies against SARS-CoV-2 variants. Identification of antibodies cross-reactive to various variants will be useful for development of therapeutic antibodies and vaccines.

Poster presentation
P-10

HIV-Tocky system to visualize proviral expression dynamics

***Omnia Reda*[1,2], *Kazuaki Monde*[3], *Kenji Sugata*[1], *Akhinur Rahman*[1], *Wajihah Sakhor*[1], *Samiul Alam Rajib*[1], *Sharmin Nahar Sithi*[1], *Benjy Jek Yang Tan*[1], *Koki Niimura*[4], *Chihiro Motozono*[5], *Kenji Maeda*[6], *Masahiro Ono*[7], *Hiroaki Takeuchi* [8], *Yorifumi Satou*[1]**

[1]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[2]*Microbiology Department, High Institute of Public Health, Alexandria University, Egypt*
[3]*Department of Microbiology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan*
[4]*School of Medicine, Kumamoto University, Kumamoto, Japan*
[5]*Division of Infection and Immunology, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[6]*Division of Antiviral Therapy, Joint Research Center for Human Retrovirus Infection, Kagoshima University, Kagoshima, Japan*
[7]*Department of Life Sciences, Imperial College London, London, UK*
[8]*Department of High-risk Infectious Disease Control, Tokyo Medical and Dental University (TMDU), Tokyo, Japan*

The stably integrated pool of HIV-1 proviruses in the host genome stands against curative strategies. This reservoir is extremely heterogeneous with respect to host cell type, anatomical location, integration site, and replication fitness. During the initial phase of infection, only a few infected cells can resist host immune clearance or cytopathic effect and establish this resistant pool. The mechanisms underlying HIV latency initiation are not fully resolved yet. In the current study, we propose and validate a new reporter model for monitoring HIV-1 provirus silencing and reactivation using Timer of cell kinetics and activity (Tocky). HIV-Tocky system uses a fluorescent Timer protein whose emission spectrum spontaneously shifts from blue to red to reveal HIV-1 provirus dynamics. We dissected provirus transcriptional phases into early, persistent, recently silenced, and latent. To our knowledge, this is the first report to distinguish two latent subsets: a directly non-expressing population and a recently silenced after brief expression. In-depth integration site analysis suggested that the distribution of proviruses in directly latent cells was similar to that in actively transcribing cell population, whereas recently silenced cells tended to harbor proviruses integrated into heterochromatin. Furthermore, we established a library of various single integration clones which we utilized to demonstrate the efficiency of the block-and-lock strategy by capturing the fast dynamics of silencing that were overlooked in previous models. In summary, we propose HIV-Tocky system to serve as a time-sensitive model that can capture the dynamics of provirus expression, making it a useful tool for HIV latency research.

Poster presentation
P-11

Intelli-OVI: A new-generation clinical tool for monitoring emerging viral infections

Md Belal Hossain[1], *Yoshikazu Uchiyama*[2], *Samiul Alam Rajib*[1], *Mami Nagashima*[3], *Kenji Sadamasu*[3], *Yasuhiro Ogi*[4], *Tatsuya Kawaguchi*[4], *Tomokazu Tamura*[5], *Takasuke Fukuhara*[5], *Masahiro Ono*[6], *Kazuhisa Yoshimura*[3] *and Yorifumi Satou*[1]

[1]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto 860-8556, Japan*
[2]*Department of Information and Communication Technology, Faculty of Engineering, University of Miyazaki, Miyazaki, Japan*
[3]*Department of Microbiology, Tokyo Metropolitan Institute of Public Health, Tokyo, Japan*
[4]*Clinical Laboratory Center of Kumamoto City Medical Association, 5-15-12 Honjo, Chuo-ku, Kumamoto 860-0811, Japan*
[5]*Department of Microbiology and Immunology, Faculty of Medicine, Hokkaido University, Sapporo, Japan*
[6]*Department of Life Sciences, Imperial College London, London, UK*

High transmissible viruses including SARS-CoV-2 frequently accumulate novel mutations that are detected via high-throughput sequencing. However, there is a need to develop an alternative rapid and non-expensive approach. Here we developed a novel multiplex DNA detection method Intelli-OVI for analysing existing and novel mutations of SARS-CoV-2. Intelli-OVI uses the micro-disc-based method IntelliPlex and computational algorithms of objective variant identification (OVI). IntelliPlex uses micro-discs printed with a unique pictorial pattern as a labelling conjugate for DNA probes, and OVI allows simultaneous identification of several variants using multidimensional data obtained by the IntelliPlex method. Importantly, de novo mutations can be identified by decreased signals, which prompts the need to design additional primers and probes. Thus, Intelli-OVI can be upgraded to keep up with rapidly evolving viruses. We believe that Intelli-OVI will be useful in establishing a world better equipped to tackle emerging novel pathogens.

Poster presentation
P-12

Recombinant HIV model system joined with parallel integration site, transcriptomic and epigenomic analysis to discover factor regulating the fate of HIV activation and latency

Wajihah Sakhor[1], *Kenji Sugata*[1], *Benjy Tan Jek Yang*[1], *Kazuoki Monde*[2], *Chihiro Motozono*[1], *Ryusho Kariya*[1], *Omnia Reda*[1,3], *Akhinur Rahman*[1], *Misaki Matsuo*[1], *Hitomi Nakamura*[4], *Seiji Okada*[1], *Takamasa Ueno*[1], *Yasuko Sagara*[4], *Hiroaki Takeuchi*[5], *Masahiro Ono*[6], *Kenji Maeda*[7], *Yorifumi Satou*[1]

[1]*Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan.*
[2]*Department of Microbiology, Faculty of Life Sciences, Kumamoto University, Japan.*
[3]*Microbiology Department, High Institute of Public Health, Alexandria University, Egypt.*
[4]*Department of Quality, Japanese Red Cross Kyushu Block Blood Center, Chikushino, Japan.*
[5]*Department of Molecular Virology, Tokyo Medical and Dental University, Tokyo, Japan.*
[6]*Department of Life Sciences, Imperial College London, United Kingdom.*
[7]*Division of Antiviral Therapy, Joint Research Center for Human Retrovirus Infection, Kagoshima University, Japan.*

The presence of transcriptionally silent but replication competent provirus in latent reservoir forms major barrier towards HIV cure. This study utilizes HIV-Fluorescence Timer model to monitor the dynamics of HIV gene transcription over time coupled with single cell multiomic (scRNA-seq and scATAC-seq; Assay for Transposase-Accessible Chromatin) to obtain parallel information on the transcriptome as well as epigenetic profile of the same cell at single cell level. Fluorescent Timer is a mCherry-derived monomeric fluorescent protein that initially emits blue (t ½ ~ 4h) followed by red fluorescence (t ½ ~ 120h) which allow us to observe progressions of infected cells from productive (blue), persistence (violet), just silenced (red) to latent infection (no color) with time. Primary $CD4^+$ T cells were activated prior to infection with single round NL43-Timer. Following detection of Timer expressions by flow cytometry analysis, each $Timer^+$ cell populations were sorted and processed using 10x Chromium's single cell multiome kit. Integration site were discovered from scATAC-seq chimeric fragments, in which only intact provirus with single integration is considered for downstream analysis. scRNA-seq transcriptomic profile together with RNA velocity analysis predicts two major routes after acute infection which is either going into apoptosis or latency. Pseudo bulk motif activity analysis of scATAC-seq data allows us to examine for transcription factors that are enriched in accessible chromatin from each $Timer^+$ population. We observed a trend in which productive (blue) and persistence (violet) populations are enriched with AP-1 family, latent population (no color) is enriched with CTCF while the just silenced population (red) are enriched with RFX family. Currently we are looking at the dynamics of integration site, epigenomics and transcriptomics to discover potential factor regulating the fate of HIV activation and latency.

Poster presentation
P-13

HTLV-1 specific immune responses in latently HTLV-1 infected individuals

***Theodore Worlanyo Asigbee*[1,2]*, Midori Nakamura-Hoshi*[2]*, Nozomi Kuse*[1,2]*, Tetsuro Matano*[1,2,3]

[1]*Division of Vaccine, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*

[2]*AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan*

[3]*Institute of Medical Science, University of Tokyo, Tokyo, Japan*

Human T-cell leukemia virus-1 (HTLV-1) causes severe diseases such as adult T cell leukemia in a minority of infected individuals with long latency. Upon infection, host cytotoxic T lymphocytes (CTLs) mount immune responses to control viral oligoclonal proliferation. Concurrently, the immune system produces specific antibodies against HTLV-1 antigens. However, the relationship between HTLV-1 specific immune response and HTLV-1 proviral loads (PVLs), especially among asymptomatic carriers, remains unclear. Thus, this study aims to assess the association among PVLs, Tax-specific T-cell responses, and anti-HTLV-1 antibody responses in HTLV-1-infected individuals.

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood collected from consented HTLV-1-infected individuals. Heat-inactivated plasma was subjected to line immunoassay using INNO-LIA HTLV I/II Score kit to evaluate antibody responses. PBMCs were subjected to intracellular cytokine staining to evaluate HTLV-1-specific T cell responses and antigen (Gag p19) expression.

Individuals with high antibody responses had significantly high PVLs compared to those with low antibody responses. A positive correlation was observed between the PVLs and p19-expressing $CD4^+$ cell frequencies. Also, the ratio of p19 expressing $CD4^+$ T cell frequencies to PVLs inversely correlated with the Tax-specific $CD8^+$ T cell frequencies.

Taken together, these findings highlight a relationship between PVLs and viral-specific immune responses and strongly argue for further studies to evaluate the relationship between immune response against HTLV-1 persistence.

Poster presentation
P-14(SP-1)

Correlation of some virological characteristics of SARS-CoV-2 variants of concern and variants of interest with spike protein-mediated fusogenicity

***Mst Monira Begum*[1,2]*, Kimiko Ichihara*[1]*, Otowa Takahashi*[1]*, Nasser Hesham*[1,3]*, Michael Jonathan*[1,2]*, The Genotype to Phenotype Japan (G2P-Japan) Consortium, Kei Sato*[4]*, Terumasa Ikeda*[1]

[1]*Division of Molecular Virology and Genetics, Joint research center for Human Retrovirus infection, Kumamoto University, Kumamoto, Japan*
[2]*Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan*
[3]*Department of Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*
[4]*Division of Systems Virology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the global pandemic of the coronavirus disease 2019 (COVID-19). The spike (S) protein of SARS-CoV-2 plays an essential role in mediating membrane fusion of the virus with the target cells, triggering viral entry into target cells. Several reports demonstrated that the fusogenicity of the S protein in SARS-CoV-2 variants is closely associated with the intrinsic pathogenicity of the virus determined by a hamster model. However, the association of S fusogenicity with other virological parameters is unclear. Therefore, we investigate the correlation of the virological parameters of eleven previous variants of concern (VOCs) and variants of interest (VOIs) with S protein-mediated fusogenicity. The fusion activity mediated by S protein is strongly correlated with the S1/S2 cleavage of S protein in the transfected HEK293 cells and plaque size formed by clinical isolates in VeroE6/TMPRSS2 cells. However, the fusogenicity of S protein is not associated with pseudovirus infectivity measured in HOS-ACE2/TMPRSS2 cells, S protein-mediated entry efficiency into HOS-ACE2/TMPRSS2 cells, and viral replication kinetics in VeroE6/TMPRSS2 cells. Altogether, our data suggest that similar to fusion activity obtained by S protein-mediated membrane fusion assay, S1/S2 cleavage efficiency and plaque size may be a potential indicator to predict the intrinsic pathogenicity of newly emerged SARS-CoV-2 variants.

Poster presentation
P-15

The role of dysregulated extracellular vesicles' miRNAs in the modulation of chronic Inflammation during HIV infection

***Mussa Bago*[1]**, *Godfrey Barabona,*[1] *Doreen Kamori*[2], *Lilian Nkinda*[2] *and Takamasa Ueno*[1]

[1]*Division of Infection and Immunity, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[2]*Depertment of Microbiology and immunology, Muhimbili University of Health and Allied Sciences, Tanzania*

miRNAs are non-coding RNAs of ~22 nucleotide in length, that regulate gene expression by binding to mRNA and induce either degradation or translational repression. They play a vital role in different cellular activities such as proliferation, apoptosis, and differentiation. Recently, miRNAs have emerged as vital regulators of inflammatory responses. miRNAs can be secreted into extracellular space via extracellular vesicles (EVs) through which they can be delivered to a diversity of target cells and exert their effect. Intriguingly, HIV has been reported to mediate dysregulation of both cellular and circulating miRNAs within EVs. Thus we hypothesize that, these dysregulated EVs miRNAs play a role in the systemic nature of HIV-related chronic inflammation. Accordingly, this study aims to elucidate the role of dysregulated EVs' miRNAs in the modulation of chronic inflammation during HIV infection.

We isolated exosomes from plasma samples obtained from Tanzania comprising of 30 HIV patients (treated=12; untreated=18) and 9 HIV uninfected individuals and analyzed the expression levels of 10 immunoregulatory miRNAs in the exosomes including miR-9-5p, miR-16-5p, miR-18a-5p, miR-20a-5p, miR-142-3p, miR-144-3p, miR-146a-5p, miR-155-5p, miR-382-5p, miR-615-5p. Our analysis shows that a number of immunoregulatory miRNAs including miR-16-5p, miR-20-5p, miR-142-3p, miR-146a-5p are differentially expressed between the two groups ($p<0.05$). Furthermore, the levels of miR-144-3p and miR-382 were significantly correlated with plasma inflammatory markers in HIV infected individuals. These results suggest that dysregulated exosomes' miRNAs might have a role to play in HIV-related chronic inflammation. Therefore, we plan to utilize THP-1 derived macrophages to study the effect of these dysregulated exosome delivered miRNAs in secretion of pro-inflammatory cytokines.

Poster presentation
P-16

Characterization of self-renewing murine macrophages with different origins

Sara A. Habash, *Naofumi Takahashi, Shinya Suzu*

Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan.

Tissue macrophages are maintained by a constant replenishment by peripheral blood monocytes that extravasate into tissues, and thought to serve as a cellular reservoir of HIV-1. However, recent studies revealed that macrophages in many adult tissues originate from yolk sac and fetal liver precursors rather than monocytes, and the embryonic macrophages have a self-renewing capacity that monocyte-derived macrophages lack. Therefore, basic understanding of the origins and functions of macrophages forms the foundation for understanding the myeloid HIV reservoir. In this study, we therefore attempted to clarify why embryonic macrophages have the self-renewing capacity.

We isolated and expanded self-renewing macrophages from bone marrow (BM), fetal liver (FL), and yolk sac (YS) of C57BL/6 mice, through the long-term culture with repeated passages in the presence of M-CSF, a key cytokine for macrophage development. Three lines from each origin were compared in their phenotypes by flow cytometry and proliferation/survival using trypan blue exclusion or MTT reagent. Bulk RNA sequencing was done using Illumina NextSeq500. Apoptotic assay was done using the PE Annexin V apoptosis detection kit with 7-AAD.We added three GMCSF expanded FL lines and compared their response to LPS with M-CSF expanded ones. Phosphorylated M-CSF receptor was assessed by Western Blotting. Two lines per each origin were subjected to bulk ATAC Seq.

All the lines tested were positive for CD45, Mac-1, and F4/80, indicating that they were macrophages. They showed a stable proliferation in the presence of M-CSF. Interestingly, after MCSF-depletion, YS lines tended to survive for longer time than FL or BM lines. Moreover, the proliferative response of YS lines to sub-optimal concentrations of M-CSF was more obvious than that of FL or BM lines. M-CSF expanded FL lines showed strong response to LPS when compared to BM, YS and GM-CSF expanded FL lines. Gene set enrichment analysis (GSEA) showed upregulation of genes related to cell proliferation such as Myc targets, E2F targets, G2/M checkpoint and enrichment of P53 pathway in BM lines compared to FL and YS lines. These results suggest YS lines have a higher survival and proliferation potential than other lines, which may explain the longest residence of YS-originated macrophages in tissues. Our ongoing ATAC-seq analysis may provide additional mechanisms explaining the unique survival and proliferative feature of yolk sac lines.

Poster presentation
P-17(SP-2)

A new function of M-Sec, the host factor for an efficient cell-to-cell transmission of HIV-1 and HTLV-1

***Randa Abd-ElNasser**, Youssef M. Eltalkhawy, Naofumi Takahashi, Shinya Suzu*

Division of Infection & Hematopoiesis, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto

We have demonstrated that a cellular protein M-Sec (also known as tnfaip2) contributes to an efficient cell-to-cell transmission of both HIV-1 and HTLV-1. M-Sec is originally identified as a critical regulator of the F-actin^{+} long membrane extensions connecting distant cells referred to as tunneling nanotubes (TNTs), which is beneficial for the cell-to-cell transmission of those viruses. In addition, our recent study revealed that M-Sec facilitates the assembly of the viral structural protein Gag of HTLV-1, which is requisite for the formation of viral particles. Although this function of M-Sec more directly contributes to the cell-to-cell viral transmission, the underlying molecular basis remains unclear. In this study, we show that M-Sec regulates not only viral Gag protein but also the cell surface receptor for M-CSF, the most important cytokine for monocytes/macrophages. M-Sec knockdown in macrophages reduced the number and size of TNTs, as expected. Meanwhile, we found that M-Sec knockdown or pharmacological M-Sec inhibition also reduced the cellular responses to M-CSF, such as M-CSF-promoted migration, phagocytosis, nitric oxide production, and osteoclast differentiation. This inhibition appeared to be specific to M-CSF since the M-Sec knockdown did not affect the response to another cytokine GM-CSF or bacterial lipopolysaccharide (LPS). Consistent with the poor response to M-CSF, M-Sec knockdown/inhibition reduced the activation of M-CSF receptor and downstream cascades. Of interest, M-Sec knockdown did not affect the basal expression level of M-CSF receptor, but significantly reduced M-CSF-triggered internalization of M-CSF receptor and their re-expression on cell surface, the process of which is required for maximal signaling by receptor tyrosine kinases including M-CSF receptor. Exogenous expression of M-Sec in HEK 293 cells accelerates not only downregulation but also re-expression of M-CSF receptor on cell surface. These results suggest that M-Sec regulates the stability or intracellular trafficking M-CSF receptor, and thereby maximizes the response of macrophages to M-CSF. Given that M-Sec regulates plasma membrane structure exemplified by TNTs, our findings imply that M-Sec also regulates intracellular structures involved in endocytic trafficking of proteins, including M-CSF receptor and viral Gag protein.

Poster presentation
P-18(SP-3)

HLA-C-restricted CTLs specific for SARS-CoV-2 nucleocapsid display potent antiviral activity across viral variants and are maintained as a long-lived memory cells

***Yoshihiko Goto*[1,3]*, Mako Toyoda*[1]*, Toong Seng Tan*[1]*, Hiroshi Hamana*[2]*, Takeshi Nakama*[1]*, Hanyu Li*[1]*, Yoshiki Aritsu*[1]*, Mizuki Kitamatsu*[4]*, Hiroyuki Kishi*[2]*, Yusuke Tomita*[3]*, Takuro Sakagami*[3]*, Takamasa Ueno*[1] *Chihiro Motozono*[1]

[1]*Division of Infection and immunity, Joint Research Center for Human Retrovirus infection, Kumamoto University*
[2]*Department of Immunology, Faculty of Medicine, Academic Assembly, University of Toyama*
[3]*Department of Respiratory Medicine, Faculty of Life Sciences, Kumamoto University*
[4]*Department of Applied Chemistry, Faculty of Science and Engineering, Kindai University*

Regardless of the progressive emergence of Variant of Concerns (VOCs), SARS-CoV-2 virus remains susceptible to CTL recognition. However, we previously reported that a spike mutation in several VOCs can confer escape from immunodominant CTL responses. Therefore, it is becoming important to unveil potent CTL responses targeting conserved epitopes across SARS-CoV-2 VOCs. Here, we focused on T cell responses to SARS-CoV-2 nucleocapsid (N) antigen due to the most abundant and highly conserved protein. IFN-γ ELISpot assay using N overlapping peptide pools revealed several immunodominant regions in convalescents with a haplotype *HLA-A24-B52-C12* which is the most prevalent in Japan. The N-specific T cell lines were responded in the context of HLA-C*12:02, but not other alleles, indicating N protein preferentially induce HLA-C-restricted T cells. Based on epitope prediction database, we identified the KF9 as an immunodominant epitope restricted by HLA-C*12:02. In fact, ex vivo KF9/HLA-C12 tetramer assay showed the higher frequency of KF9/C12-specific T cells in convalescents with HLA-C*12:02 and in vitro-expanded T cell lines were found to produce IFN-γ, TNFα and IL-2 and upregulate CD107a, suggesting multifunctional nature and cytotoxic potential. Moreover, we demonstrated that the T cell lines comparably suppressed viral replication of prototype and Omicron variants when the target cells expressed HLA-C*12:02 KF9-specific memory $CD8^+$ T cells were maintained as effector memory and TEMRA phenotype 6 months and 1 year after infection and were proliferated as effector memory subsets in *in vitro* stimulation, suggesting a potent cytotoxic potential upon reinfection. Taken together, we identified SARS-CoV-2 N-specific CTLs showing the potent antiviral activity across VOCs and being maintained as a high qualitative memory with the rapid proliferative capacity in the context of HLA-C*12:02, highlighting the importance of HLA-C restriction molecules to control newly emerging SARS-CoV-2 VOCs, in addition of HLA-A and HLA-B and providing the great insights into the future vaccine development including N antigen.

Poster presentation
P-19

Cross-reactivity of HLA-A*24:02-restricted T-cell receptors towards SARS-CoV-2 mutations

***Takeshi Nakama*[1]**, *Aaron Wall*[2], *Hiroshi Hamana*[3], *Huanyu Li*[1], *Yoshihiko Goto*[1], *Yoshiki Aritsu*[1], *Mako Toyoda*[1], *Toong Seng Tan*[1], *Mizuki Kitamatsu*[4], *Keiko Udaka*[5], *Pierre J Rizkallah*[2], *Hiroyuki Kishi*[3], *Andrew K. Sewell*[2], *Takamasa Ueno*[1], *Chihiro Motozono*[1]

[1]*Division of Infection and immunity, Joint Research Center for Human Retrovirus infection, Kumamoto University, Kumamoto, Japan*

[2]*Division of Infection and Immunity, Cardiff University School of Medicine, CF14 4XN Cardiff, Wales, UK*

[3]*Department of Immunology, Faculty of Medicine, Academic Assembly, University of Toyama, Toyama, Japan*

[4]*Department of Applied Chemistry, Faculty of Science and Engineering, Kindai University, Osaka, Japan*

[5]*Department of Immunology, Kochi University, Kochi 783-8505, Japan*

Although the SARS-CoV-2 variants retains susceptibility to cellular immunity, the cross-reactivity and molecular basis of antigen-specific T cells towards variants remain unclear. In this study, we analyzed PBMCs isolated from Japanese convalescents and vaccinated donors, and revealed that the NF9 ($S_{448-456}$: NYNYLYRLF) was an immunodominant epitope presented by HLA-A*24:02 *ex vivo* and *in vitro*. NF9 epitope stood out as a notably dominant antigenic determinant encompassing the entire spike protein, discerned through the utilization of the IFN-γ ELISpot with overlapping SARS-CoV-2 spike peptides. Single cell TCR analysis of NF9-specific T cells revealed that public TCR clonotypes comprised of TRAV12 and TRBV6 with unique CDR3β motifs were identified in both of convalescents and vaccinated-donors. It is reported that a naturally-occurring mutation, L452 mutation in Lambda (Q), Delta (R) and Omicron BA.2 (Q/R) and BA.5 (R) variant, is located at 5th position within the NF9 epitope. To understand the implications of these mutations, we established TCR-transfected NFAT-Luciferase reporter cells and tested the recognition of NF9-specific $TRAV12^{+}TRBV6^{+}$ TCRs towards NF9-5X variant peptides. We observed that there was no difference in HLA binding among the peptides in HLA-stabilization assay, indicating that the fifth amino acid of the NF9 epitope play a crucial role in TCR-peptide interaction. Interestingly, $TRAV12^{+}TRBV6^{+}$ TCRs displayed partial recognition toward mutations such as W, V, I, and T within the NF9 peptide, but not towards Q and R. The crystal structure of the public TCR-NF9/A24 complex will elucidate the molecular mechanisms of TCR recognition towards the mutant. Taken together, these findings underscore the significance of public TCR responses in individuals with HLA-A*24:02 and their susceptibility to emerging SARS-CoV-2 variants featuring L452 mutations.

Poster presentation
P-20

APOBEC3 degradation is the primary function of HIV-1 Vif determining virion infectivity in the myeloid cell line THP-1

Ryo Shimizu[1], *Hesham Nasser*[1,2], *Michael Jonathan*[1], *Michael A. Carpenter*[3,4], *Adam Z. Cheng*[5,6], *William L. Brown*[5,6], *Daniel Sauter*[7], *Reuben S. Harris*[3,4], *Terumasa Ikeda*[1]

[1]*Division of Molecular Virology and Genetics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[2]*Department of Clinical Pathology, Faculty of Medicine, Suez Canal University, Egypt*
[3]*Department of Biochemistry and Structural Biology, University of Texas Health San Antonio, USA*
[4]*Howard Hughes Medical Institute, University of Texas Health San Antonio, USA*
[5]*Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, USA*
[6]*Institute for Molecular Virology, University of Minnesota, USA*
[7]*Institute for Medical Virology and Epidemiology of Viral Diseases, University Hospital Tübingen, Germany*

HIV-1 must overcome multiple innate antiviral mechanisms to replicate in $CD4^+$ T lymphocytes and macrophages. Previous studies have demonstrated that the APOBEC3 (A3) family of proteins (at least A3D, A3F, A3G, and stable A3H haplotypes) contribute to HIV-1 restriction in $CD4^+$ T lymphocytes. Virus-encoded virion infectivity factor (Vif) counteracts this antiviral activity by degrading A3 enzymes allowing HIV-1 replication in infected cells. In addition to A3 proteins, Vif also targets other cellular proteins in $CD4^+$ T lymphocytes, including PPP2R5 proteins. However, whether Vif primarily degrades only A3 proteins during viral replication is currently unknown. Herein, we describe the development and characterization of *A3F*-, *A3F/A3G*-, and *A3A*-to-*A3G*-null THP-1 cells. In comparison to Vif-proficient HIV-1, Vif-deficient viruses have substantially reduced infectivity in parental and *A3F*-null THP-1 cells, and a more modest decrease in infectivity in *A3F/A3G*-null cells. Remarkably, disruption of A3A–A3G protein expression completely restores the infectivity of Vif-deficient viruses in THP-1 cells. These results indicate that the primary function of Vif during infectious HIV-1 production from THP-1 cells is the targeting and degradation of A3 enzymes.

Poster presentation
P-21

Spike protein-mediated membrane fusion assay enables tracing the evolution of SARS-CoV-2 and variants

***Hesham Nasser*[1]*, Rigel Suzuki*[2]*, Ryo Shimizu*[1]*, The Genotype to Phenotype Japan (G2P-Japan) Consortium, Akatsuki Saito*[3]*, Kei Sato*[4]*, Keita Matsuno*[5]*, Takasuke Fukuhara*[2] *and Terumasa Ikeda*[1]

[1]*Division of Molecular Virology and Genetics, Joint Research Center for Human Retrovirus infection, Kumamoto University, Kumamoto, Japan*
[2]*Department of Microbiology and Immunology, Graduate School of Medicine, Hokkaido University, Sapporo, Japan*
[3]*Department of Veterinary Science, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan*
[4]*Division of Systems Virology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan*
[5]*International Collaboration Unit, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan*

SARS-CoV-2, the causative virus of the global pandemic COVID-19, continues to evolve giving rise to several variants with diverse virological and pathological characteristics. In addition, SARS-CoV-2 possesses a relatively large genome (30 kb), however, it is evidenced that the SARS-CoV-2 *Spike* (*S*) gene accumulates the highest number of mutations compared to other viral genes. Since S protein mediates target cell recognition, viral fusion and entry, amino acid changes of S protein indeed diversify pathological outcomes. Therefore, we developed a cell-based assay system to investigate variable fusion activities of S proteins in the emerged variants. The assay utilizes a dual split protein (DSP) encoding *Renilla* luciferase (*RL*) and *GFP* genes, where the generated signal is proportional to spike/target cell fusion. Results showed the P681R mutation in the S protein significantly enhanced the fusogenicity of the Delta variant, which is associated with aggravated pathogenicity. Likewise, the diminished fusogenicity of the Omicron BA.1 variant S protein is associated with attenuated pathogenicity. We detected variations of S-mediated viral fusogenicity with the emergence of multiple Omicron subvariants. S protein of Omicron BA.2.75 acquired amino acid substitutions that enhanced fusogenicity and fitness. Lately, omicron subvariant XBB emerged with a recombined mutation breakpoint located in the receptor-binding domain (RBD) of S protein, rendering recombinant spike confers immune evasion and increases fusogenicity. Collectively, our assay system successfully quantifies the fusion activities of SARS-CoV-2 emerging variants, thus enabling tracing viral evolution.

Poster presentation
P-22

Development of altered peptide ligands containing non-natural amino acids that efficiently induce antiviral T cell responses.

***Yoshiki Aritsu*[1]**, *Amiri Kurose*[2], *Takeshi Nakama*[1], *Takamasa Ueno*[1], *Mizuki Kitamatsu*[2], *Chihiro Motozono*[1]

[1]*Division of Infection and Immunity for Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto*
[2]*Department of Applied Chemistry, Faculty of Science and Engineering, Kindai University, Osaka*

Cytotoxic T cells play an important role in the control of viral infection. Epitope peptides, consisting of 8-14 amino acids degraded from viral proteins, are presented by human leukocyte antigens (HLA) on infected cells and recognized by virus-specific T cells through their T cell receptors (TCRs), leading to the killing of these cells directly and contributing to the control of viral infection. Although the development of artificially modified peptides that efficiently recognized by TCRs has been done by random amino acid substitutions, a rational design method has not been established yet. In this study, we focused on non-natural amino acids as a tool for the precise modification of orientation and functional groups of the peptides based on TCR-peptide/HLA (pHLA) structure. Here, we focused on two HLA-A*24:02-restricted immunodominant epitopes, NF9 ($S_{448-456}$: NYNYLYRLF) and QI9 ($S_{1208-1216}$: QYIKWPWYI) derived from the spike protein of SARS-CoV-2 as model antigens. Using TCR-reporter cells, we evaluated the antigen recognition of antigen-specific TCRs for modified peptides. As the crystal structure of NF9-speicific TCR/peptide/HLA-A24 complex revealed that NF9-specific TCR interacts with Tyr at position 4 and position 6 of the NF9, we investigated modifications to the hydroxyl group positions and functional groups. The substitutions at position 4 of NF9 resulted in high sensitivity at low concentrations. In fact, peptides to fluorine substitutions at the ortho or meta-position exhibited 2.8 and 1.5 times higher sensitivity than the wild-type response, respectively, suggesting that the decreased electron density of the aromatic ring potentially facilitated interaction of TCR with the modified peptides. The substitutions at position 6 of NF9 did not show enhanced recognition than the wild type. Moreover, during the synthesis of the QI9 peptide, we found N-terminal pyroglutamination of the QI9 that recognized potently by QI9-specific TCRs rather than wild-type peptide. In the future, we plan to develop artificially modified peptides with higher responsiveness based on crystal structural structure and modeling by supercomputer (Fugaku) and evaluate the functionality of peptide-induced T cells through *in vivo* infection experiments using HLA-A24 transgenic mice.

Poster presentation
P-23

Establishment of SARS-CoV-2 infected Nr4a3-Tocky mice model with different severity

Chatherine Silas Mtali[1], *Rise Kurokawa*[1], *Wajihah Sakhor*[2], *Omnia Reda*[2], *Yorifumi Sato*[2], *Masahiro Ono*[3,4], *Takushi Nomura*[1,5]

[1]*Division of Virology and Pathology, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan*

[2]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan*

[3]*Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, UK*

[4]*Collaboration Unit for Infection, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan*

[5]*AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan*

Background: COVID-19 pneumonia is prevalent in older adults, and disease severity is associated with aging. The dysfunction of innate, humoral and cellular immunity is suggested to be involved in the exacerbations of COVID-19, but the detailed mechanism of the pathogenesis remains unclear. This study aims to investigate T-cell dynamics in the subacute phase of SARS-COV-2 infection using infected mouse models with severe/mild pneumonia to elucidate the adaptive immune responses associated with SARS-COV-2 severity. **Materials and methods:** Nr4a3-Timer mice, which enables in vivo analysis of the antigen-presenting activation of T cells, were subjected to the following infection experiment. Old and middle-aged male mice were inoculated intranasally with 1.0×10^5 $TCID_{50}$ (30μl) of SARS-CoV-2/QHmusX. Old and middle-aged mock-infected mice were inoculated with 2% FBS containing DMEM. All mice were followed up with daily weight measurements and euthanized under anesthesia at five dpi. Lung mononuclear cells were isolated by enzymatic treatment. The obtained cells were subjected to flow cytometric analysis to evaluate inflammatory cell infiltration and antigen-specific T-cell dynamics in the lung. **Results:** Middle-aged animals exhibited recovery of body weight after temporal reduction with mild pneumonia. Old infected mice indicated severe pneumonia and slower recovery of body weight. The middle-aged mice induced significantly higher levels of antigen-specific reactive T cells in the lungs than the old mice. In both middle-aged and older mice, inflammatory cells infiltrated the lungs. **Conclusion:** We established SARS-CoV-2 infected Nr4a3-Tocky mice model with different severity depending on the age. T-cell responses induced in the lungs in the middle-aged mice may contribute to the viral control and suppression of SARS-CoV-2 pneumonia. The dysfunction of T-cell functions in the old mice may be associated with the severity of SARS-CoV-2 pneumonia, indicating a role of immune aging in COVID-19 pathogenesis.

Poster presentation
P-24

Analysis of virological and immunological characteristics of SARS-CoV-2 spike variants with mutations at position L452

***Mako Toyoda*[1]**, *Toong Seng Tan[1], Hirotaka Ode[2], Chihiro Motozono[1], Yasumasa Iwatani[2], Takamasa Ueno[1]*

[1]Division of Infection and Immunity, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan
[2]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Aichi, Japan

Mutations at spike protein L452 are recurrently observed in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOC) including omicron lineages. It remains elusive how amino acid substitutions at L452 are selected in VOC. Here, we characterized all 19 possible mutations at this site and revealed that five mutants expressing the amino acids Q, K, H, M and R gained greater fusogenicity and pseudovirus infectivity, whereas other mutants failed to maintain steady-state expression levels and/or pseudovirus infectivity. Moreover, the five mutants showed decreased sensitivity toward neutralization by vaccine-induced antisera and conferred escape from T cell recognition. According to the sequence data obtained from the Global Initiative on Sharing All Influenza Data (GISAID) revealed that the naturally occurring L452 mutations with a frequency of over 0.001 were R, M, and Q, all of which can arise from a single nucleotide change (Updated on 25 August 2023). The L452R mutation appeared in the Delta and Omicron strains BA.5 and BQ.1.1, the L452Q in the Lambda strain, and the L452M in the BA.2 sublineage. Our results suggest that changes in virological and immunological characteristics due to the acquisition of mutations at the L452 site may contribute to the rapid spread of the virus.

Poster presentation
P-25

Isolation of resistant mutants against Lenacapavir using the Capsid Library System

Wright Andrews Ofotsu Amesimeku, *Joyce Appiah-Kubi, Nyame Perpetual, Nami Monde, Hiromi Terasawa, Md. Jakir Hossain, Tomohiro Sawa, Yosuke Maeda, Kazuaki Monde*

Department of Microbiology, Graduate School of Medical Sciences, Kumamoto University

The life-long burden associated with the daily taking of anti-retroviral drugs has been a concern expressed by people living with HIV (PLWH). PLWH experiences medication fatigue that might lead to sub-optimal treatment adherence and the emergence of drug-resistant variants. Lenacapavir, a novel capsid inhibitor approved in 2022 in Europe and North America but not in Japan, has been shown to have an exceptional potency against HIV-2 and all major HIV-1 types. The daily burden of taking HIV drugs has been reduced to a single oral or sub-cutaneous shot in six months. Drug mechanism has also been shown to disrupt the viral capsid core, prevent capsid-mediated nuclear import and affect HIV particle production. Resistance selections experiment *in vivo* and *in vitro* by other researchers identified some resistance-associated mutations (RAMs) in HIV-1 capsid (CA) when treated with lenacapavir (L56I, M66I, Q67H, K70N, N74D, N74S, and T107N). Further investigations are needed to identify other RAMs and to clarify the resistance acquisition mechanism of mutants.

A library system designed in our laboratory was used in the making of the capsid library based on the Los Alamos database. Using this system, a unique mutant was isolated in the presence of lenacapavir. The isolated mutant encoded RAMs with some mutations in and near the cyclophilin A binding loop. The RAMs are L56I, M66I, M68I, E71, E79D, V83M, I91V, M96I and E98D. Lenacapavir inhibited the wt HIV-1_{JRFL} replication but not the HIV-1 mutant at 100pM. However, the virus release of the isolated HIV-1 mutant was 100-fold lower than the wt HIV-1. The infectivity of the isolated mutant was 3-fold lower the wt HIV-1. On the other hand, lenacapavir induced the cell death in the infected-T cells. Interestingly, the mutant accelerated the cell death regardless of lenacapavir-treatment. This suggests that lenacapavir-resistant variants lose the ability to replicate and are more likely to lose infected cells by the cell death. As a result, one dose every 6 months may be enough for the HIV-1 therapy. Further experiments are ongoing to determine other phenotypic characteristics of the isolated resistant variant and to clarify the mechanism leading to the acceleration of the cell death in the lenacapavir treated conditions infected with the HIV-1 mutant virus.

Poster presentation
P-26

Characterization of antigen-specific T cell responses in third-dose SARS-CoV-2 vaccinated Japanese with HIV-1 infection.

Ndubi Mark [1], *Mako Toyoda* [1], *Chihiro Motozono* [1], *Rumi Minami* [2], *Takamasa Ueno* [1]

[1]*Division of Infection and Immunity, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan*
[2]*Internal Medicine, Clinical Research Institute, National Hospital Organization, Kyushu Medical Center, Fukuoka, Japan*

Background: T cell functionality in persons living with HIV (PLWH) differs from healthy individuals due to immune dysfunction caused by HIV, even with application of antiretroviral therapy (ART). This may lead to impaired vaccine responses in this population. In this study, we want to quantitatively analyse the intricate antigen-specific T cell responses in PLWH elicited by the third-dose SARS-CoV-2 vaccine.

Methodology: T cell responses specific to SARS-CoV-2 spike were evaluated by IFN-γ ELISPOT and the activation-induced marker (AIM) assays in response to the quadruplicate consecutive peptide pools covering the entire spike protein (Pool 1 [$S_{1\text{-}82}$], Pool 2 [$S_{80\text{-}169}$], Pool 3 [$S_{168\text{-}S244}$] and Pool 4 [$S_{243\text{-}S315}$]) toward PBMC isolated from ART-treated PLWH (n=28) at the median of 42 days (IQR, 26-68) after the 3rd-dose of SARS-CoV-2 mRNA vaccine at Kyushu Medical Center, Japan. In AIM assay, the antigen-specific activation was measured by $CD137^+OX40^+$ in $CD4^+$ T cells and $CD137^+CD25^+$ in $CD8^+$ T cells.

Results: Notably, the most prominent antigen-specific IFN-γ^+ cell responses were observed in favor of Spike Pool 3 and Pool 4. The total count of IFN-gamma+ cells showed a negative association with CD4/CD8 ratio in ART-treated PLWH ($p<0.01$) and a positive association with days after the 3rd dose ($p<0.01$), with no significant associations with plasma viral load, age, or CD4 T cell number. The AIM assay findings indicated a noteworthy negative association between the combined proportions of activated $CD4^+$ ($CD137^+CD134^+$) and $CD8^+$ ($CD137^+CD25^+$) T cell subsets and age ($p < 0.05$), but no significant associations with CD4/CD8 ratio, post-vaccination duration, viral load, or CD4 T cell count.

Conclusion: We have successfully established the quantitative assays for T cell responses specific to SARS-CoV-2 spike. The negative association between IFN-γ^+ cells and CD4/CD8 ratio suggest that immune recovery of PLWH may be an important factor for vaccine although greater numbers of participants are needed and are currently underway.

Poster presentation
P-27

HIV-1 minigene system to establish an in vitro latency model

***Dechuan Kong*[1,2]*, Seiya Ozono*[1]*, Masanori Kameoka*[3]*, Takamasa Ueno*[2]*, Kenzo Tokunaga*[1,2]

[1]*Department of Pathology, National Institute of Infectious Diseases, Tokyo*
[2]*Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto*
[3]*Department of Public Health, Kobe University Graduate School of Health Sciences, Hyogo*

The goal for HIV/AIDS is not only a functional cure but also a sterilizing cure that can completely eliminate latently infected cells in the body. To achieve this, it is desirable to develop a versatile in vitro platform to reflect HIV-1 latency by simplifying a whole infection system, which enables us to precisely perform quantitative assays with no requirement for live viruses. In this study, we newly generated an in vitro HIV-1 latency model by generating an HIV-1 minigene (by deleting or disrupting *gag-pol*, *vif*, and *vpr* genes) that harbors two reporter genes (encoding a novel fluorescent protein StayGold and a codon-optimized firefly luciferase protein Luc2), resulting in a Tat-Rev-dependent Env and dual-reporter expression plasmid to mimic a nonproductive state of HIV-1 infection. We confirmed the establishment of latently infected cells within four weeks postinfection with the HIV-1 minigene-based virus, by detecting gradually reduced StayGold and Luc2 activity. During a persistent state of infection (before the latency), we also proved that viruses produced after reintroduction of the Gag-Pol expression plasmid into the cells remained infectious. CRISPR-KO targeting the tat gene efficiently disrupted proviral DNAs in latently infected cells, whereas the effect of LTR-targeting CRISPRa is currently under investigation. We conclude that the HIV-1 minigene-based virus system can reproduce a latent phase of HIV-1 infection and can be widely utilized for evaluating various HIV-1 knockout and reactivation methods.

Poster presentation
P-28

HIV-1 broadly Neutralizing antibodies in Newly diagnosed Tanzanians

Isaac Ngare[1]*, Takeo Kuwata*[1]*, Godfrey Barabona*[1]*, Takamasa Ueno*[1]

[1]*Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan*

BACKGROUND: Failed attempts to elicit HIV-1 broadly neutralizing antibodies (bNAbs) via vaccination underscore the need to further investigate bNAb elicitation in previously undercharacterized populations. Here, we investigated HIV-1 bNAb elicitation in newly diagnosed Tanzanians of an indeterminate duration of infection.

METHODS: Preliminary bNAb screening assessed plasma neutralization of a smaller panel of tier 2 envelopes of subtype B and AC extraction. Potential bNAb plasmas had their polyclonal IgG fractions isolated and additionally screened on a broader global panel of 12 envelopes spanning various HIV-1 subtypes. bNAb breadth was defined as the percentage of envelopes on the global panel neutralized; whereas bNAb potency was the mean of IgG IC_{50} values across the panel. Plasma cytokines were quantified by Cytometric bead array.

RESULTS: bNAbs (defined as IgG fractions that could neutralize ≥ 5 envelopes on the global panel) were identified in 14 (6%) of the 230 participants screened. Contrary to other cohorts, moderate HIV pathogenicity appeared to associate with bNAb elicitation given participants with bNAbs had significantly lower viral load (p= 0.03) and that high levels of IL-21 in the periphery associated with higher bNAb potency (r= -0.47, p= 0.02). Further, in a subset of 4 participants with bNAbs, and whose IgG was available after >3 years of antiretroviral therapy (ART) and suppressed viremia, bNAb breadth was surprisingly maintained while bNAb potency marginally decreased (p> 0.56).

CONCLUSION: Our findings suggest atypical viral and immune conditions associating with HIV bNAb elicitation in newly diagnosed Tanzanians with indefinite durations of infection.

Poster presentation
P-29

The establishment of transgenic mouse system to characterize HTLV-1-driven CD4$^+$ T cell immortalization mechanism

***M Ishrat Jahan*[1]**, *Kenji Sugata*[1], *Nobuko Irie*[2], *Masaya Baba*[2], *Masahiro Ono*[2,3], *Yorifumi Satou*[1,2]

[1]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, 860-8556, Japan*
[2]*International Research Center for Medical Sciences (IRCMS), Kumamoto University, Kumamoto, 860-0811, Japan*
[3]*Department of Life Sciences, Imperial College London*

Adult T-cell leukemia (ATL) is caused by the infection of CD4$^+$ T cells by Human T-cell leukemia virus type 1 (HTLV-1). The major pathogenic genes-HBZ, encoded by anti-sense, strand and Tax, encoded by sense strand, are responsible for this transformation.

Previous studies have reported that infected CD4$^+$ T cells receive excessive T cell activation presumably due to continuous TCR stimulation from the endogenous antigens. Additionally, the accumulation of mutations in genes related to TCR signaling pathway has been evident in transformed ATL cells. It is still yet to understand how the viral genes contribute to the commencement of the malignant transformation in vivo.

HBZ and Tax proteins target several transcription factors downstream of TCR signaling pathway (NFkB, AP-1, NFAT, CBP, Jun). HBZ is responsible for the survival of infected clones and expresses constantly throughout the latency. Tax is known to transactivate the proviral plus strand transcription which is important for infected cell proliferation.

Therefore, we are working to establish HBZ-Tg that expresses HBZ under CD4 promoter, enhancer and silencer. Currently, we have got 14 founder generation HBZ-Tg. We are planning to analyze the CD4$^+$ T cell phenotype in these mice as well as TCR clonality and compare with the non-Tg littermate.

In our study, we are also trying to develop a new transgenic mouse model targeting Tax to express under Nr4a3 promoter (an immediate target of TCR signaling). To track the transient Tax expression, Timer fluorescence protein was placed downstream of Tax with P2A under the same promoter.

We got four founder generation mice. Among them in two lines, few Timer red cells were detected from thymocytes only. However, we were unable to detect Timer blue in the splenocytes upon TCR signal stimulation compared to positive control. Also, Tax protein was below detection level by flow cytometry in any of the lines. Currently we are trying to understand the underlying reasons regarding Timer expression in the unstimulated and stimulated splenocytes and if the construct is working.

Poster presentation
P-30(SP-4)

Pro-inflammatory cytokine production after SARS-CoV-2 infection in people living with HIV.

***Alitzel Anzurez*[1,2]*, Lucky Runtuwene*[1]*, Thao Thi Thu Dang*[1,2]*, Kaori Hosoya-Nakayama*[1]*, Aki Tanabe*[1]*, Michiko Koga*[4]*, Yukihiro Yoshimura*[5]*, Natsuo Tachikawa*[5]*, Tetsuro Matano*[1,2,3]*, Ai Kawana-Tachikawa*[1,2,3]

[1]*AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan*
[2]*Joint Research Center for Human Retrovirus Infection, Kumamoto, Japan*
[3]*Department of AIDS Vaccine Development, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[4]*Division of Infectious Diseases, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan*
[5]*Department of Infectious Diseases, Yokohama Municipal Citizens' Hospital, Kanagawa, Japan*

Introduction. Dysregulated inflammatory responses after SARS-CoV-2 infection cause severe COVID-19. Although HIV infection is considered to be one of the risk factors for severity, inflammatory responses during acute SARS-CoV-2 infection have yet to be evaluated in people living with HIV (PLWH). In this study, levels of multiple cytokines were measured in PLWH during the acute and recovery phases of COVID-19.

Materials and Methods: Nineteen PLWH and 18 HIV-seronegative individuals with SARS-CoV-2 infection were enrolled in this study. Plasma samples were collected at 1-14 days (acute phase) and 1-2 months (recovery phase) post-symptom onset. Levels of 20 pro-inflammatory cytokines were measured using multiplex Luminex assay system (Invitrogen): GM-CSF, IFNα, IFNγ, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, TNFα, IP-10, MCP-1, MIP-1α, MIP-1β, ICAM-1, CD62E, and CD62P. Plasma samples at 6-12 months after SARS-CoV-2 infection were also measured to define the basal levels of these cytokines.

Results. The levels of most measured cytokines in the acute phase were significantly elevated compared to the basal levels and declined towards the recovery phase. The levels of IL-1α, IL-8, and IP-10 in the acute phase were significantly higher in severe cases than in mild cases. Although the basal levels of CD62E, ICAM-1, and IP-10 were significantly higher in PLWH, there was no significant difference in the level of each cytokine between the groups in the acute phase.

Conclusion. Inflammatory responses are induced in PLWH at a similar level to HIV-seronegative individuals after SARS-CoV-2 infection, despite their chronic pro-inflammatory status.

Poster presentation
P-31

Effect of CXCR4 oligomeric states on HIV-1 infectivity

***Mayu Okumura*[1]**, *Tomofumi Nakamura*[1], *Masao Matsuoka*[1], *Junichiro Yasunaga*[1] *and Hirotomo Nakata*[1]

[1]*Department of Hematology, Rheumatology and Infectious Diseases, Kumamoto University School of Medicine, Kumamoto, Japan*

Chemokine receptors have some functions such as the signal transduction of leukocyte migration. It has been reported that the oligomeric states of chemokine receptors are associated with the functional regulation. Therefore, we expected that the oligomeric or monomeric states of chemokine receptors similarly affect the entry step of HIV-1 infection. In this study, we investigate the effect of a chemokine receptor, CXCR4 oligomerization on HIV-1 infectivity.

At first, we selected several amino acid residues such as N192A, W195A, F201A, M205A, L210A, L267A, and E268A which are potentially important for CXCR4 dimerization through the crystal structure of a CXCR4 dimer reported by Wu *et al*. To examine effect of CXCR4 oligomeric states on HIV-1 infectivity, we generated cell-expression plasmids carrying $CXCR4_{N192A}$, $CXCR4_{W195A}$, $CXCR4_{F201A}$, $CXCR4_{M205A}$, $CXCR4_{L210A}$, $CXCR4_{L267A}$ or $CXCR4_{E268A}$, and performed fusion assay, TZM-$bl_{CXCR4\Delta}$ assay, FRET assay, and Western blotting (WB) with the crosslinker BS^3.

In the fusion assay, the fusion activity of the cells expressing $CXCR4_{N192A}$ decreased by 50%, similar to $CXCR4_{M205A}$ and $CXCR4_{L210A}$ decreased by 30% compared to the $CXCR4_{WT}$. In the FRET assay, the FRET ratio to see oligomeric states of CXCR4 increased in wild-type $CXCR4_{WT}$ and variant $CXCR4_{MT}$, showing that $CXCR4_{WT}$ and $CXCR4_{MT}$ form oligomeric states. Whereas, all FRET ratios of $CXCR4_{MT}$ were lower than that of $CXCR4_{WT}$, suggesting that CXCR4 oligomeric states may be diminished by these mutations. We are also investigating whether WB cross-linking with BS^3 to see CXCR4 oligomeric states similar to these FRET result. Next, we examined the susceptibility of $HIV\text{-}1_{NL4\text{-}3}$ to TZM-$bl_{CXCR4\Delta}$ cells transiently expressing the variant $CXCR4_{MT}$. The susceptibility to TZM-$bl_{CXCR4\ MT}$ was not significantly different from that of $CXCR4_{WT}$ because of the large variation.

In $CXCR4_{N192A}$, $CXCR4_{M205A}$, and $CXCR4_{M210A}$, the fusion activity and FRET ratio decreased similarly, suggesting that reduction of CXCR4 oligomeric states may lead to reduced HIV-1 infection. To clarify the detailed association between CXCR4 oligomerization and HIV-1 infectivity, we plan to perform further experiments in the future.

Poster presentation
P-32

Development of Biomarker for predicting hepatocarcinogenesis after Hepatitis C virus elimination.

***Khaled Elgeshy**, Katsuya Nagaoka, Takehisa Watanabe, Takayuki Tokunaga, Kentaro Tanaka, Satoshi Narahara, Hiroki Inada, Sotaro Kurano, Yoko Yoshimaru, Hiroko Setoyama, Yasuhito Tanaka*

Department of Gastroenterology and Hepatology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Vast majority of these tumors result from the biological consequences of persistent viral infection. HCV is a major cause of acute and chronic liver diseases. HCV infection usually induces chronic hepatic inflammation, which favors the development of liver cancer. However, the molecular mechanisms underlying this process are not well understood.

MicroRNAs (miRNAs) are small, endogenous, highly evolutionary conserved, noncoding RNAs that direct posttranscriptional regulation of gene expression by binding to partially complementary sites within the 3′untranslated region of target messenger RNAs (mRNAs), resulting in translational repression or mRNA deadenylation and degradation. MiRNAs have been implicated in the regulation of a wide range of important biologic processes, such as cellular growth and differentiation, developmental timing, apoptosis, and modulation of host response to viral infection.

MicroRNA-x3 has been recently identified. Few recent studies showed it to be mostly a tumor suppressor microRNA. Gain-of-function/loss of function studies are being performed to determine the regulatory roles of the microRNA on proliferation, apoptosis, and invasion before and after direct acting antivirals agent.

Different studies showed that HCV eradication, induced by direct acting antivirals, significantly reduces, but does not eliminate, the risk of developing HCC and, therefore, cirrhotic patients should continue HCC surveillance after sustained virologic response (SVR).

Our main objective is to identify miRNAs that lead to elucidation of their developmental mechanism and prediction of prognosis by conducting comprehensive expression analysis of miRNAs and genes related to HCV post-SVR hepatocarcinogenesis.

Poster presentation
P-33

Analysis of immune responses associated with age-dependent severity in SARS-CoV-2-infected Nr4a3-Tocky mice

***Rise Kurokawa*[1]*, Chatherine Silas Mtali [1], Omnia Reda[2], Wajihah Sakhor[2], Yorifumi Satou[2], Masahiro Ono[3,4], Takushi Nomura[1,5]**

[1]*Division of Virology and Pathology, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan*
[2]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan*
[3]*Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, UK*
[4]*Collaboration Unit for Infection, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan*
[5]*AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan*

Background: The dysfunction of innate, humoral, and cellular immunity are associate with the severity of COVID-19, but the detailed mechanisms that determine the pathogenesis remain unclear. Recently, we constructed a SARS-CoV-2 infected Nr4a3-Timer mouse model that exhibited different disease severity depending on the age of the mice. In this study, we analyzed the immune activation in the lungs in SARS-CoV-2 infected Nr4a3-Timer mouse to investigate the determinants of severity of pneumonia. **Methods**: Old and middle-aged Nr4a3-Timer mice were infected with SARS-CoV-2/QHmusX. We performed the autopsy at day five post-infection and collected right and left lung lobes separately. The right lungs were injected with 4% PFA and subjected to histopathological analysis. The lung viable cells were isolated from the left lung and subjected to gene expression analysis by single-cell RNA sequencing technique. **Results:** HE staining of paraffin-embedded sections of the PFA-fixed right lung demonstrated thickening of the alveolar wall and the appearance of exfoliated cells into the alveoli in old infected mice. On the other hand, the middle-aged infected mice appeared exfoliated cells in the alveoli as in the old mice. However, the thickening of the alveolar wall was milder, indicating a limited lung inflammation. CD3+ T/Macrophage lineage cells were aggregated perivascularly and exfoliated into the alveoli in both old and middle-aged infected mice. Old mice exhibited SARS-CoV2 N+ cells in the bronchioles and alveoli, whereas middle-aged infected mice showed N-positive phagocytes in the alveoli but not in the bronchioles. The gene expression pattern analysis exhibited enhanced expression of activation marker genes such as *Cd69*, *PD-1*, and *PD-L1* in both old and middle-aged animal. However, clonal expansions of T cells were detected only in the middle-aged animal. **Conclusion**: This study revealed the immunological background in the lungs associated with different pneumonia levels in SARS-CoV-2-infected old and middle-aged mice. Our findings will contribute to understanding the determinants of age-dependent COVID-19 pneumonia.

Poster presentation
P-34

The anti-tumor effect of Andrographolide against Primary effusion lymphoma

Itnarin Mongkon*[1]*, *Ryusho Kariya[1], Piyanard Boonnate[1], Gunya Sittithumcharee[1], Rungnapha Saeeng[2], Seiji Okada[1]*

[1]Division of Hematopoiesis, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Japan

[2]Department of Chemistry, Faculty of Science, Burapha University, Chonburi, Thailand

Primary effusion lymphoma (PEL) is a rare and aggressive non-Hodgkin lymphoma (NHL) that mostly occurs in AIDS patients. PEL has a very poor prognosis with an overall of 6 months. Therefore, new chemotherapy regimens have been required. Andrographolide (AG) is a natural compound extracted from *Andrographis paniculata*. The anti-tumor effects of AG in various malignancies have been recently reported with the underlying mechanism of NF-κB, PI3K/Akt, and JAK/STAT pathway suppression. These mechanisms are also important for PEL survival, but there is no study on PEL. Therefore, the purpose of this study is to investigate the anti-tumor effect of AG on PEL, both *in vitro* and *in vivo.*

First, we assessed the efficacy of AG on cell proliferation using PEL cell lines, BCBL-1, BC-1, BC-3, GTO, and TY-1 by MTT assay. Next, apoptosis was detected by Annexin V/PI staining, and the role of AG-induced cell death via ROS activation was investigated by N-acetyl-L-cysteine (NAC) and PI staining. Caspase activation was confirmed by broad-spectrum caspase inhibitor (Q-VD-OPh). The mechanism of AG inducing PEL cell death was examined by Western blotting. Finally, we affirmed the tumor suppression of AG using the PEL xenograft model.

The results showed that AG has an anti-proliferative effect against all PEL cell lines. The IC_{50} of AG on BCBL-1 and TY-1 was 25.50 ± 3.11 μM and 6.52 ± 1.64 μM, respectively. AG treatment in BCBL-1 and TY-1 increased the percentages of Annexin V/PI positive cells whereas pre-treated with NAC or Q-VD-OPh suppressed AG induced-cell death. Western blotting showed that AG treatment activated caspase -8, -9, and -3, and decreased the phosphorylation level of p-p65, p-Akt (S473), and p-STAT3 without altering the total level of NF-κB p65, Akt, and STAT3. In BCBL-1 xenograft model, AG at the dose of 500 mg/kg/day, oral gavage, significantly suppressed ascites without observable toxicity.

These results indicated that AG increased ROS production, induced apoptosis via the caspase-dependent, decreased the activation of NF-κB, Akt, and STAT3 pathways, and led to the suppression of PEL development and ascites. The results referred that AG has an anti-tumor effect and may serve as a potential agent for PEL treatment.

Poster presentation
P-35

In vitro priming of HLA-A*24:02-restricted variants-specific CD8$^+$ T cells in SARS-COV-2 infection

***Huanyu Li**, Takeshi Nakama, Yoshihiko Goto, Yoshiki Aritsu, Mako Toyoda, Takamasa Ueno, and Chihiro Motozono*

Division of Infection and immunity, Joint Research Center for Human Retrovirus infection, Kumamoto University, Kumamoto, Japan

Although the SARS-CoV-2 variants retains susceptibility to the cellular immunity, the characteristics of antigen-specific T cells toward variants remain unclear. We previously demonstrated that Spike-derived NF9 ($S_{448-456}$: NYNYLYRLF) was an immunodominant epitope presented by HLA-A*24:02 but NF9-specific T cells did not recognize L452R mutation located at the 5th position within the NF9 epitope in Delta and Omicron BA.5 variant. In this study, we investigated whether variant (NF9-5R) specific T cells are induced in convalescents infected with Delta and Omicron BA.5 variant. *Ex vivo* analysis of the convalescents using HLA-tetramers demonstrated the absence of NF9-5R-specific T cells. Moreover, NF9-5R-specific T cells were not induced with *in vitro* stimulation with NF9-5R peptide, indicating that NF9-5R-specific T cells are not efficiently induced in the convalescents. To investigate *in vitro* priming of naïve CD8$^+$ T cells specific for NF9-5R peptide, PBMCs from non-vaccinated and non-infected individuals were stimulated with NF9-5R peptide and STING ligand 3′3′-cGAMP. We detected NF9-5R-specific CD8$^+$ T cell lines by tetramer staining and showed CCR7$^-$CD45RA$^-$ effector memory phenotype. Moreover, NF9-5R-specific T cells were also detected in convalescents infected with Omicron BA.5 by *in vitro* priming, indicating the presence of naïve and memory pools specific for NF9-5R peptide. TCR single cell analysis revealed that NF9-5R-specific TCRs were comprised of unique TCRα chains with conserved CDR3β motifs similar to those in NF9-specific TCRs. In conclusion, we successfully generated NF9-5R-specific T cells by *in vitro*-priming and identified unique TCR clonotypes different from NF9-specific TCRs. We will characterize the specificity and cross-reactivity of variant-specific TCRs, providing a better insight for rational vaccine design for viral variants.

Poster presentation
P-36

Human endogenous retrovirus-K (HERV-K) antisense RNA is induced by SOX4 in the teratocarcinoma cells

Md. Jakir Hossain*, Nami Monde, Hiromi Terasawa, Wright Andrews Ofotsu Amesimeku, Nyame Perpetual, Joyce Appiah-Kubi, Tomohiro Sawa, Yosuke Maeda, Kazuaki Monde*

Department of Microbiology, Graduate School of Medical Sciences, Kumamoto University

Human endogenous retroviruses (HERVs) are part of the human genome (8%) as ancestral relics of previous germline retroviral infections. Albeit, over time HERVs have mislaid their innate capability of being a virulent or active form of a virus. Nevertheless, they are actively involved in different disease progression, especially cancer. For instance, the long non-coding RNA of HERVs is associated with breast cancer. On the other hand, depending on the cell type SRY-related HMG-box (SOX) genes are expressed. One of those SOX is SOX4 predominantly expressed in almost all cancer cells. Currently, we reported that the SOX2 protein in NCCIT cells is crucial for the HERV-K expression. However, the association between HERV-K LTR5Hs and SOX proteins notably SOX4 yet under investigation in different cancer cell cells. This study was designed to explore the aspect of SOXs in the activation of HERV-K LTR5Hs in HeLa and NCCIT cells. Firstly, we determined the transcriptional activation of HERV from either side of both LTRs in presence of SOXs. To execute the study, we constructed pHERV-K Venus as reporter gene in both forward (5' LTR to 3' LTR) and reverse (3' LTR to 5' LTR) orientations between the two LTRs. Then both of the constructs independently were co-transfected with SOX4 into the HeLa and without SOX4 into the NCCIT cells. The result showed that the Venus expression on the sense strand from 5'LTR is activated by SOX2 but not by SOX4. On the other hand, both SOX2 and SOX4 activates the Venus expression on the antisense strand from 3'LTR. Afterward, we determined the presence of endogenous SOX4 in NCCIT and NCCIT-KO-SOX2 cells. Furthermore, we found the expression of the antisense RNA in NCCIT and NCCIT-KO-SOX2 cells. Altogether, these results suggest that the endogenous SOX2 and SOX4 induces the HERV-K antisense RNA from the 3'LTR. As SOX4 is predominantly found in almost all cancer cells, therefore, the antisense RNA of HERV-K might be involved in several tumor progression.

Poster presentation
P-37

RESEARCH ON THE MECHANISM OF ACCELERATION OF HIGHLY REPLICATION-COMPETENT HIV-1 MATRIX (MA) MUTANTS

***Joyce Appiah-Kubi**, Wright Andrews Ofotsu Amesimeku, Nyame Perpetual, Nami Monde, Hiromi Terasawa, Md. Jakir Hossain, Tomohiro Sawa, Yosuke Maeda, Kazuaki Monde*

Department of Microbiology, Graduate School of Medical Sciences, Kumamoto University

Gag polyproteins of HIV-1 play a key role in the virus particle assembly, release, and maturation, it is also vital in the establishment of a productive infection. The Gag precursor polyprotein of HIV-1 is directed by the matrix (MA) domain to the plasma membrane during the late events of virus replication. The capsid (CA) and nucleocapsid (NC) domains are responsible for Gag multimerization and RNA packaging and the p6 domain recruits cellular factors required for virus budding. HIV-1 MA also plays an essential role in the early stages of the virus replication cycle. It has been reported that the MA mutations impact and alter Gag membrane binding on the phenotypes of newly produced virus particles. However, the effects of the MA mutations on the core morphogenesis and details of the process remain to be fully understood. Further studies on HIV-1 Gag specifically the MA domain and its regulatory mechanism could be useful to properly understand and help develop potential therapies that target the MA-related stages in the virus lifecycle.

In this study, we monitor replication kinetics in PM1_CCR5/Vpr-HiBiT cells infected with HIV-1 Gag (MA) library constructed based on the Los Alamos Database. The cell line was established on the concept that HIV-1 Vpr accessory protein is recruited by the p6 domain of the Gag polyprotein during virus assembly and thus employs the Vpr-tagged HiBiT reporter peptide into the growing virion. The Vpr-HiBiT gene was transfected into PM1 cells by electroporation and successfully selected with puromycin. Virus replication was monitored by measuring the amount of HiBiT-tagged proteins captured in the NanoGlo HiBiT Lytic Detection System. Extracted DNA was obtained from infected cells, and amplified by RT-qPCR using specific primers targeting the MA domain of the Gag polyprotein. Amplicons were cloned using a TOPO cloning kit, transformed into DH5-alpha-competent cells, cultured, prepped, and sequenced for further analysis. Successful mutants were molecularly cloned based on sequence analysis and have been transduced into PM1_CCR5/Vpr-HiBiT cells to determine critical regions of acceleration.

Isolated mutants adapted well to our newly developed PM1_CCR5/Vpr-HiBiT cells. Sequence analysis reveals common mutations in all extracted DNA specifically in the Helix 1, Membrane Binding Motif, Nuclear Localization Signal 1, PI (4,5) P2 interaction, and Highly Basic Region of the MA domain. The observed mutations from HIV-1 Gag Library are known to be involved in both the early and late stages of the virus life cycle. Designed molecular clones are currently being monitored for their replication kinetics to help elucidate and identify critical regions of acceleration.

Poster presentation
P-38(SP-5)

A derivative compound, HT-7, inhibits HIV-1 release by inducing BST2/tetherin on the cell surface

***Perpetual Nyame*[1]*, Akihiro Togami*[2]*, Tomofumi Yoshida*[1]*, Takuya Masunaga*[2]*, Hiromi Terasawa*[1]*, Nami Monde*[1]*, Yurika Tahara*[2]*, Tomohiro Sawa*[1]*, Yorifumi Satou*[3]*, Mikako Fujita*[2]*, Yosuke Maeda*[1]*, Hiroshi Tateishi*[2]*, Kazuaki Monde*[1]

[1]Department of Microbiology, Faculty of Life Sciences, Kumamoto University, Kumamoto 860-8556, Japan
[2]Medical and Biological Chemistry Science Farm Joint Research Laboratory, Faculty of Life Sciences, Kumamoto University 862-0973, Kumamoto, Japan
[3]Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto 860-8556, Japan

The zeal to end the HIV-1 epidemic has led to the discovery of potent antiviral therapy that hinders different processes in HIV-1 replication. Although the overwhelming approval of novel ART is promising, discovering a new antiviral drug that has a repressive effect on the late stages of HIV-1 replication. In this respect, the HIV-1 Gag protein, which drives the HIV-1 assembly has lately been of great interest. In this study, a new high-performance screening system using T cell lines, the Vpr-HiBiT technique, was established to indirectly quantify the amount of HIV-1 release. Candidate compounds from the Ono Pharmaceutical Compound Library were tested for their efficacy in obstructing HIV-1 release by infecting Jurkat T cells expressing Vpr-HiBiT with a single round VSV-G pseudo typed NL4-3. The HT-7 derivative compound was designed with EC50 and CC50 of 95.8μM and 418.9μM respectively. In our search to investigate the process/processes targeted by HT-7 that impair viral release, the expression of BST2/tetherin and Gag punctate formation were evaluated by flow cytometry and confocal laser imaging. In addition to increasing GagVenus signal intensity in the infected HT-7 treated cells, partial disruption of Gag accumulation on the plasma membrane was also visible. In particular, cell surface expression of the host factor (BST2/tetherin), which impedes HIV-1 release, increased significantly with HT-7 treatment. The absence of Vpu that counteracts the BST2/tetherin prevented HT-7 from suppressing the release of HIV-1. The implications are that the upregulation of BST2/tetherin by HT-7 led to HIV-1 release inhibition. The HT-7, which was discovered by the Vpr-HiBiT technology, is a potential antiviral agent that suppresses the release of HIV-1 by inducing BST2/tetherin expression on the cell surface. The affordable, short turnaround time and high performance of the Vpr-HiBiT technique highlight it as a test tool for viral research.

Poster presentation
P-39

Implementation of an artificial antigen presenting cell system for HTLV-1 Tax-specific peptides to evaluate HLA-restricted CD8+ T cell exhaustion

Daniel Enriquez-Vera[1]*, Kenji Sugata*[2]*, Yorifumi Satou*[2]*, Shingo Nakahata*[1]

[1]*Division of HTLV-1/ATL Carcinogenesis and Therapeutics, Joint Research Center for Human Retrovirus Infection, Kagoshima University, Kagoshima, 890-8544, Japan.*
[2]*Division of Genomics and Transcriptomics, Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, 860-8556, Japan.*

Background: Adult T-cell leukemia/lymphoma (ATL) is an aggressive lymphoid malignancy associated with HTLV-1 infection. All currently available treatments exhibit short-term responses, and allogeneic stem-cell transplantation (allo-HSCT) remains as the only long-term curative alternative for a limited number of patients. Interestingly, strong HTLV-1 Tax-specific CD8+ T cell responses have been linked to successful allo-HSCT or chemotherapy. Although the mechanism underlying differences in CD8+ T cell responsiveness between patients remains unclear, recent studies have suggested T cell exhaustion as a mechanism that impairs Tax-specific CD8+ T cell responses. Therefore, in this study, we attempted to establish an ex vivo model of T cell activation to explore the mechanisms of Tax-specific responses and exhaustion in CD8+ T cells and for useful platform for drug screening to reprogram exhausted T cell.
Methods: K562 is a chronic myelogenous leukemia cell line which can be primmed and present peptides to effector cells. K562 HLA-null cell line was transduced with full length HLA-A24 (A*2402) cDNA to generate K562-HLA-A24. TCR-sequences from primary samples of HAM/TSP patients with HLA-A24 restricted genotypes were isolated and transduced into TCR-null Jurkat T cell line to generate HAM125-1 cell line.
Results: To establish an artificial antigen presenting cell (aAPC) system for Tax specific peptides, Jurkat T cells transduced with TCR with high affinity for HLA/Tax 301-309 was used for establishing ex vivo assays for T cell activation. As higher expansion of effector cells has been shown to be achieved by treating K562 with formaldehyde fixation compared to classical irradiation, we used formaldehyde-fixed K562 to induce T cell responses. To explore adequate conditions to induce efficient T-cell activation, K562-HLA-A24 was pulsed with Tax 301-309 peptide for different times and fixed with different concentrations of formaldehyde. Then, peptide-pulsed K562 cells was cocultured with HAM125-1 cell line for 48 h and expression of T cell activation markers was analyzed by flow cytometry. T cell activation was readily detectable only in the presence of Tax peptide and K562-HLA-A24, suggesting that activation and recognition of Tax peptide by K562-HLA were specific. Robust T cell activation was achieved by fixation of the antigen-presenting cells with 1% formaldehyde and pulsing with antigen for 24 h.
Conclusions: An aAPC system for Tax peptide was validated with restriction to a specific HLA and functional TCR, and this model can be potentially useful for drug screening, CD8+ T cell exhaustion, and CAR-T cell exhaustion.

Poster presentation
P-40(SP-6)

Characterisation of the HTLV-1-specific cytotoxic T-lymphocyte response and viral gene expression in HTLV-1 carriers at high risk of developing Adult T-cell Leukaemia/Lymphoma

***Devon Weterings*[1]*, Lisa Lam Chiou Yee*[1]*, Graham P. Taylor*[1,2]*, Lucy B. Cook*[1,3]*, Aileen Rowan*[1]

[1]*Section of Virology, Department of Infectious Disease, Imperial College London, London, UK,*
[2]*National Centre for Human Retrovirology, Imperial College Healthcare NHS Trust, London, UK,*
[3]*Department of Haematology, Imperial College Healthcare NHS Trust, London, UK*

Adult T-cell leukaemia/lymphoma (ATL) is caused by chronic infection with the retrovirus human T-cell leukaemia virus type 1 (HTLV-1). Despite the constitutive expression of viral genes by malignant cells, little is known about how the antiviral immune response contributes to oncogenesis. We recently reported that clonally expanded ATL-like HTLV-1 infected cells circulate in the blood up to 10 years before the onset of ATL symptoms, and that HTLV-1-carriers with circulating ATL-like clones have a high risk of developing ATL. Here, we characterized antiviral cytotoxic T-lymphocyte (CTL) function and viral gene expression in HTLV-1 carriers who have suspected premalignant lesions, ATL-like clones, circulating in their blood.

We studied peripheral blood mononuclear cells from three groups of patients: HTLV-1-carriers who had circulating ATL-like clones but no clinical symptoms of malignancy (high-risk carriers, n=12), HTLV-1-carriers with matched proviral loads but without detectable ATL-like clones (controls, n=12) and patients with ATL (n=9). CTL killing and viral gene expression was assayed by co-culture of *ex vivo* infected $CD4^+$ T-cells with autologous $CD8^+$ T-cells, followed by flow cytometric analysis of cell survival. ATL-like clones were identified by staining for T-cell receptor Vβ subunits, and HTLV-1-infected cells were identified by staining extracellularly for CADM1 and intracellularly for viral protein Tax. IFN-γ production by CD8+ T cells in response to overlapping 15mer peptides corresponding to Tax and HBZ was assayed by ELISpot.

CD8+ T-cells from high-risk HTLV-1-carriers and patients with ATL were significantly less efficient at killing Tax-expressing HTLV-1-infected cells and produced significantly lower levels of IFN-γ in response to Tax peptides than CD8+ T-cells from the control group. Furthermore, in 50% of high-risk HTLV-1-carriers the ATL-like clones did not express immunodominant viral protein Tax. Overall, these findings show early indications of both inefficient HTLV-1-specific CTLs and immune evasion in HTLV-1 carriers with circulating ATL-like clones.

Poster presentation
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Understanding ATL oncogenesis by detecting driver mutations in HTLV-1 carriers

Watber P[1], *Melamed A[1], Wolf S[1], Dhasmana D[2], Greiller C.L[1], Taylor G.P[1,2], Cook, L.B.M[2], Rowan A.G[1]*

[1]Section of Virology, Department of Infectious Disease, Imperial College London, St Mary's Hospital, Praed Street, London W2 1NY. London, UK
[2]National Center for Human Retrovirology, St. Mary's Hospital, Imperial College Healthcare NHS Trust, London, UK

Human T-cell Leukaemia Virus type 1 (HTLV-1) is the causative agent of Adult T-cell Leukaemia (ATL), an aggressive neoplasm that affects ~5% of HTLV-1 carriers. However, the order and timing of events that drive oncogenesis are poorly understood. ATL tumours carry hundreds of somatic mutations in the host and proviral genomes, with ~56 recurrently mutated human genes identified as probable ATL-drivers. Recently, our group has shown that prior to developing symptomatic ATL, premalignant cells carrying ATL-driver mutations circulate the blood. We aim to investigate ATL evolution by characterising ATL-driver mutations in HTLV-1 carriers with suspected premalignant lesions, using next-generation sequencing (NGS). Here we present preliminary data concerning the performance of our sequencing protocol.

A ~160kbp targeted sequencing panel (Agilent SureSelect-XT-HS2) was designed to capture 42 human genes and HTLV-1. Paired peripheral blood mononuclear cell (PBMC) genomic DNA samples from four HTLV-1-carriers pre- and post-diagnosis with ATL were compared with three PBMC genomic DNA samples from HTLV-1-carriers with no detectable evidence of premalignancy. 150bp paired-end reads were sequenced using a MiSeq. Adaptors were removed using TrimGalore, data quality was evaluated by FASTQC/MultiQC, reads were aligned to HG19 using BWA-MEM, and variants were called and annotated using an in-house computational pathway.

The panel covered 99% of targeted loci to a depth of ~150X. Previously obtained whole exome sequencing data confirmed that driver mutations that were expected to be present in the sample were detected by targeted sequencing – (n=2 mutations in the pre-ATL sample and 5 mutations in the ATL sample). Preliminary variant calling identified ~62 somatic mutations across the whole cohort and analysis of HTLV-1 identified single nucleotide variants and deletions within the provirus. To assess clinical utility, sensitivity and specificity will be established. Further work is underway to extend and confirm our observations and provide vital insights into ATL development, disease management, and treatment discovery.