RESEARCH ACTIVITIES

Department of Microbiology and Immunology

Division of Bacterial Infection
細菌感染分野

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Research in this division is directed toward understanding the complex interactions that occur between pathogenic bacteria and their human hosts at very early stage of bacterial infectious processes. Our special interest is focused upon the molecular pathogenicity of enteropathogenic bacteria, such as Shigella, Helicobacter pylori, enteropathogenic E. coli and enterohemorrhagic E.coli. We are also searching for effective methods to protect or regulate bacterial infection by using knowledge accumulated.

1. A BACTERIAL EFFECTOR TARGETS MAD 2L2, AN APC INHIBITOR, TO MODULATE HOST CELL CYCLING.

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The gut epithelium self-renews every several days, providing an important innate defense system that limits bacterial colonization. Nevertheless, many bacterial pathogens, including Shigella, efficiently colonize the intestinal epithelium. Here, we show that the Shigella effector IpaB, when delivered into epithelial cells, causes cell-cycle arrest by targeting Mad2L2, an anaphase-promoting complex/cyclosome (APC) inhibitor. Cyclin B1 ubiquitination assays revealed that APC undergoes unscheduled activation due to IpaB interaction with the APC inhibitor Mad2L2. Synchronized HeLa cells infected with Shigella failed to accumulate Cyclin B1, Cdc20, and Plk1, causing cell-cycle arrest at the G2/M phase in an IpaB/Mad2L2-dependent manner. IpaB/Mad2L2-dependent cell-cycle arrest by Shigella infection was also demonstrated in rabbit intestinal crypt progenitors, and the IpaB-mediated arrest contributed to efficient colonization of the host cells. These results strongly indicate that Shigella employ special tactics to influence epithelial renewal in order to promote bacterial colonization of intestinal epithelium.

2. *Helicobacter pylori* dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach.

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Colonization of the gastric pits in the stomach by Helicobacter pylori (Hp) is a major risk factor for gastritis, gastric ulcers, and cancer. Normally, rapid self-renewal of gut epithelia, which occurs by a balance of progenitor proliferation and pit cell apoptosis, serves as a host defense mechanism to limit bacterial colonization. To investigate how Hp overcomes this host defense, we use the Mongolian gerbil model of Hp infection. Apoptotic loss of pit cells induced by a proapoptotic agent is suppressed by Hp. The ability of Hp to suppress apoptosis contributed to pit hyperplasia and persistent bacterial colonization of the stomach. Infection with WT Hp but not with a mutant in the virulence effector cagA increased levels of the prosurvival factor phospho-ERK and antiapoptotic protein MCL1 in the gastric pits. Thus, CagA activates host cell survival and antiapoptotic pathways to overcome self-renewal of the gastric epithelium and help sustain Hp infection.

3. New Animal Model of Shigellosis in the Guinea Pig: Its Usefulness for Protective Efficacy Studies

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Shigella possess 220-kb plasmid, and the major virulence determinants, called effectors, and the type III secretion system (TTSS) are exclusively encoded by the plasmid. The genome sequences of *S. flexneri* strains indicate that several *ipaH* family genes are located on both the plasmid and the chromosome, but whether their chromosomal IpaH cognates can be secreted from *Shigella* remains unknown. Here we report that *S. flexneri* strain, YSH6000 encodes seven *ipaH* cognate genes on the chromosome and that the IpaH proteins are secreted via the TTSS. The secretion kinetics of IpaH proteins by bacteria, however, showed delay compared with those of IpaB, IpaC, and IpaD. Expression of the each mRNA of *ipaH* in *Shigella* was increased after bacterial entry into epithelial cells, and the IpaH proteins were secreted by intracellular bacteria. Although individual chromosomal *ipaH* deletion mutants showed no appreciable changes in the pathogenesis in a mouse pulmonary infection model, the Δ*ipaH-null* mutant, whose chromosome lacks all *ipaH* genes, was attenuated to mice lethality. Indeed, the histological examination for mouse lungs infected with the Δ*ipaH-null* showed a greater inflammatory response than induced by wild-type *Shigella*, suggesting that the chromosomal IpaH proteins act synergistically as effectors to modulate the host inflammatory responses.

Publications

Iizumi, Y., Sagara H, Kabe Y, Azuma M, Kume K, Ogawa M, Nagai T, Gillespie PG, Sasakawa C, Handa H. The enteropathogenic E. coli -effector EspB facilitates microvilli effacing and antiphagocytosis by inhibiting myosin function. Cell Host Microbe 2: 383-92, 2007.

Mimuro, H., Suzuki, T., Nagai, S., Rieder, G., Suzuki, M., Nagai, T., Fujita, Y., Nagamatsu, K., Ishijima, N., Koyasu, S., Haas, R., and Sasakawa, C. Helicobacter pylori Dampens Gut Epithelial Self-Renewal by Inhibiting Apoptosis, a Bacterial Strategy to Enhance Colonization of the Stomach. Cell Host & Microbe 2: 250-263, 2007.

Iwai, H., Kim, M., Yoshikawa, Y., Ashida, H., Ogawa, M., Fujita, Y., Muller, D., Kirikae, T., Jackson, P.K., Kotani, S., and Sasakawa, C. A Bacterial Effector Targets Mad2L2, an APC Inhibitor, to Modulate Host Cell Cycling. Cell 130: 611-623, 2007.

Suzuki, T., Franchi, L., Toma, C., Ashida, H., Ogawa, M., Yoshikawa, Y., Mimuro, H., Inohara, N., Sasakawa, C., and Nunez, G. Differential Regulation of Caspase-1 Activation, Pyroptosis, and Autophagy via Ipaf and ASC in Shigella-Infected Macrophages. PLoS Pathog. 3: e111, 2007.

Nagai, S., Mimuro, H., Yamada, T., Baba, Y., Moro, K., Nochi, T., Kiyono, H., Suzuki, T., Sasakawa, C, and Koyasu, S. Role of Peyer's patches in the induction of Helicobacter pylori-induced gastritis. Proc Natl Acad Sci USA 104: 8971-8976, 2007.

Shim, D.H., Suzuki, T., Chang, S.Y., Park, S.M., Sansonetti, P.J., Sasakawa, C. and Kweon, M. N. New animal model of shigellosis in the Guinea pig: its usefulness for protective efficacy studies. J. Immunol. 178: 2476-2482, 2007.

Department of Microbiology and Immunology

Division of Host-Parasite Interaction
宿主寄生体学

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The goal of our Department is to elucidate the cellular defense system and the counteracting viral strategy at the level of gene regulation and to establish new approaches for suppression of human pathogenic viruses. Cellular mechanisms for the surveillance and transcriptional suppression of DNA parasites such as retroviruses are now being recognized as an important host cell defense system through epigenetical regulation of chromosome. We have been concentrating on epigenetical regulation of retrovirus including HIV by chromatine remodeling factor, SWI/SNF complex and on genome-wide networks formed between transcriptional factors and microRNAs. Using these results, we also develop new retrovirus vectors for human gene therapy and basic researches.

1. SWI/SNF chromatin remodeling factor and retroviral gene expression.

Since retroviruses that are once integrated into host chromosomes cannot be excised, host cells inevitably use epigenetical regulation systems to shut-off virus gene expression. In this post-genome era, therefore, elucidation of this epigenetical mechanisms would be essential to understand host defence strategies. At the same time, if we can elucidate molecular mechanisms for stochastic reactivation of latent HIV, it would strongly contribute to the desired, but difficult, goal of complete virus eradication in HIV-infected patients.

In cellular nuclei, DNA methylation, histone modification and chromatin remodeling are the major molecular basis for epigenetical regulation. In 2002, we showed that Murine leukemia virus (MLV)-based retrovirus vector transgene expression is rapidly silenced in human tumor cell lines lacking expression of Brm, a catalytic submit of SWI/SNF chromatin remodeling complex, even though these vectors can successfully enter, integrate, and initiate transcription. We detected this gene silencing as a reduction in the ratio of cells expressing the exogenous gene rather than a reduction in the average expression level, indicating that down-regulation occurs in an all-or-none manner. Retroviral gene expression was protected from silencing and maintained in Brm-deficient host cells by exogenous expression of Brm but not BRG1, an alternative ATPase subunit in the SWI/SNF complex. Introduction of exogenous Brm to these cells suppressed recruitment of protein complexes containing YY1 and histone deacetylase (HDAC) 1 and 2 to the 5′-LTR region of the integrated provirus, leading to the enhancement of acetylation of specific lysine residues (Lys-5 and Lys-8) in histone H4 located in this region. These results suggest that the Brm-containing SWI/SNF complex subfamily (trithorax-G) is essential for preventing the recruitment of a complex including YY1, EZH2, EED and HDACs (Polycomb-G) to the 5′-LTR region to maintain

transcription of exogenously introduced genes.

This year, we have shown that the above observation in MLV-based vector is essentially applicable to HIV-based vector (a). Importantly, we also show that gene silencing of these retroviruses can be reversible, and more than a half population of the integrants is reactivated stochastically. Whereas SWI/SNF complex has been recognized as regulator at the transcriptional level, we have also demonstrated that this complex in conjugation with $p54^{nrb}$ can also function as a splicing regulator for the the transcript products (b). Considering that $p54^{nrb}$/PSF was previously reported to regulate HIV mRNA stability, this finding would be quite important to elucidate post-transcriptional regulation of HIV. We also have demonstrated anti-oncogenic potential of the *Brm* gene by constructing efficient retrovirus vectors harboring expression unit for shRNA against *Brm* (c).

a. Sustained HIV expression require Brm containing SWI/SNF complex.

Taketoshi Mizutani, Aya Ishizaka, Nobutake Yamamichi, Takuya Okazaki and Hideo Iba

We constructed a replication defective lentivirus vector driven by intact HIV LTR and have isolated many cellular clones that has expressed the reporter gene, *GFP*, one day after transduction by FACS. In SWI/SNF competent HeLaS3 cells, all the isolated clones showed sharp unimodal patterns, indicating stable expression of all the integrants. When human tumor cell lines derficient in Brm expression (SW13 and C33A) were used instead, significant clones showed either gene silencing or expression patterns with a broad peak. Transient exogenous *Brm* introduction reactivated expression in most of these silenced clones, indicating that Brm containing SWI/SNF complex is crucial for stable HIV transcription when Tat is absent. Resorting of subcellular fractions of the broad peaks showed that silenced subcellular fractions can be stochastically reactivated, indicating that gene silencing is reversible in these clone.

b. Brm transactivates the *telomerase reverse transcriptase* (*TERT*) gene and modulates the splicing patterns of its transcripts in concert with $p54^{nrb}$

Taiji Ito, Hirotaka Watanabe, Nobutake Yamamichi, Shunsuke Kondo, Toshio Tando, Takeshi Haraguchi, Taketoshi Mizutani, Kouhei Sakurai, Shuji Fujita, Tomonori Izumi[1] Toshiaki Isobe[1] and Hideo Iba: [1]Division of Proteomics Research, Institute of Medical Science, University of Tokyo.

We report that a DBHS (for *Drosophila* behavior, human splicing) family protein, $p54^{nrb}$, binds both BRG1 and Brm, catalytic subunits of SWI/SNF chromatin remodeling complex, and also another core subunit of this complex, BAF60a. The N-terminal region of $p54^{nrb}$ is sufficient to pull-down other core subunits of the SWI/SNF complex, suggesting that $p54^{nrb}$ binds SWI/SNF-like complexes. Polypyrimidine tract-binding protein (PTB)-associated splicing factor (PSF), another DBHS family protein known to directly bind $p54^{nrb}$, was also found to associate with the SWI/SNF-like complex. When short hairpin (sh) RNAs targeting Brm were retrovirally expressed in a BRG1-deficient human cell line (NCI-H 1299), the resulting clones showed downregulation of the *telomerase reverse transcriptase (TERT)* gene and an enhancement of ratios of exon-7-and-8-excluded *TERT* mRNA that encodes an inactive protein. All of these clones display growth arrest within two months of the Brm-knockdown. In NCI-H1299 cells, Brm, $p54^{nrb}$, PSF, and RNA polymerase II phosphorylated on CTD serine 2, specifically co-localize at a region incorporating an alternative splicing acceptor site of *TERT* exon7. These findings suggest that at the *TERT* gene locus in human tumor cells containing functional SWI/SNF complex, Brm, and possibly BRG1, in concert with $p54^{nrb}$ would initiate efficient transcription and could be involved in the subsequent splicing of *TERT* transcripts by accelerating exon-inclusion, which partly contributes to the maintenance of active telomerase.

c. Frequent Loss of Brm Expression in Gastric Cancer Correlates with Histological Features and Differentiation State.

Nobutake Yamamichi, Ken-ichi Inada[2], Masao Ichinose[3], Mitsue Yamamichi-Nishina, Taketoshi Mizutani, Hirotaka Watanabe, Kazuya Shiogama[2], Mitsuhiro Fujishiro[3], Takuya Okazaki, Naohisa Yahagi[4], Takeshi Haraguchi, Shuji Fujita, Yutaka Tsutsumi[4], Masao Omata[4], and Hideo Iba: [2]1st Department of Pathology, Fujita Health University School of Medicine, [3]Second Department of Internal Medicine, Wakayama Medical College, [4]Department of Gastroenterology, Faculty of Medicine, University of Tokyo.

The mammalian SWI/SNF chromatin remodeling complex, an essential epigenetic regulator, contains either a single Brm or BRG1 molecule as its catalytic subunit. We observed frequent loss of Brm expression but not of BRG1 in hu-

man gastric cancer cell lines. Treatment with HDAC inhibitor rescued Brm expression indicating epigenetic regulation of this gene, and an RNAi-based colony formation assay revealed anti-oncogenic properties of Brm. Brm immunostaining of 89 primary gastric cancers showed an obvious reduction in 60 cases (67%) and a severe decrease in 37 cases (42%). Loss of Brm is frequent in the major gastric cancer types (well or moderately differentiated tubular adenocarcinoma and poorly differentiated adenocarcinoma), and positively correlates with the undifferentiation state. Among the minor gastric cancer types, Brm expression persists in signet-ring cell carcinoma and mucinous adenocarcinoma, but a marked decrease is observed in papillary adenocarcinoma. Intestinal metaplasia never shows decreased expression, indicating that Brm is a valid marker of gastric oncogenesis. In contrast BRG1 is retained in most cases; a concomitant loss of BRG1 and Brm is rare in gastric cancer, contrary to other malignancies. We further show that Brm is required for *villin* expression, a definitive marker of intestinal metaplasia and differentiation. Via regulating such genes important for gut differentiation, Brm should play significant roles in determining the histological features of gastric malignancy.

2. Regulatory networks formed between transcriptional regulation and RNA interference.

Recently new layers of molecular mechanisms are shown to be involved in the gene regulation; many RNA transcripts of exogenous as well as endogenous genes are regulated at post-transcriptional level by a mechanism designated as RNA interference (RNA silencing). In human cells, major players of RNA interference are endogenous micro (mi)RNAs and short interfering (si)RNAs, which are produced from either host or viral aberrant RNAs rich in dsRNA regions. Furthermore, it has been suggested that in plant and yeast, chromosomal regulation and RNA silencing are interconnected through a putative pathway designated as RNA-directed transcriptional gene silencing (RdTS). In plants, for example, double-stranded RNA that targets the promoter regions of coding genes causes their transcriptional silencing, and this is associated with the methylation of the proximal DNA sequences. It is known also that double-stranded RNA generated from yeast centromeric transcripts contributes to the formation of heterochromatin within this region. Importantly, such RNA-dependent transcriptional silencing (RdTS) has been reported to operate in human cell lines transiently transfected with siRNAs. Since retroviral transcripts have a significant characteristic in that they inevitably contain a sequence corresponding to its own promoter in its 3′-UTR (U3 region), we can speculate enhanced frequencies of dsRNA formation in this region, which could trigger RNA interference. Therefore this year we tested whether retroviral gene silencing could be induced by RdTS (a).

As for miRNAs, a significant body of evidence has accumulated that they are involved in cellular development, differentiation, innate immunity induction, and anti-viral responses by post-transcriptional regulation of important mRNA. To elucidate genome-wide interplay between miRNAs and transcriptional factors, this year, we developed an algorithm that predicts promoter region of human miRNA genes that are involved in important regulatory systems evolutionarily conserved among vertebrates (b). We have further scrutinized an important regulatory system involving, miR-21, AP-1, NF-1 and SWI/SNF complex (c).

a. siRNAs do not induce RNA-dependent transcriptional silencing of retrovirus in human cells

Takeshi Haraguchi, Taketoshi Mizutani, Taiji Ito, Shigeru Minoguchi, Nobutake Yamamichi and Hideo Iba

In plants and yeasts, double-stranded RNAs that target chromosome DNA cause transcriptional silencing of the corresponding genes. Such RNA-dependent transcriptional silencing (RdTS) has been reported to operate in human cell lines transiently transfected with siRNAs. It is tempting to speculate that RdTS plays a role in retroviral gene silencing, considering that retroviral RNA transcripts harbor a U3 promoter sequence that is a potentially good source of double-stranded RNAs. To test this possibility, we here constructed several model HeLa cell lines expressing GFP driven by the murine leukaemia virus (MLV)-LTR and transduced them with lentivirus vectors expressing a series of shRNAs that target the U3 region of the MLV-LTR as a precursor of siRNA. However transcriptional gene silencing was not induced in most instances, in spite of the fact that processed shRNA was found in cellular nuclei as well as cytoplasm. These results indicate that RdTS does not generally contribute to MLV gene silencing in host cells.

b. Putative promoter regions of miRNA genes involved in evolutionarily conserved regulatory systems among vertebrates.

Shuji Fujita and Hideo Iba

Just as transcription factors, miRNA genes modulate global patterns of gene expression during differentiation, metabolic activation, stimulus response and also carcinogenesis. However, little is currently known how the miRNA gene expression itself is regulated owing to lack of basic information of their gene structure. Global prediction of promoter regions of miRNA genes would allow us to explore the mechanisms underlying gene-regulatory mechanisms involving these miRNAs. We speculate that if specific miRNA molecules are involved in evolutionarily conserved regulatory systems in vertebrates, this would entail a high level of conservation of the promoter of miRNAgene as well as the miRNA molecule. By our current screening of putative promoter regions of miRNA genes (miPPRs) on this base, we identified 59 miPPRs that would direct production of 79 miRNAs. We present both biochemical and bioinformatical verifications of these putative promoters.

c. *miR-21* gene expression triggered by AP-1 is sustained through a double negative feedback mechanism.

Shuji Fujita, Taiji Ito, Taketoshi Mizutani, Shigeru Minoguchi, Nobutake Yamamichi, Kouhei Sakurai and Hideo Iba

miR-21 has been reported to be highly expressed in various cancers and also to be inducible in a human promyelocytic cell line, HL-60, after PMA treatment. To examine molecular mechanisms involved in miR-21 expression, we analyzed structure of the *miR-21* gene by determining its promoter and primary transcripts. We show that AP-1 activates the *miR-21* transcription in conjugation with SWI/SNF complex, after PMA stimulation, through the conserved AP-1 and PU.1 binding sites in the promoter identified here. The previous findings of enhanced miR-21 expression in several cancers likely reflect the elevated AP-1 activity in these carcinomas. A single precursor RNA containing miR-21 was transcribed just downstream from the TATA box in this promoter, which is located in an intron of a coding gene *TMEM49*.

Importantly, expression of this overlapping gene is completely PMA-independent and all its transcripts are polyadenylated before reaching the miR-21 hairpin embedding region, indicating that *miRNAs* could have their own promoter even if overlapped with other genes. By available algorithms that predict miRNA target using a conservation of sequence complementary to the miRNA seed sequence, we next predicted and confirmed that the *NFI-B* mRNA is a target of miR-21. NFI-B protein usually binds the *miR-21* promoter in HL-60 cells as a negative regulator and is swept off from the *miR-21* promoter during PMA-induced macrophage differentiation of HL-60. Since exogenous miR-21 expression moderately induced endogenous miR-21, an evolutionarily conserved double negative feedback regulation would be operating as a mechanism to sustain miR-21expression.

Publications

Yamamichi N., Inada, K., Ichinose, M., Yamamichi-Nishina M., Mizutani, T., Watanabe, H., Shiogama, K., Okazaki, T., Fujishiro, M., Yahagi, N., Haraguchi, T., Fujita, S., Tsutsumi, Y., Omata, M., and Iba H. Frequent loss of Brm expression in gastric cancer correlates with histological features and differentiation state. *Cancer Research*, 67 (22): 10727-10735 (2007).

Haraguchi, T., Mizutani, T., Yamamichi, N., Ito, T., Minoguchi, S., and Iba, H. RNA-dependent transcriptional silencing does not contribute to retroviral gene silencing in human cells. *FEBS Letter*, 581: 4949-4954 (2007).

Fujita, S., and Iba, H. Putative promoter regions of *miRNA* genes involved in evolutionarily conserved regulatory systems among vertebrates. *Bioinformatics*, doi: 10. 1093/bioinformatics/btm 589.

Ito, T., Watanabe, H., Yamamichi, N., Kondo, S., Tando, T., Haraguchi, T., Mizutani T., Sakurai, K., Fujita, S., Izumi, T., Isobe, T., and Iba, H. Brm transactivates the *telomerase reverse transcriptase (TERT)* gene and modulates the splicing patterns of its transcripts in concert with p 54^{nrb}. *Biochemical J*. doi 10. 1042/BJ20071075.

Department of Microbiology and Immunology

Division of Virology
ウイルス感染分野

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Viruses can cause devastating diseases. The long-term goal of our research is to understand the molecular pathogenesis of viral diseases, using influenza and Ebola virus infections as models. Interactions between viral and host gene products during viral replication cycles determine the consequences of infection (i.e., the characteristics of disease manifestation, whether limited or widespread); hence, our research has centered on such interactions in these viral infections.

1. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus

Kobasa D, Jones, SM, Shinya K, Kash JC, Copps J, Ebihara H, Hatta Y, Kim JH, Halfmann P, Hatta M, Feldmann F, Alimonti JB, Fernando L, Li Y, Katze MG, Feldmann H, Kawaoka Y.

The 1918 influenza pandemic was unusually severe, resulting in about 50 million deaths worldwide. The 1918 virus is also highly pathogenic in mice, and studies have identified a multigenic origin of this virulent phenotype in mice. However, these initial characterizations of the 1918 virus did not address the question of its pathogenic potential in primates. Here we demonstrate that the 1918 virus caused a highly pathogenic respiratory infection in a cynomolgus macaque model that culminated in acute respiratory distress and a fatal outcome. Furthermore, infected animals mounted an immune response, characterized by dysregulation of the antiviral response, that was insufficient for protection, indicating that atypical host innate immune responses may contribute to lethality. The ability of influenza viruses to modulate host immune responses, such as that demonstrated for the avian H5N1 influenza viruses, may be a feature shared by the virulent influenza viruses.

2. Protective efficacy of neutralizing antibodies against Ebola virus infection

Takada A, Ebiharab H, Jones S, Feldmannd H, Kawaoka Y.

Ebola virus causes lethal hemorrhagic fever in humans and nonhuman primates, but no effective antiviral compounds are available for the treatment of this infection. The surface glycoprotein (GP) of Ebola virus is an important target of neutralizing antibodies. Although passive transfer of GP-specific antibodies has been evaluated in mouse and guinea pig models, protection was achieved only by treatment shortly before or after virus challenge. Using these animal models,

we evaluated the protective efficacy of two monoclonal antibodies whose epitopes are distinct from those of the antibodies tested by others. Treatment of mice with these antibodies 2 days after challenge completely protected most of the animals; even treatment 3 or 4 days after challenge was partially effective. Although antibody treatment in the guinea pig model was not as effective as in the mouse model, single-dose treatment of guinea pigs 1 day before, or 1 or 2 days after challenge did protect some animals. Interestingly, the protective effects seen in these animal models did not correlate with the in vitro neutralizing activity of the antibodies, suggesting different mechanisms of the neutralization by these antibodies. These results underscore the potential therapeutic utility of monoclonal antibodies for postexposure treatment of Ebola virus infections.

3. Contributions of two nuclear localization signals of influenza A virus nucleoprotein to viral replication

Ozawa M, Fujii K, Muramoto Y, Yamada S, Yamayoshi S, Takada A, Goto H, Horimoto T, Kawaoka Y.

The RNA genome of influenza A virus, which forms viral ribonucleoprotein complexes (vRNPs) with viral polymerase subunit proteins (PA, PB1, and PB2) and nucleoprotein (NP), is transcribed and replicated in the nucleus. NP, the major component of vRNPs, has at least two amino acid sequences that serve as nuclear localization signals (NLSs): an unconventional NLS (residues 3 to 13; NLS1) and a bipartite NLS (residues 198 to 216; NLS2). Although both NLSs are known to play a role in nuclear transport, their relative contributions to viral replication are poorly understood. We therefore investigated their contributions to NP subcellular/subnuclear localization, viral RNA (vRNA) transcription, and viral replication. Abolishing the unconventional NLS caused NP to localize predominantly to the cytoplasm and affected its activity in vRNA transcription. However, we were able to create a virus whose NP contained amino acid substitutions in NLS1 known to abolish its nuclear localization function, although this virus was highly attenuated. These results indicate that while the unconventional NLS is not essential for viral replication, it is necessary for efficient viral mRNA synthesis. On the other hand, the bipartite NLS, whose contribution to the nuclear transport of NP is limited, was essential for vRNA transcription and NP's nucleolar accumulation. A virus with nonfunctional NLS2 could not be generated. Thus, the bipartite NLS, but not the unconventional NLS, of NP is essential for influenza A virus replication.

4. Proteolytic Processing of the Ebola Virus Glycoprotein Is Not Critical for Ebola Virus Replication in Nonhuman Primates

Neumann G, Geisbert TW, Ebihara H, Geisbert JB, Daddario-Dicaprio KM, Feldmann H, Kawaoka Y.

Enveloped viruses often require cleavage of a surface glycoprotein by a cellular endoprotease such as furin for infectivity and virulence. Previously, we showed that Ebola virus glycoprotein does not require the furin cleavage motif for virus replication in cell culture. Here, we show that there are no appreciable differences in disease progression, hematology, serum biochemistry, virus titers, or lethality in nonhuman primates infected with an Ebola virus lacking the furin recognition sequence compared to those infected with wild-type virus. We conclude that glycoprotein cleavage by subtilisin-like endoproteases is not critical for Ebola virus infectivity and virulence in nonhuman primates.

5. Mapping of theVP40-binding regions of the Nucleoprotein of Ebola virus

Noda T, Watanabe S, Sagara H, Kawaoka Y.

Expression of Ebola virus nucleoprotein (NP) in mammalian cells leads to the formation of helical structures, which serve as a scaffold for the nucleocapsid. We recently found that NP binding with the matrix protein VP40 is important for nucleocapsid incorporation into virions. To identify the region(s) on the NP molecule required for VP40 binding, we examined the interaction of a series of NP deletion mutants with VP40 biochemically and ultrastructurally. We found that both termini of NP (amino acids 2 to 150 and 601 to 739) are essential for its interaction with VP40 and for its incorporation into virus-like particles (VLPs). We also found that the C terminus of NP is important for nucleocapsid incorporation into virions. Of interest is that the formation of NP helices, which involves the N-terminal 450 amino acids of NP, is dispensable for NP incorporation into VLPs. These findings enhance our understanding of Ebola virus assembly and in so doing move us closer to the identification of targets for the development of antiviral compounds to combat Ebola virus infection.

6. Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors

Hatakeyama S, Sugaya N, Ito M, Yamazaki M, Ichikawa M, Kimura K, Kiso M, Shimizu H, Kawakami C, Koike K, Mitamura K, Kawaoka Y.

Very little is known about the frequency of generation and transmissibility of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. Furthermore, transmission of resistant virus, whether influenza A or B, has not been recognized to date. To assess the prevalence and transmissibility of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. Investigation of the neuraminidase inhibitor sensitivity of influenza B isolates from 74 children before and after oseltamivir therapy and from 348 untreated patients with influenza (including 66 adults) seen at 4 community hospitals in Japan during the 2004-2005 influenza season. Four hundred twentytwo viruses from untreated patients and 74 samples from patients after oseltamivir therapy were analyzed. Sialidase inhibition assay was used to test the drug sensitivities of influenza B viruses. The neuraminidase and hemagglutinin genes of viruses showing reduced sensitivity to neuraminidase inhibitors were sequenced to identify mutations that have the potential to confer reduced sensitivity to these drugs. In 1 (1.4%) of the 74 children who had received oseltamivir, we identified a variant with reduced drug sensitivity possessing a Gly402Ser neuraminidase substitution. We also identified variants with reduced sensitivity carrying an Asp198Asn, Ile222Thr, or Ser 250Gly mutation in 7 (1.7%) of the 422 viruses from untreated patients. Review of the clinical and viral genetic information available on these 7 patients indicated that 4 were likely infected in a community setting, while the remaining 3 were probably infected through contact with siblings shedding the mutant viruses. In this population, influenza B viruses with reduced sensitivity to neuraminidase inhibitors do not arise as frequently as resistant influenza A viruses. However, they appear to be transmitted within communities and families, requiring continued close monitoring.

7. Enhanced growth of seed viruses for H5N1 influenza vaccines

Horimoto T, Murakami S, Muramoto Y, Yamada S, Fujii K, Kiso M, Iwatsuki-Horimoto K, Kino Y, KawaokaY.

Seed viruses used to produce inactivated H5N 1 influenza vaccines are recombinant viruses with modified avirulent-type hemagglutinin (HA) and intact neuraminidase (NA) genes, both derived from an H5N1 isolate, and all remaining genes from the PR8 strain, which grows well in eggs. However, some reassortants grow suboptimally in eggs, imposing obstacles to timely, cost-efficient vaccine production. Here, we demonstrate that our PR8 strain supports better in ovo growth than the PR8 strain used for the WHO-recommended seed virus, NIBRG-14. Moreover, inclusion of an alternative NA protein further enhanced viral growth in eggs. These findings suggest that our H5N1 vaccine candidates would increase the availability of H5N1 vaccine doses at the onset of a new pandemic.

8. An adenoviral vector-mediated reverse genetics system for influenza A virus generation

Ozawa M, Goto H, Horimoto T, Kawaoka Y.

Plasmid-based reverse genetics systems allow the generation of influenza A virus entirely from cloned cDNA. However, since the efficiency of virus generation is dependent on the plasmid transfection efficiency of cells, virus generation is difficult in cells approved for vaccine production that have low transfection efficiencies (e.g., Vero cells). Here we established an alternative reverse genetics system for influenza virus generation by using an adenovirus vector (AdV) which achieves highly efficient gene transfer independent of cell transfection efficiency. This AdV-mediated reverse genetics system will be useful for generating vaccine seed strains and for basic influenza virus studies.

9. Growth of H5N1 influenza A viruses in the upper respiratory tracts of mice

Hatta M, Hatta Y, Kim JH, Watanabe S, Shinya K, Kawaoka Y.

Highly pathogenic avian H5N1 influenza A viruses have spread throughout Asia, Europe, and Africa, raising serious worldwide concern about their pandemic potential. Although more than 250 people have been infected with these viruses, with a consequent high rate of mortality, the molecular mechanisms responsible for the efficient transmission of H5N1 viruses among humans remain elusive. We used a mouse model to examine the role of the amino acid at position 627 of the PB2 viral protein in efficient replication of H5N1 viruses in the

mammalian respiratory tract. Viruses possessing Lys at position 627 of PB2 replicated efficiently in lungs and nasal turbinates, as well as in cells, even at the lower temperature of 33 8C. Those viruses possessing Glu at this position replicated less well in nasal turbinates than in lungs, and less well in cells at the lower temperature. These results suggest that Lys at PB2-627 confers to avian H5N1 viruses the advantage of efficient growth in the upper and lower respiratory tracts of mammals. Therefore, efficient viral growth in the upper respiratory tract may provide a platform for the adaptation of avian H5N1 influenza viruses to humans and for efficient person-to-person virus transmission, in the context of changes in other viral properties including specificity for human (sialic acid a-2,6-galactose containing) receptors.

Publications

Original papers

Kobasa D, Jones, SM, Shinya K, Kash JC, Copps J, Ebihara H, Hatta Y, Kim JH, Halfmann P, Hatta M, Feldmann F, Alimonti JB, Fernando L, Li Y, Katze MG, Feldmann H, Kawaoka Y. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature 445: 23, 2007.

Takada A, Ebiharab H, Jones S, Feldmannd H, Kawaoka Y. Protective efficacy of neutralizing antibodies against Ebola virus infection. Vaccine 25: 993-999, 2007.

Ozawa M, Fujii K, Muramoto Y, Yamada S, Yamayoshi S, Takada A, Goto H, Horimoto T, Kawaoka Y. Contributions of two nuclear localization signals of influenza A virus nucleoprotein to viral replication. J Virol 81: 30-41, 2007.

Neumann G, Geisbert TW, Ebihara H, Geisbert JB, Daddario-Dicaprio KM, Feldmann H, Kawaoka Y. Proteolytic Processing of the Ebola Virus Glycoprotein Is Not Critical for Ebola Virus Replication in Nonhuman Primates. J Virol 81, 2995-2998, 2007.

Noda T, Watanabe S, Sagara H, Kawaoka Y. Mapping of theVP40-binding regions of the Nucleoprotein of Ebola virus. J Virol 81, 3554-3562, 2007.

Garulli B, Meola M, Stillitano MG, Kawaoka Y, Castrucci MR. Efficient vagina-to-lower respiratory tract immune trafficking in a murine model of influenza A virus infection. Virology 361, 274-282, 2007.

Kurosaki Y, Takada A, Ebihara H, Grolla A, Kamo N, Feldmann H, Kawaoka Y, Yasda J. Rapid and simple detection of Ebola virus by reverse transcription-loop-mediated isothermal amplification. J Virol Methods 141, 78-83, 2007.

Sugaya N, Mitamura K, Yamazaki M, Tamura D, Ichikawa M, Kimura K, Kawakami C, Kiso M, Ito M, Hatakeyama S, Kawaoka Y. Lower clinical effectiveness of oseltamivir against influenza B contrasted with influenza A infection in children. Clin Infect Dis 44: 197-202, 2007.

Hatakeyama S, Sugaya N, Ito M, Yamazaki M, Ichikawa M, Kimura K, Kiso M, Shimizu H, Kawakami C, Koike K, Mitamura K, Kawaoka Y. Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. JAMA 297, 1435-1442, 2007.

Urata S, Noda T, Kawaoka Y, Morikawa S, Yokosawa H, Yasuda J. Interaction of Tsg101 with Marburg virus VP40 depends on the PPPY motif, but not the PT/SAP motif as in the case of Ebola virus, and Tsg101 plays a critical role in the budding of Marburg virus-like particles induced by VP40, NP, and GP. J Virol 81: 4895-4899, 2007.

Horimoto T, Murakami S, Muramoto Y, Yamada S, Fujii K, Kiso M, Iwatsuki-Horimoto K, Kino Y, KawaokaY. Enhanced growth of seed viruses for H5N1 influenza vaccines. Virology 366: 23-27, 2007.

Ozawa M, Goto H, Horimoto T, Kawaoka Y. An adenoviral vector-mediated reverse genetics system for influenza A virus generation. J Virol 81: 9556-9559.

Hatta M, Hatta Y, Kim JH, Watanabe S, Shinya K, Kawaoka Y. Growth of H5N1 influenza A viruses in the upper respiratory tracts of mice PLoS Pathog 3: 1374-1379.

Rigoni M, Shinya K, Toffan A, Milani A, Bettini F, Kawaoka Y, Cattoli G, Capua I. Pneumo- and eurotropism of avian origin Italian highly pathogenic avian influenza H7N1 isolates in experimentally infected mice. Virology 364, 28 -35, 2007.

Guo CT, Takahashi N, Yagi H, Kato K, Takahashi T, Yi SQ, Chen Y, Ito T, Otsuki K, Kida H, Kawaoka Y, Hidari KI, Miyamoto D, Suzuki T, Suzuki Y. The quail and chicken intestine have sialyl-galactose sugar chains responsible for the binding of influenza A viruses to human type receptors. Glycobiology 17, 713-724, 2007.

Sawada T, Hashimoto T, Nakano H, Suzuki T, Suzuki Y, Kawaoka Y, Ishida H, Kiso M. Influenza viral hemagglutinin complicated shape is advantageous to its binding affinity for sialosaccharide receptor. Biochem Biophys

Res Commun 355: 6-9, 2007.
Shinya K, Watanabe S, Ito T, Kasai N, Kawaoka Y. Adaptation of an H7N7 equine influenza A virus in mice. J Gen Virol 88, 547-532, 2007.
Takada A, Ebihara H, Feldmann H, Geisbert TW, Kawaoka Y. Epitopes required for antibodydependentenhancement of Ebola virus infection. J Infect Dis 196 Suppl 2: S347-356, 2007.
Ebihara H, Theriault S, Neumann G, Alimonti JB, Geisbert JB, Hensley LE, Groseth A, Jones SM, Geisbert TW, Kawaoka Y, Feldmann H. In vitro and in vivo characterization of recombinant Ebola viruses expressing enhanced green fluorescent protein. J Infect Dis 196 Suppl 2: S313-322, 2007.
Yamayoshi S, Kawaoka Y. Mapping of a region of Ebola virus VP40 that is important in the production of virus-like particles. J Infect Dis 196 Suppl 2: S291-295, 2007.
Watanabe S, Noda T, Halfmann P, Jasenosky L, Kawaoka Y. Ebola virus (EBOV) VP24 inhibits transcription and replication of the EBOV genome. J Infect Dis 196 Suppl 2: S284-290, 2007.
Shimojima M, Ikeda Y, Kawaoka Y. The mechanism of Axl-mediated Ebola virus infection. J Infect Dis 196 Suppl 2: S259-263, 2007.
Noda T, Halfmann P, Sagara H, Kawaoka Y.Regions in Ebola virus VP24 that are important for nucleocapsid formation. J Infect Dis 196 Suppl 2: S247-250, 2007.
Nochi T, Yuki Y, Matsumura A, Mejima M, Terahara K, Kim DY, Fukuyama S, Iwatsuki-Horimoto K, Kawaoka Y, Kohda T, Kozaki S, Igarashi O, Kiyono H. A novel M cell-specific carbohydrate-targeted mucosal vaccine effectively induces antigen-specific immune responses. J Exp Med 204: 2789-2796, 2007.
Youn H-J, Ko S-Y, Lee K-A, Ko H-J, Lee Y-S, Fujihashi K, Boyaka PN, Kim S-H, Horimoto T, Kweon M-N, Kang C-Y: A single intranasal immunization with inactivated influenza virus and alpha-galactosylceramide induces long-term protective immunity without redirecting antigen to the central nervous system. Vaccine 25: 5189-5198, 2007.

Reviews

Wright PF, Neumann G, Kawaoka Y. Orthomyxoviruses. In: Fields Virology. Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, *eds*. Wolters Kluwer, Lippincott Williams & Wilkins, Philadelphia, 2007. Chapter 48, pp. 1691-1740.
Gabriele Neumann, Kyoko Shinya, Yoshihiro Kawaoka. Molecular pathogenesis of H5N1 influenza virus infections. Antiviral Therapy 12: 617-626, 2007.

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Division of Infectious Genetics
感染遺伝学分野

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Immune cells express multiple Toll-like receptors (TLRs) that are concomitantly activated by a variety of pathogen products during microbial and viral infection. There is presumably a need to coordinate the expression function of TLRs in individual cells. Recent reports also have indicated that the balance of TLRs responses has an important role in inducing autoimmune diseases. Our research main focuses on molecular regulatory mechanism to coordinate pathogen recognition by TLRs.

1. A Protein associated with Toll-like receptor 4 (PRAT4A) is required for TLR-dependent immune responses

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Immune cells express multiple Toll-like receptors (TLRs) that are concomitantly activated by a variety of pathogen products. While there is presumably a need to coordinate the expression function of TLRs in individual cells, little is known about the mechanisms governing this process. Here we show that a protein associated with TLR4 (PRAT4A) is required for multiple TLR responses. PRAT4A resides in the endoplasmic reticulum (ER) and PRAT4A knockdown inhibited trafficking of TLR1 and TLR4 to the cell surface and ligand-induced trafficking of TLR9 to lysosomes. Other cell surface molecules were expressed normally on immunocytes from PRAT4A$^{-/-}$ mice. There was impaired cytokine production to TLR ligands except to TLR3 ligand poly (I:C), and to whole bacteria. Activation of antigen-specific T helper type 1 responses was also defective. Moreover, PRAT4 A$^{-/-}$ bone marrow chimeric mice were resistant to LPS-induced sepsis. These results suggest that PRAT4A regulates the subcellular distribution and response of multiple TLRs and is required for both innate and adaptive immune responses.

2. Pathogenic roles for tonic B cell activation by Toll-like receptor 2/4 and Radioprotective105 in lupus-prone MRL/lpr mice

Toshihiko Kobayashi[1], Koichiro Takahashi,

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Toll-like receptors (TLRs) have a crucial role in sensing microbial products and triggering immune responses. Recent reports have indicated that TLR7 and TLR9 have an important role in activating autoreactive B cells. In addition to TLR7 and TLR9, mouse B cells express TLR2, TLR4, and structurally related RadioProtective 105 (RP105). We have previously showed that RP105 work in concert with TLR2/4 in antibody response to TLR2/4 ligands. We here report that B cells are constitutively activated by TLR2/4 and RP105. Tonic B cell activation was revealed by γ^3 germline transcript and serum IgG3 production, both of which were impaired by the lack of RP105 or of TLR2/4. Serum IgG3 was not altered in germ-free or antibiotics-treated mice, suggesting that the microbial flora hardly contributes to the tonic activation of B cells. The lack of tonic B cell activation due to RP105 mutation ameliorated disease progression in lupus-prone MRL/lpr mice. $RP105^{-/-}$ MRL/lpr mice showed less lymphoadenopathy/splenomegaly and longer survival than MRL/lpr mice. Whereas glomerulonephritis and autoantibody production were not altered, improvement in blood urea nitrogen (BUN) and lower incidence of renal arteritis indicated that renal function was ameliorated in the absence of RP105. These results suggest that tonic B cell activation dependent on TLR2/4 and RP105 has a pathogenic role in MRL/lpr mice.

3. Roles for LPS-dependent interaction and relocation of TLR4 and TRAM in TRIF-signaling

Natsuko Tanimura, Shinichiroh Saitoh, Fumi Matsumoto, Sachiko Akashi-Takamura, and Kensuke Miyake

Toll-like receptor 4 (TLR4) activates two distinct signaling pathways depending on two adaptor molecules, MyD88 or TRIF. These signaling pathways lead to production of proinflammatory cytokines or type I interferons (IFNs), respectively. TIRAP is a sorting adaptor molecule recruiting MyD88 to activated TLR4 in the plasma membrane. TRAM serves as a signaling molecule between TLR4 and TRIF, and was shown to associate with TLR4 or TRIF. Little is known, however, how LPS influences TRAM-interaction with TLR4 or with TRIF. Here we show that LPS induces association of TLR4 with TRAM, TRIF recruitment to the plasma membrane, and their subsequent internalization into endosome/lysosome. The internalized signaling complex consisting of TLR4 and TRAM co-localizes with TRAF3, a signaling molecule working downstream of TRIF, in endosome/lysosome. These results suggest that TLR4 activates TRIF-signaling pathway in endosome/lysosome.

4. Induction of IgE antibody production and Toll-like receptor 4 stimulation by malarial peroxiredoxin

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In this study, 2-Cys *Plasmodium berghei* ANKA (PbA) peroxiredoxin (Prx) was identified as an antigenic protein recognized by an anti-PbA IgE antibody using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and proteomic analysis. Innate immune responses to PbAPrx were examined using cells from mice deficient in TLRs or related molecules, and it was demonstrated that responses were severely impaired in $TLR4^{-/-}$, $MyD88^{-/-}$ and $MD\text{-}2^{-/-}$ mice, but not Toll/IL-1 receptor domain-containing adaptor-inducing IFNβ $(TRIF)^{-/-}$, $TLR2^{-/-}$ or radioprotective $(RP)105^{-/-}$ mice. An association between PbAPrx and TLR4 was observed following immunoprecipitation and immunoblotting, suggesting that PbAPrx was associated with TLR4/MD-2. Interactions between Prx and TLR4/MD-2 were also examined by FACS using TLR4/MD-2- or TLR2-expressing cells. NFκB/GFP activity was observed in TLR4/MD-2- but not in TLR2-expressing cells following stimulation with Prx. However, this effect was not observed after treatment with proteinase K, suggesting that

PbAPrx is a protein ligand for TLR4 and that PbAPrx activity observed in this study is not due to contamination with LPS. These findings indicate that malarial Prx induces IgE-mediated protection through FcεRI on mast cells and innate immunity thorough TLR4 with MyD88 and MD-2, suggesting a novel function for malarial Prx in innate and acquired immune responses in malaria.

5. Cathepsins are required for Toll-like receptor 9 responses

Fumi Matsumoto, Shin-Ichiroh Saitoh, Ryutaroh Fukui, Toshihiko Kobayashi, Natsuko Tanimura, Kazunori Konno, Yutaka Kusumoto, Sachiko Akashi-Takamura, Kensuke Miyake

Toll-like receptors (TLR) recognize a variety of microbial products and activate defense responses. Pathogen sensing by TLR2/4 requires accessory molecules, whereas little is known about a molecule required for DNA recognition by TLR9. After endocytosis of microbes, microbial DNA is exposed and recognized by TLR9 in lysosome. We here show that cathepsins, lysosomal cysteine proteases, are required for TLR9 responses. A cell line Ba/F3 was found to be defective in TLR9 responses despite enforced TLR9 expression. Functional cloning with Ba/F3 identified cathepsin B/L as a molecule required for TLR9 responses. The protease activity was essential for the complementing effect. TLR9 responses were also conferred by cathepsin S or F, but not by cathepsin H. TLR9-dependent B cell proliferation and CD86 upregulation were apparently downregulated by cathepsin B/L inhibitors. Cathepsin B inhibitor downregulated interaction of CpG-B with TLR9 in 293T cells. These results suggest roles for cathepsins in DNA recognition by TLR9.

6. A tyrosine-based motif and a di-leucine motif are essential for TLR4 endocytosis

CaiHua Xuan, Shin-Ichiroh Saitoh and Kensuke Miyake

TLR4 plays an important role for the inflammatory responses against lipopolysaccharide (LPS) derived from Gram-negative bacteria. TLR 4 recognizes LPS and dimerizes on the cell surface to activate the signaling molecules. The activated TLR4 is transported from the plasma membrane to endosomes and lysosomes for degradation that is one of negative regulatory mechanisms to shut off signaling. The TLR4 endocytosis is dependent on clathrin-coated vesicle. The tyrosine-based motif, YXXΦ, and the di-leucine motif have been reported to drive a clathrin dependent endocytosis. On the basis of the reports, we investigated the functional endocytosis motif by substituting the tyrosine-based motifs and a di-leucine motif for alanines in TLR4. Finally, we identified a tyrosine-based motif, YRDF and a di-leucine motif, ELYRLL as the functional endocytosis motif. Our results support that the TLR4 endocytosis is dependent on the formation of a clathrin-coated vesicle.

Publications

Takahashi K, Shibata T, Akashi-Takamura S, Kiyokawa T, Wakabayashi Y, Tanimura N, Kobayashi T, Matsumoto F, Fukui R, Kouro T, Nagai Y, Takatsu K, Saitoh S, Miyake K. A protein associated with Toll-like receptor (TLR) 4 (PRAT4A) is required for TLR-dependent immune responses. J Exp Med 204: 2963-76, 2007.

Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, Kotani H, Yamaoka S, Miyake K, Aoe S, Kamei Y, Ogawa Y. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. Arterioscler. Thomb. Vasc. Biol. 2007, 27: 84-91.

Miyake K. Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. Semin Immunol. 2007, 19: 3-10.

Ohto U, Fukase K, Miyake K, Satow Y. Crystal structures of human MD-2 and its complex with antiendotoxic lipid IVa. Science 2007, 316: 1632-1634

Saijo, S., Fujikado, N., Furuta, T., Kotaki, H., Seki, K., Sudo, K., Adachi, Y., Ohno, N., Kinjo, T., Nakamura, K., Kawakami, K., and Iwakura, Y. Dectin-1 plays an important role host defense, against the pathogenic fungus *Pneumocystis carinii* under immunocompromised condition. Nature Immunol. 8: 39-46, 2007.

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Division of Mucosal Immunology
炎症免疫学分野

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The mucosal immune system not only plays an important role as the first line of immunological defense for preventing the host from invasion of microorganisms, but also contributes to the establishment and maintenance of mucosal homeostasis. Our major focus is the elucidation and understanding of molecular and cellular nature of the mucosal immune system for the development of mucosal vaccine against infectious diseases and mucosal immune therapy for mucosa-associated diseases, such as food allergy and inflammatory diseases.

1. M cell Biology

Tomonori Nochi[1], Osamu Igarashi[1], Kazutaka Terahara[1], Masato Yoshida[1], Takaharu Taketomi[1], Mio Mejima[1], Akiko Matsumura[1], Gemilson S. Pontes[1], Yuko Takahashi[1], Steven E. Domino[2], Yoshikazu Yuki[1] and Hiroshi Kiyono[1]: [1]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, [2]Department of Obstetrics and Gynecology, University of Michigan

Membranous or microfold cells (M cells) located in the follicle-associated epithelium (FAE) of Peyer's patches (PPs) play a pivotal role in the uptake of luminal antigens for the induction of antigen-specific immune responses in both systemic and mucosal compartments. We previously found alternative antigen-sampling cells in intestinal villi and designated villous M cells. To investigate relationship between PP-associated and villous M cells, we examined expression level of fucosyltransferase (FUT)1 and FUT2 as specific enzymes for the α(1,2) fucosylation because α(1,2) fucose has been considered to be the specific marker of these M cells in mice. The histochemical analyses with FUT1 or FUT2 deficient mice showed that the synthesis of fucosylated glycans of PP-associated and villous M cells was regulated by FUT1 and FUT2, respectively. Independently, we recently established a novel M cell-specific monoclonal antibody (NKM 16-2-4) that recognized both PP-associated and villous M cells. In this time, we confirmed that the mAb reacted with M cell-specific carbohydrate moiety with α(1,2) fucose modulated by FUT1 or FUT2. Interestingly, the expression of FUT2 in intestinal epithelium was enhanced by environmental stresses and the fucosylated epithelial cells possessed intermediate cellular traits between M cells and absorptive epithelial cells. Therefore, we categorized and designated these cells as induced M-like cells. Our current efforts are aimed to understand the differentiation processes of these M cells for the elucidation of antigen-sampling network in the gastrointestinal tract.

2. Development of Mucosal Vaccine

Yoshikazu Yuki[1], Tomonori Nochi[1], Daisuke

Tokuhara[1], Nobuhiro Kataoka[1], Mio Mejima[1], Shiho Kurokawa[1], Yuko Takahashi[1], Ushio Nakanishi[1], Tomoko Kohda[2], Shunji Kozaki[2], Fumio Takaiwa[3], Takehiro Masumura[4], Kunisuke Tanaka[4] and Hiroshi Kiyono[1]: [1]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, [2]Laboratory of Veterinary Epidemiology, Department of Veterinary Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, [3]Transgenic Crop Research and Development Center, National Institute of Agrobiological Sciences, [4]Laboratory of Genetic Engineering, Graduate School of Agriculture, Kyoto Prefectural University

Mucosal vaccination, which can induce the antigen-specific immune responses in both systemic and mucosal compartments, is considered an ideal strategy for the global control of infectious diseases. We developed rice-based oral vaccine possessing many practical advantages over most traditional vaccines. It was stable at room temperature for several years and was protected from digestive enzymes. When ingested, this vaccine induced antigen-specific antibodies with neutralizing activities. These results show that the rice-based oral vaccine offers a highly practical global strategy for cold-chain- and needle-free vaccination against mucosal infectious diseases. In addition, we recently developed M cell-targeted mucosal vaccine using newly established M cell-specific monoclonal antibody (NKM 16-2-4). Oral administration of low doses of tetanus toxoid (TT)- or botulinum toxoid (BT)-conjugated NKM 16-2-4 together with mucosal adjuvant cholera toxin (CT) resulted in the induction of high-levels antigen-specific serum IgG and mucosal IgA responses, whereas TT- or BT-conjugated control rat IgG induced no- or very low-levels antigen-specific immune responses. Furthermore, oral vaccine with BT-conjugated NKM 16-2-4 induced protective immunity against 10000x LD_{50} dose of botulinum toxin challenge. A combination of these two novel vaccine systems is considered the best practical strategy for developing effective oral vaccine. Thus, our current efforts are aiming at the creation of rice-expressed single-chain fragment variable (scFv) of NKM 16-2-4 for the development of M cell-targeted rice-based mucosal vaccine.

3. Mucosal Trafficking

Jun Kunisawa, Masashi Gohda, Yosuke Kurashima, Morio Higuchi, Yuki Kagiyama, Atsuhiro Matsumoto, Ushio Nakanishi, Tomokazu Ishizuka, Izumi Ishikawa, and Hiroshi Kiyono: Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo

It is generally considered that lymphocyte trafficking is regulated by chemokine and adhesion molecules. In addition to these molecules, accumulating evidence has indicated that sphingosine 1-phosphate (S1P), a lipid mediator, is also involved in the regulation of lymphocyte trafficking, but its role in the regulation of mucosal immune system remains to be investigated. In this project, we have revealed pivotal roles of S1P in the regulation of mucosal lymphocyte trafficking. For instance, we found that naïve intraepithelial lymphocytes (IEL) is principally regulated by S1P for the migration from the thymus into the intestine, but unconventional thymic IEL precursors including TCR^+ double negative thymocytes do not require S1P for their trafficking into the intestine. In addition, our study showed that S1P regulates peritoneal B cell trafficking into the intestine and subsequent intestinal IgA production. Furthermore, it was revealed that pathogenic T and mast cells causing allergic diarrhea used S1P in their trafficking from the systemic compartment into the large intestine. Thus, disruption of S1P-mediated pathway resulted in the inhibition of allergic diarrhea. Our current efforts are aimed to elucidate the molecular and cellular mechanisms of the S1P-mediated mucosal lymphocyte trafficking and to apply them to the development of mucosal immune therapy.

4. Mucosal Allergy 1 -Food Allergy-

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To establish novel and effective strategies to regulate intestinal allergic diseases, we have elucidated molecular and cellular mechanisms of the development of food allergy using ovalbumin-specific food allergy murine model. In this year, we have revealed the involvement of regulatory-type T cells and a lipid mediator in the development of food allergy. In the former case, we found that Peyer's patches (PPs) possessed regulatory function in allergic diarrhea by showing that mice lacking PPs were more susceptible to the allergy development.

Additional analyses revealed that PPs contained IL-10 producing regulatory $CD4^+CD25^+$ $Foxp3^+$ T cells (Treg) and inhibited the development of allergic diarrhea. Indeed, the treatment with anti-CD25 or anti-IL-10 antibody resulted in the rapid and severe development of allergic diarrhea. These studies demonstrate that PP is the site to induce IL-10-producing Treg cells for the control of intestinal allergic responses. In the latter study, we showed that sphingosine 1-phosphate (S1P), a lipid mediator, played important roles in the development of food allergy by regulating the trafficking of pathogenic T and mast cells. When mice were treated with FTY720, a S1P receptor modulator, the incidence of allergic diarrhea was markedly inhibited. In those mice, the number of mast cells and activated $CD4^+$ Th2 cells was decreased in the intestinal compartment. These findings suggest that S1P-mediated trafficking of mast cells and activated T cells could be an effective target for the prevention and treatment of food allergy. Based of these findings, we are now trying to elucidate the detailed molecular and cellular mechanisms of induction, activation, and trafficking of allergen-specific $CD4^+$ Th2 cells, mucosal mast cell and Treg cells to develop the immunotherapy against intestinal allergic diseases.

5. Mucosal Allergy 2 -Allergic Rhinitis-

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The lymphoid chemokines CCL19 and CCL21 are known to be crucial for lymphoid cell trafficking. We shed light on the role of CCL19 and CCL21 in the development of allergic rhinitis. After nasal challenge with OVA, OVA-sensitized *plt* mice (paucity of lymph node T cells) mice, which are deficient in CCL19 and CCL21, showed more severe allergic symptoms than did identically treated wild-type mice. OVA-specific IgE production, eosinophil infiltration, and Th2 cytokine responses were enhanced in the upper airway of *plt* mice. Moreover, in the allergic condition of *plt* mice, the number of $CD4^+CD25^+$ regulatory T cells decreased in the secondary lymphoid tissues, whereas the number of Th2-inducer-type $CD8^-CD11b^+$ myeloid dendritic cells (m-DCs) increased in cervical lymph nodes and NALT. Nasal administration of the plasmid-encoding DNA of CCL19 resulted in the reduction of m-DCs in the secondary lymphoid organs and the suppression of allergic responses in *plt* mice. These results suggest that CCL19 and CCL21 act as regulatory chemokines for the control of airway allergic disease and so may offer a new strategy for the control of allergic disease.

6. Non-canonical second lymphoid organogenesis

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Secondary lymphoid tissue development is initiated by the interaction of $CD3^-CD4^+CD45^+$ lymphoid tissue inducer cells (LTi) with stromal organizer cells. The differentiation of LTi requires several molecules such as inhibitor of basic helix-loop-helix transcription factor 2 (Id2) and retinoic acid receptor-associated orphan receptor (ROR)γt. Intriguingly, $Id2^{-/-}$ mice showed an impairment of almost all secondary lymphoid organs including Peyer's patch (PP) and nasopharynx-associated lymphoid tissue (NALT), whereas $ROR\gamma t^{-/-}$ mice lack most secondary lymphoid organs including PP, but not NALT. In addition, tear duct-associated lymphoid tissue (TALT), we have recently discovered in murine lacrimal sac, developed normally in both $Id2^{-/-}$ and $ROR\gamma t^{-/-}$ mice. These findings suggest that although the development of mucosa-associated lymphoid tissues (MALT) depends on $CD3^-CD4^+CD45^+$ LTi, those LTi can be divided into several subsets by the dependency on transcriptional factors. Our comprehensive analyses identified several key molecules discriminating each LTi. For example, NALT and TALT inducer cells were identified as $CD3^-CD4^{low}CD45^+$ cells whereas PP inducer cells were characterized by $CD3^-CD4^{high}CD45^+$ cells. In addition, we revealed that interferon regulatory factor (IRF) 1 was selectively expressed in NALT inducer cells. Therefore, $IRF1^{-/-}$ mice

showed impaired NALT organogenesis with normal development of other secondary lymphoid organs including PP and TALT. These findings collectively indicate the presence of versatile differentiation pathways of MALT inducer cells and IRF1 is a key and specific molecule specifically regulating NALT inducer cells. We are currently trying to reveal further molecular and cellular mechanisms to determine PP, NALT and TALT organogenesis.

7. Molecular and Cellular Analysis of Host-Microflora Interaction

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The host is continuously exposed to the external environment via the large surface area covered by mucosal epithelium. Enormous numbers of indigenous microflora are colonizing symbiotically at mucosal surface by creating appropriate homeostatic conditions and they have an important role in shaping and maintaining host mucosal immunity. Although it has been reported that gut microflora are taken up by Peyer's patches and induce host immune responses by using GFP-*Enterobacter cloacae*, it is still unclear what kinds of bacteria are uptaken by Peyer's patchs/other mucosal compartments in the normal conditions. In this study, we revealed the bacterial composition in the various intestinal compartments by using metagenomic analysis. For example, *Lactobacillius* and *Segmented filamentous bacteria* (*SFB*) were predominantly present on the surface of Peyer's patches, however inside Peyer's patches, completely different bacteria were predominant. We also revealed that gut microflora is involved in the induction of fucosylation to intestinal epithelial cells. Germ-free mice had low numbers of fucosylated epithelial cells compared with conventional mice. Furthermore, these fucosylation was observed more in ileum than in duodenum. And we found that the bacterial populations of these two different parts are quite different. For instance, *Burkholderia* was predominantly existed in duodenum and SFB was in ileum. In addition, SFB has already been reported to induce fucosylation to intestinal epithelia by SFB-associated mice model. Now we are analyzing the molecular and cellular interaction between host mucosal immune system and these gut microflora in more detail to find out what's going on.

Publications

Kim, NJ., Kunisawa, J., Kweon, MN, Eog, JG. and Kiyono, H. Oral feeding of *Bifidobacterium bifidum* (BGN4) prevents $CD4^+$ CD45RB (high) T cell-mediated inflammatory bowel disease by inhibition of disordered T cell activation. Clin. Immunol, 123: 30-39, 2007.

Kunisawa, J., Kurashima, Y., Gohda, M., Higuchi, M., Ishikawa, I., Ogahara, I. and Kiyono, H. Sphingosine 1-phosphate regulates peritoneal B cell trafficking for subsequent intestinal IgA production. Blood 109: 3749-3756, 2007.

Tamagawa, H., Hiroi, T., Mizushima, T., Ito, T., Matsuda, H. and Kiyono, H. Therapeutic effects of roxithromycin in interleukin-10-deficient colitis. Inflamm. Bowel Dis. 13: 547-556, 2007.

Tanaka, N., Fukuyama, S., Fukuiwa, T., Kawabata, M., Sagara, Y., Ito, HO., Miwa, Y., Nagatake, T., Kiyono, H. and Kurono, Y. Intranasal immunization with phosphorylcholine induces antigen specific mucosal and systemic immune responses in mice. Vaccine 25: 2680-2687, 2007.

Kunisawa. J., Takahashi. I. and Kiyono. H. Intraepithelial lymphocytes: their shared and divergent immunological behaviors in the small and large intestine. Immunol Rev. 215: 136-153, 2007.

McGhee. JR., Kunisawa. J. and Kiyono H. Gut lymphocyte migration: we are halfway 'home'. Trends Immunol. 28: 150-153, 2007.

Takayama, N., Igarashi, O., Kweon, MN. and Kiyono, H. Regulatory role of Peyer's patches for the inhibition of OVA-induced allergic diarrhea. Clin. Immunol. 123: 199-208, 2007.

Makita, S., Kanai, T., Nemoto, Y., Totsuka, T., Okamoto, R., Tsuchiya, K., Yamamoto, M., Kiyono, H. and Watanabem M. Intestinal lamina propria retaining $CD4^+CD25^+$ regulatory T cells is a suppressive site of intestinal inflammation. J. Immunol. 178: 4937-4946, 2007.

Fukuyama. S. and Kiyono. H. Neuroregulator RET initiates Peyer's-patch tissue genesis. Immunity. 26: 393-395, 2007.

Nagai, S., Mimuro, H., Yamada, H., Baba, Y.,

Moro, K., Nochi, T., Kiyono, H., Suzuki, T., Sasakawa, C. and Koyasu, S. Role of Peyer's patches in the induction of Helicobacter pylori -induced gastritis. Proc. Natl. Acad. Sci. USA. 104: 8971-8976, 2007.

Ohka, S., Igarashi, H., Nagata, N., Sakai, M., Nochi, T., Kiyono, H. and Nomoto, A. Establishment of a poliovirus oral infection system in human poliovirus receptor-expressing transgenic mice that are deficient in alpha/ beta interferon receptor. J. Virol. 81: 7902-7912, 2007.

Nochi, T., Takagi, H., Yuki, Y., Yang, L., Matsumura, T., Mejima, M., Nakanishi, U., Matsumura, A., Uozumi, A., Hiroi, T., Morita, S., Tanaka, K., Takaiwa, F. and Kiyono, H. Rice-based mucosal vaccine: a new global strategy for cold-chain and needle free vaccination. Proc. Natl. Acad. Sci. USA. 104: 10986-10991, 2007.

Kurashima, Y., Kunisawa, J., Higuchi, M., Gohda, M., Shimizu, M., Takayama, N. and Kiyono, H. Sphingosine 1-phosphate- mediated trafficking of pathogenic Th2 and mast cells for the control of food allergy. J. Immunol. 179: 1577-1585, 2007.

Yuki. Y., Nochi. T. and Kiyono. H. Progress towards an AIDS mucosal vaccine: an overview. Tuberculosis (Edinb). 87: S35-44, 2007.

Kunisawa, J., Kurashima, Y., Higuchi, M., Gohda, M., Ishikawa, I., Ogahara, I., Kim, N., Shimizu, M. and Kiyono, H. Sphingosine 1-phosphate dependence in the regulation of lymphocyte trafficking to the gut epithelium. J. Exp. Med. 204: 2335-2348, 2007.

Takamura, K., Fukuyama, S., Nagatake, T., Kim, DY., Kawamura, A., Kawauchi, H. and Kiyono, H. Regulatory role of lymphoid chemokine CCL19 and CCL21 in the control of allergic rhinitis. J. Immunol. 179: 5897-5906, 2007.

Nochi, T., Yuki, Y., Matsumura, A., Mejima, M., Terahara, K., Kim, DY., Fukuyama, S., Igarashi, O. and Kiyono, H. New generation of mucosal vaccine: Novel M cell specific carbohydrate-targeted vaccination is effective for the induction of antigen-specific immune responses. J. Exp. Med. 204: 2789-2796, 2007.

Terahara, T., Yoshida, M., Igarashi, O., Nochi, T., Pontes, GS., Hase, K., Ohno, H., Mejima, M., Kurokawa, S., Takayama, N., Yuki, Y., Low, AW. and Kiyono, H. Comprehensive gene expression analysis among Peyer's patch M cells, villous M-like cells and intestinal epithelial cells by DNA microarray analysis. J. Immunol. 2008. (in press)

Hashizume, T., Togawa, A., Nochi, T., Igarashi, O., Kweon, MN., Kiyono, H. and Yamamoto, M. Peyer's patches are required for intestinal IgA responses to *Salmonella*. Infect. Immun. 76: 927-934, 2007.

Kunisawa. J., McGhee. JR. and Kiyono H. Mucosal S-IgA enhancement: development of safe and effective mucosal adjuvants and mucosal antigen delivery vehicles. Mucosal Immune Defense: Immunoglobulin A. Edited by Kaetzel, CS. (Kluwer Academic/Plenum Publishers, NY). pp. 345-389, 2007.

Kiyono H., Kunisawa. J., McGhee. JR. and Mestecky J. The mucosal immune system. Fundamental Immunology. Edited by Paul, WE. (Lippincott-Raven, Philadelphia). 2007. (in press)