Japan Academy Prize to:

Hiroshi KIYONO
Director, Synergy Institute for Futuristic Mucosal Vaccine Research and Development (cSIMVa), Chiba University
Head of Division, Division of Infectious Disease Vaccine R&D, Research Institute of Disaster Medicine, Chiba University
Adjunct Professor, Department of Medicine, UC San Diego School of Medicine, University of California, San Diego, USA
Professor Emeritus, The University of Tokyo
Adjunct Professor, La Jolla Institute for Immunology, USA

for "Starting with Caries Vaccine Research, Establishing Mucosal Immunology and Developing Oral and Nasal Vaccines"

Outline of the work:

Following the identification of *Streptococcus mutans* as the causative agent behind dental caries (one of the two major oral diseases, alongside periodontal diseases) in the 1970s, Dr. Hiroshi Kiyono embarked on his research career. Fresh out of dental school in Japan, he joined a research team in the United States studying the role of *S. mutans* in the development of caries vaccines. While the initial goal of caries vaccine development was not achieved, Dr. Kiyono's work with his colleagues demonstrated that oral immunization using haptenized *S. mutans* could induce antigen (Ag)-specific IgA and IgG antibodies. This pioneering research not only laid the foundation for his future exploration of mucosal immunity regulation but also contributed to the advancement of the field itself.

Given the prevailing belief that Peyer's patches (PPs) in the intestine were responsible for initiating Ag-specific IgA responses, Dr. Kiyono and his colleagues focused their research on these issues. They developed a groundbreaking enzymatic cell isolation method from PPs, enabling the demonstration of antigen-presenting cells within them. These cells play a vital role in initiating Ag-specific immune responses. He further leveraged T cell cloning technology to identify IgA isotype-specific Th cells within the PPs. These specialized T helper cells preferentially induce and regulate Ag-specific IgA antibody responses. This finding definitively established PPs as a site for initiating Ag-specific immune responses.

Dr. Kiyono's team further elucidated the critical role of antigen uptake through the development of a monoclonal antibody, NKM16-2-4, targeting antigen-sampling M cells within the PP epithelium. This antibody was then used to create a chimeric Ag by coupling it with botulinum toxoid (BT). Oral administration of the chimeric antigen consisting with NKM-16-2-4 and BT

to mice resulted in efficient uptake by M cells, subsequently inducing Ag-specific IgA and IgG antibodies that neutralize botulinum toxin. The M cell-specific antibodies revealed their presence beyond PPs, also residing within the villous epithelium. This finding not only underscored the efficacy of Ag delivery to M cells but also highlighted the diversity of mucosal antigen sampling systems, contributing to the scientific foundation for oral vaccine development.

Dr. Kiyono's research extended beyond the well-studied intestine's mucosal immune system to the less-explored respiratory system. The nasopharyngeal-associated lymphoid tissue (NALT), a target for nasal immunization, mirrors the function of PPs in the gut. Interestingly, M cells were also found in the NALT's epithelial layer. Furthermore, Dr. Kiyono's work revealed the presence of M cells not only in the NALT epithelium but also in the nasal cavity epithelium, similar to their distribution in the intestinal villous layer. Dr. Kiyono's research further revealed the presence of immunocompetent cells within the NALT, essential for inducing and regulating Ag-specific immune responses. These findings significantly contributed to establishing a strategic foundation for the development of nasal vaccines.

Using an interdisciplinary approach encompassing medicine, agriculture, and plant engineering, Dr. Kiyono's team pioneered a novel rice-based oral vaccine platform called "MucoRice." This innovative approach offers a cold-chain and needle-free solution for mucosal vaccine delivery. MucoRice-engineered rice expressing the B subunit of cholera toxin (CTB), essential for the toxin binding to epithelial cells, effectively induces antigen-specific IgA antibodies with the toxin-neutralizing capability upon oral administration in the experimental animals. A subsequent Phase I clinical trial in healthy Japanese volunteers employed a dose-escalation design to evaluate MucoRice-CTB. This study demonstrated a dose-dependent increase of Ag-specific antibodies, supporting its immunogenicity as a human oral vaccine. To further confirm these findings and assess safety in a broader population, a separate Phase I trial was conducted in healthy, racially diverse U.S volunteers.

Dr. Kiyono's research also encompassed nasal vaccine development. To address the negatively charged nature of the nasal mucosa's epithelial layer, his team, in collaboration with biomaterial engineers, developed a cationic cholesteryl-group-bearing pullulan nanogel (cCHP) capable of encapsulating proteins. This innovative carrier system allowed the encapsulated antigen to adhere to the nasal epithelium for a prolonged period after administration, facilitating efficient delivery to the immunocompetent cells residing beneath the epithelial layer. Intriguingly, nasal immunization with the encapsulated pneumococcal surface protein A from *Streptococcus pneumonia* within cCHP elicited Ag-specific IgA and IgG antibodies in experimental animals, effectively inhibiting the development of pneumonia.

Dr. Kiyono's research initiated dentistry and led to groundbreaking discoveries in the mucosal immune system and the development of innovative mucosal vaccines. His influence extends beyond national borders, as evidenced by his leadership positions in prestigious scientific societies, serving as president of the Japanese Society for Vaccinology, the Japanese Society for Immunology, the Society for Mucosal Immunology, and the Intestinal Microbiology Society. A tireless advocate for mucosal immunology research, Dr. Kiyono has not only shaped academic fields and research directions but also championed the development of practical applications. His achievements and dedication to the field are highly respected worldwide.

List of Main Publications

1. **Kiyono, H.**, McGhee, J.R. and Michalek, S.M. 1980. Lipopolysaccharide regulation of the immune response: comparison of responses to LPS in germfree, *Escherichia coli* - monoassociated and conventional mice. J. Immunol. 124: 36–41.
2. **Kiyono, H.**, McGhee, J.R., Wannemuehler, M.J., Frangakis, M.V., Spalding, D.M., Michalek, S.M. and Koopman, W.J. 1982. In vitro responses to a T-dependent antigen by disassociated Peyer's patch cultures. Proc. Natl. Acad. Sci. U.S.A. 79: 596–600.
3. **Kiyono, H.**, McGhee, J.R., Mosteller, L.M., Eldridge, J.H., Koopman, W.J., Kearney, J.F. and Michalek, S.M. 1982. Murine Peyer's patch T cell clones: characterization of antigen-specific helper T cells for immunoglobulin A responses. J. Exp. Med. 156: 1115–1130.
4. **Kiyono, H.**, Cooper, M.D., Kearney, J.F., Mosteller, L.M., Michalek, S.M., Koopman, W.J. and McGhee, J.R. 1984. Isotype-specificity of helper T cell clones: Peyer's patch Th cells preferentially collaborate with mature IgA B cells for IgA responses. J. Exp. Med. 159: 798–811.
5. Fukuyama, S., Hiroi, T., Yokota, Y., Rennert, P.D., Yanagita, M., Kinoshita, N., Terawaki, S., Shikina, T., Yamamoto, M., Kurono, Y. and **Kiyono, H.** 2002. Initiation of NALT organogenesis is independent of the IL-7R, LTßR and NIK signaling pathways, but requires the Id2 gene and $CD3^{-}CD4^{+}CD45^{+}$ cells. Immunity 17: 31–40.
6. Jang, M.H., Kweon, M.N., Iwatani, K., Yamamoto, M., Terahara, K., Sasakawa, C., Suzuki, T., Nochi, T., Yokota, Y., Hiroi, T., Tamagawa, H., Iijima, H., Kunisawa, J., Yuki, Y. and **Kiyono, H.** 2004. Intestinal villous M cells: A new antigen entry site in the mucosal epithelium. Proc. Natl. Acad. Sci. U.S.A. 101: 6110–6115.
7. **Kiyono, H.** and Fukuyama, S. 2004. NALT-versus Peyer's patch-mediated mucosal immunity. Nat. Rev. Immunol. 4: 699–710.
8. Nochi, T., Yuki, Y., Matsumura, A., Mejima, M., Terahara, K., Kim, D.Y., Fukuyama, S., Iwatsuki-Horimoto, K., Kawaoka, Y., Igarashi, O. and **Kiyono, H.** 2007. A novel M-cell-specific carbohydrate-targeted mucosal vaccine induces effectively antigen-specific immune responses. J. Exp. Med. 204: 2789–2796.
9. Terahara, K., Yoshida, M., Igarashi, O., Nochi, T., Pontes, G.S., Hase, K., Ohno, H., Kurokawa, S., Mejima, M., Takayama, N., Yuki, Y., Lowe, A.W. and **Kiyono, H.** 2008. Comprehensive gene expression analysis among Peyer's patch M cells, villous M cells and intestinal epithelial cells by DNA microarray analysis. J. Immunol. 180: 7840–7846.
10. Nagatake, T., Fukuyama, S, Kim. D.Y., Gohda, K., Igarashi. O., Sato, S., Nochi, T., Sagara, H., Yokota, Y., Jetten, A.M., Kaisho, T., Akira, A., Mimuro, H., Sasakawa, C., Fukui, Y., Fujihashi, K., Akiyama, T., Inoue, J., Penninger, J.M., Kunisawa, J. and **Kiyono, H.** 2009. Id2-, RORγt-, and LTßR-independent lymphoid organogenesis in ocular immunity. J. Exp. Med. 206: 2351–2364.
11. Kim, D.Y., Sato, A., Fukuyama, S., Sagara, H, Nagatake, T., Kong, I.G., Goda, K., Nochi, T., Kunisawa, J., Sato, S., Yokota, Y., Lee, C.H. and **Kiyono, H.** 2011. The airway antigen sampling system: Respiratory M cells as an alternative gateway for inhaled antigens. J. Immunol. 186: 4253–4262 (Cover page article).

12. Kurashima, Y., Amiya, T., Nochi, T., Fujisawa, K., Haraguchi, T., Iba, H., Tsutsui, H., Sato, S., Nakajima, S., Iijima, H., Kunisawa, J. and **Kiyono, H.** 2012. Extracellular ATP mediates mast cell–dependent intestinal inflammation through P2X7 purino receptors. Nat. Commun. 3: 1034.
13. Obata, T., Goto, Y., Kunisawa, J., Sato, S., Sakamoto, M., Setoyama, H., Matsuki, T., Nonaka, K., Shibata, N., Gohda, M., Kagiyama, Y., Nochi, T., Yuki, Y., Fukuyama, Y., Mukai, A., Shinzaki, S., Fujihashi, K., Sasakawa, C., Iijima, H., Goto, M., Umesaki, Y., Benno, Y. and **Kiyono, H.** 2010. Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. Proc. Natl. Acad. Sci. U.S.A. 107: 7419–7424.
14. Goto, Y., Obata, T., Kunisawa, J., Sato, S., Ivanov, I., Lamichhane, A., Takeyama, N., Kamioka, M., Sakamoto, M., Matsuki, T., Setoyama, H., Imaoka, A., Uematsu, S., Akira, S., Domino, S., Kulig, P., Becher, B., Renauld, J-C., Sasakawa, C., Umesaki, Y., Benno, Y. and **Kiyono, H.** 2014. Innate lymphoid cells regulate intestinal epithelial cell glycosylation. Science 345: 1254009.
15. Goto,Y., Uematsu, S. and **Kiyono, H.** 2016. Epithelial glycosylation in gut homeostasis and inflammation. Nat. Immunol. 17: 1244–1251.
16. Kurashima, Y. and **Kiyono, H.** 2017. Mucosal ecological network of epithelium and immune cells for gut homeostasis and tissue healing. Annu. Rev. Immunol. 35: 119–147.
17. Kurashima, Y., Kigoshi, T., Murasaki, S., Arai, F., Shimada, K., Seki, N., Kim, Y.G., Hase, K., Ohno, H., Kawano, K., Ashida, H., Suzuki, T., Morimoto, M., Saito, Y., Sasou, A., Goda, Y., Yuki, Y., Inagaki, Y., Iijima, H., Suda, W., Hattori, M. and **Kiyono, H.** 2021. Pancreatic glycoprotein 2 is a first line of defense for mucosal protection in intestinal inflammation. Nat. Commun. 12: 1067.
18. Kamioka, M., Goto, Y., Nakamura, K., Yokoi, Y., Sugimoto, R., Ohira, S., Kurashima, Y., Umemoto, S., Sato, S., Kunisawa, J., Takahashi, Y., Domino, S.E., Renauld, J-C., Nakae, S., Iwakura, Y., Ernst, P.B., Ayabe, T. and **Kiyono, H.** 2022. Intestinal commensal microbiota and cytokines regulate Fut2^{+} Paneth cells for gut defense. Proc. Natl. Acad. Sci. U.S.A. 119: e2115230119.
19. Nochi, T., Takagi, H., Yuki, Y., Yang, L., Masumura, T., Mejima, M., Nakanishi, U., Matsumura, A., Uozumi, A., Hiroi, T., Morita, S., Tanaka, K., Takaiwa, F. and **Kiyono, H.** 2007. Rice-based mucosal vaccine as a global strategy for cold-chain- and needle-free vaccination. Proc. Natl. Acad. Sci. U.S.A. 104: 10986–10991.
20. Nochi, T., Yuki, Y., Katakai, Y., Shibata, H., Tokuhara, D., Mejima, M., Kurokawa, S., Takahashi, Y., Nakanishi, U., Ono, F., Mimuro, H., Sasakawa, C., Takaiwa, F., Terao, K. and **Kiyono, H.** 2009. A rice-based oral cholera vaccine induces macaque-specific systemic neutralizing Abs but does not influence pre-existing intestinal immunity. J. Immunol. 183: 6538–6544.
21. Tokuhara, D., Yuki, Y., Nochi, T., Kodama, T., Mejima, M., Kurokawa, S., Takahashi, Y., Nanno, M., Nakanishi, U., Takaiwa, F., Honda, T. and **Kiyono, H.** 2010. Secretory IgA-mediated protection against *V. cholerae* and heat-labile enterotoxin-producing enterotoxigenic *Escherichia coli* by rice-based vaccine. Proc. Natl. Acad. Sci. U.S.A. 107: 8794–

8799.

22. Nochi, T., Yuki, Y., Takahashi, H., Sawada, S. I., Mejima, M., Kohda, T., Harada, N., Kong, I.G., Sato, A., Kataoka, N., Tokuhara, D., Kurokawa, S., Takahashi, Y., Tsukada, H., Kozaki, S., Akiyoshi, K. and **Kiyono, H.** 2010. Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. Nat. Mater. 9: 572–578.
23. Kong, I.G., Sato, A., Yuki, Y., Nochi, T., Takahashi, H., Sawada, S., Mejima, M., Kurokawa, S., Okada, K., Sato, S., Briles, D.E., Kunisawa, J., Inoue, Y., Yamamoto, M., Akiyoshi, K. and **Kiyono, H.** 2013. Nanogel-based PspA intranasal vaccine prevents invasive disease and nasal colonization by *Streptococcus pneumoniae*. Infect. Immun. 81: 1625–1634.
24. Fukuyama, Y., Yuki, Y., Katakai, Y., Harada, N., Takahashi, H., Takeda, S., Mejima, M., Joo, S., Kurokawa, S., Sawada, S., Shibata, H., Park, E.J., Fujihashi, K., Briles, D.E., Yasutomi, Y., Tsukada, H., Akiyoshi, K. and **Kiyono, H.** 2015. Nanogel-based pneumococcal surface protein A nasal vaccine induces microRNA-associated Th17 cell responses with neutralizing antibodies against *Streptococcus pneumoniae* in macaques. Mucosal Immunol. 8: 1144–1153.
25. Yuki, Y., Nojima, M., Hosono, O., Tanaka, H., Kimura, Y., Satoh, T., Imoto, S., Uematsu, S., Kurokawa, S., Kashima, K., Mejima, M., Nakahashi-Ouchida, R., Uchida, Y., Marui, T., Yoshikawa, N., Nagamura, F., Fujihashi, K. and **Kiyono, H.** 2021. Assessment of oral MucoRice-CTB vaccine for the safety and microbiota-dependent immunogenicity in humans: A randomized trial. Lancet Microbe. 2: e429–e440.
26. Yuki, Y., Nojima, Kashima, K., Sugiura, K., Maruyama, S., Kurokawa, S., Yamanoue, T., Nakahashi-Ouchida, R., Nakajima, H., Hiraizumi, T., Kohno, H., Goto, E., Fujihashi, K. and **Kiyono, H.** 2022. Oral MucoRice-CTB vaccine is safe and immunogenic in healthy US adults. Vaccine 40: 3372–3379.
27. Umemoto, S., Nakahashi-Ouchida, R., Yuki, Y., Kurokawa, S., Machita, T., Uchida, Y., Mori, H., Yamanoue, T., Shibata, T., Sawada, S., Ishige, K., Hirano, T., Fujihashi, K., Akiyoshi, K., Kurashima, Y., Tokuhara, D., Ernst, P.B., Suzuki, M. and **Kiyono, H.** 2023. Cationic-nanogel nasal vaccine containing the ectodomain of RSV-small hydrophobic protein induces protective immunity in rodents. NPJ Vaccines 8: 106.
28. Nakahashi-Ouchida, R., Fujihashi, K., Kurashima, Y., Yuki, Y. and **Kiyono, H.** 2023. Nasal vaccines: solutions for respiratory infectious diseases. Trends. Mol. Med. 29: 124–140.