Profile of Akiko Iwasaki

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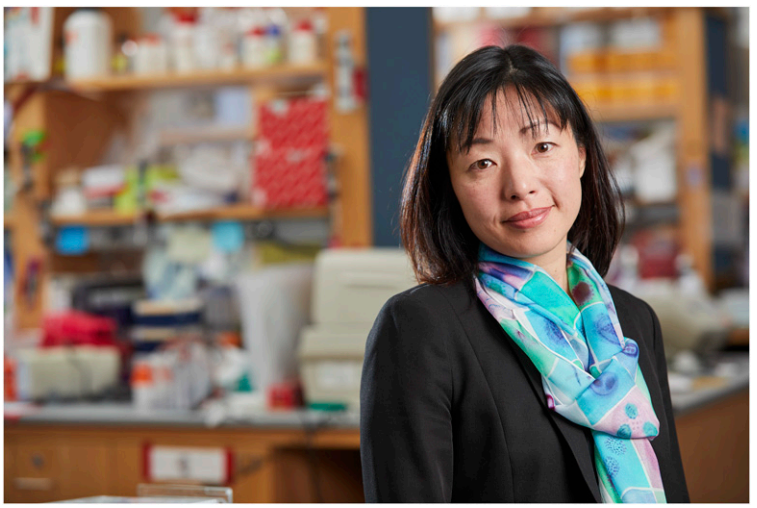
Akiko Iwasaki, an immunologist at the Yale School of Medicine and a Howard Hughes Medical Institute Investigator, has made significant contributions toward understanding innate and adaptive immunity. Her achievements include the demonstration of tissue-specific properties of dendritic cells (DCs), discovery of a pathway by which immune responses to viruses can be triggered, development of a mammalian model of a vaginal Zika infection, and formulation of the "prime and pull" vaccine strategy. The latter is being implemented in a clinical trial of a therapeutic vaccine for cervical intraepithelial neoplasia that is underway at Yale New Haven Hospital. Elected to the National Academy of Sciences in 2018, Iwasaki focuses on endogenous retroviruses (ERVs) in her Inaugural Article (1). She and her colleagues report the creation of the ERVMap, a tool for analyzing ERV expression from RNA-sequencing reads against a database of all known ERVs in the human genome.

Early Influences

Iwasaki was born and raised in Iga, Japan, where she and her two sisters enjoyed exploring nature. Her father Hiroshi, a physicist, and mother Fumiko, who fought for women's rights in the workplace, were her role models. Observing her mother's challenges, Iwasaki has become an ardent advocate for women and minorities in the sciences. Another early influence was her seventh-grade math teacher, Hajime Kajioka. She says, "What was so refreshing to me at the time was that someone outside of my family actually believed in me and my potential."

Having learned of career opportunities for women in North America, Iwasaki traveled to Canada to attend the University of Toronto, where she minored in physics and majored in biochemistry. During her third year at the university, she took an introductory immunology course taught by immunologist Brian Barber and was hooked. She says, "His lectures were eloquent, passionate, and convincing. It was impossible not to become excited about vaccines after his lectures." She joined his laboratory to do graduate work on DNA vaccines, earning her PhD in immunology in 1998. Her thesis elucidated a mechanism by which plasmid DNA vaccines induce immune responses (2). The findings garnered attention because they showed that such vaccines promote presentation of antigens to T cells through white blood cells, and not muscle cells, as was previously theorized.



Akiko Iwasaki. Image courtesy of Robert Lisak (photographer).

Formative Research

From 1998 to 2000, Iwasaki served as a postdoctoral fellow in the NIH laboratory of mucosal immunologist Brian Lee Kelsall, who specializes in the study of DCs within Peyer's patches. Iwasaki says, "He was just starting his own laboratory when I joined, and in addition to myself, there was one other postdoc and technician. This environment allowed me to develop like a seed in a small planter—lots of attention by the mentor, Dr. Kelsall, but still independent enough to explore and fail." With Kelsall, she clarified the roles of DCs and associated chemokines, with a surprising finding that DCs in the gut respond differently than those in other lymphoid organs (3–5).

After the postdoctoral stint, Iwasaki accepted a position as an assistant professor in the Department of Epidemiology and Public Health at Yale in 2000, and later in the Department of Immunobiology at Yale.

This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 12565.
Published online December 3, 2018.

www.pnas.org/cgi/doi/10.1073/pnas.1818903115

Insights on Autophagy and Toll-Like Receptors

Iwasaki and her colleagues identified the important role of autophagy, a cell biological process, and its machinery in innate recognition of viruses and antigen presentation by DCs. The researchers observed autophagy's importance in this regard while uncovering a pathway by which plasmacytoid DCs detect viruses (6). She says, "This pathway involves the use of autophagy to deliver viral genetic material to endosomes, where it can be recognized by the pattern recognition receptor, Toll-like receptor 7 (TLR7)." Iwasaki and her team subsequently analyzed mice lacking Atg5, a key autophagy gene, and determined that autophagic machinery, but not canonical autophagy itself, is used by DCs for optimal processing and presentation of microbes for the MHC class II (7).

Iwasaki has led seminal research on not only TLR7 but also other TLRs. She and her colleagues reported the first in vivo demonstration of TLR9 function, showing that it detects DNA viruses (8). They also helped to clarify bifurcation of TLR9 signaling, which primarily leads to transcription of either cytokine or type 1 IFN genes (9). The study found that plasmacytoid DCs have specialized lysosome-related organelles from which TLR7 and TLR9 trafficking and signaling stimulate IFN production. While TLRs often recognize viruses based on viral nucleic acids, Iwasaki and coworkers (10) established that a virus replication strategy itself can be a trigger for innate immune recognition.

Prime and Pull Vaccine Strategy

Iwasaki's team studies how adaptive immune responses to viruses are orchestrated at the site of virus infection. In 2009, she and her colleagues demonstrated that CD4 T cell help is important during the effector phase of CD8 T cell responses (11). She says, "This study revealed that effector CD8 cells are not by themselves able to enter infected tissue, such as the genital mucosa, and that CD4 T cells must serve as a pioneer to open access to such restricted tissues."

The findings inspired Iwasaki and postdoctoral fellow Haina Shin to design a two-stage vaccination strategy: prime and pull (12). A conventional vaccine is first given to prime the T cell immune responses, followed by a pull signal: application of chemokines to the target tissue. The second stage enables T cells to enter the tissue. The researchers created such a vaccine that successfully protected mice against genital herpes. Iwasaki says, "We currently have a clinical trial based on prime and pull to treat women with precancerous lesions in the cervix to prevent cervical cancer."

In 2014, Iwasaki and Norifumi Iijima, then a Yale associate research scientist, investigated how well T cells in circulation versus resident T cells already in a given tissue confer protection against viral infection, and found that the latter were more effective (13). The study demonstrated the need for vaccine strategies like prime and pull in establishing tissue-resident memory T cells for robust protection against viral challenges. In a follow-up study on antigen-specific memory CD4 cells, the researchers determined that circulating memory CD4 T cells promote local vascular permeability through the production of IFN-γ and allow antibodies access to infected neuronal tissue for viral control (14). Such transient access of antibodies to the brain holds promise for delivering biologics and drugs to treat infectious diseases and neurodegenerative conditions.

Pathways to Disease, Resistance

Noting that the elderly account for 90% of influenza deaths annually in the United States, Iwasaki and her colleagues analyzed influenza-infected human monocytes taken from subjects aged 20–89 years (15). The team showed that these immune cells exhibit reduced antiviral activity in individuals over 65 years of age (16). The research included analysis of influenza-infected mice, and found that two innate immune-sensing pathways work together to promote antiviral immunity against this disease. Mice lacking antiviral immunity, similar to elderly humans, often had elevated bacterial burdens in their lungs and increased inflammasome responses, which led to influenza-associated lethal consequences for some.

Iwasaki and her colleagues also led research on the human rhinovirus, and determined that mouse airway epithelial cells supporting rhinovirus replication initiate a more robust antiviral defense response when they are not subjected to cooler temperatures (17). The findings help to explain why rhinovirus outbreaks often occur during cold weather periods.

The Zika virus is additionally of interest to Iwasaki's group, in part, because it has numerous modes of transmission, including through sexual contact. To better understand the process, Iwasaki's team developed the first mouse model of a vaginal Zika infection (18). Using the model, the researchers showed that RNA virus sensors, TLR7 and RIG-I–like receptors, provide a detection mechanism that blocks viral replication in the genital mucosa. Even in immunocompetent mice, however, mother-to-fetus transmission of Zika virus occurred, leading to brain infection of the fetus. The follow-up study showed that the mother's immune response, rather than the virus itself, was causing fetal growth restriction and abortion in Zika-infected mice (19). Iwasaki hopes to study IFN and other cytokines at different times during pregnancy to further elucidate how microcephaly and other health issues associated with the Zika virus develop.

ERVMap

Up to 8% of the human genome consists of ERV DNA sequences versus the ∼2% dedicated to protein-coding genes. Given the prevalence of ERVs and their health impacts, both beneficial and detrimental, Iwasaki led efforts to create an ERVMap: a tool to map RNA-sequencing reads against a database of all known ERVs in the human genome (1). ERVs frequently are not annotated in human genome maps, so as part of the extensive project, Maria Tokuyama, an associate research scientist at Yale, manually curated a list of proviral ERVs identified by various research groups. The ERVMap revealed that each cell type within the human body has a unique ERV expression (ERVome; accessible at https://www.ervmap.com/).

Using the ERVMap, Iwasaki and her team found that many ERVs are highly elevated in peripheral blood cells from patients with systemic lupus erythematosus. The researchers also determined that ∼200 ERVs are elevated in breast cancer tissues. Iwasaki says, "We believe this tool is useful for the scientific community to find out exactly which ERVs are expressed in a given disease or cell type, for the purpose of studying them as antigens, biomarkers, or pathogenic agents."

Expansion into Cancer Research

Iwasaki has received awards from numerous medical associations, such as the Infectious Diseases Society of America Wyeth Lederle Young Investigator Award (2003–2005), the American Associations of Immunologists BD Biosciences Investigator

Award (2011), and the American Society for Microbiology's Eli Lilly and Company Research Award (2012). She is particularly proud of honors bestowed upon her by students, such as Yale's Charles W. Bohmfalk Teaching Award (2018) and the Inspiring Yale award (2017). Of the latter award ceremony, she says, "I was able to convey my passion in studying immune responses to viruses, and was blown away by the other inspiring speakers."

Iwasaki's team continues to study various aspects of antiviral immunity. Recently, they have begun to explore how immune responses of mothers affect fetal development, how ERVs are controlled by the immune system, and how the body detects and responds to cancer cells. "There are a lot of parallels—and differences—in immune response to cancer cells," Iwasaki says, "and we are excited to help tackle cancer in the next phase of our research."

1 Tokuyama M, et al. (2018) ERVmap analysis reveals genome-wide transcription of human endogenous retroviruses. *Proc Natl Acad Sci USA* 115:12565–12572.
2 Iwasaki A (1998) Cytotoxic T lymphocyte induction by plasmid DNA immunization: Mechanism and applications. PhD dissertation (University of Toronto, Toronto). Available at www.collectionscanada.gc.ca/obj/s4/f2/dsk3/ftp04/nq35195.pdf. Accessed November 20, 2018.
3 Iwasaki A, Kelsall BL (1999) Mucosal immunity and inflammation. I. Mucosal dendritic cells: Their specialized role in initiating T cell responses. *Am J Physiol* 276:G1074–G1078.
4 Iwasaki A, Kelsall BL (1999) Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* 190:229–239.
5 Iwasaki A, Kelsall BL (2000) Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3alpha, MIP-3beta, and secondary lymphoid organ chemokine. *J Exp Med* 191:1381–1394.
6 Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A (2007) Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* 315:1398–1401.
7 Lee HK, et al. (2010) In vivo requirement for Atg5 in antigen presentation by dendritic cells. *Immunity* 32:227–239.
8 Sato A, Linehan MM, Iwasaki A (2006) Dual recognition of herpes simplex viruses by TLR2 and TLR9 in dendritic cells. *Proc Natl Acad Sci USA* 103:17343–17348.
9 Sasai M, Linehan MM, Iwasaki A (2010) Bifurcation of Toll-like receptor 9 signaling by adaptor protein 3. *Science* 329:1530–1534.
10 Ichinohe T, Pang IK, Iwasaki A (2010) Influenza virus activates inflammasomes via its intracellular M2 ion channel. *Nat Immunol* 11:404–410.
11 Nakanishi Y, Lu B, Gerard C, Iwasaki A (2009) CD8(+) T lymphocyte mobilization to virus-infected tissue requires CD4(+) T-cell help. *Nature* 462:510–513.
12 Shin H, Iwasaki A (2012) A vaccine strategy that protects against genital herpes by establishing local memory T cells. *Nature* 491:463–467.
13 Iijima N, Iwasaki A (2014) T cell memory. A local macrophage chemokine network sustains protective tissue-resident memory CD4 T cells. *Science* 346:93–98.
14 Iijima N, Iwasaki A (2016) Access of protective antiviral antibody to neuronal tissues requires CD4 T-cell help. *Nature* 533:552–556.
15 Pillai PS, et al. (2016) Mx1 reveals innate pathways to antiviral resistance and lethal influenza disease. *Science* 352:463–466.
16 Molony RD, et al. (2017) Aging impairs both primary and secondary RIG-I signaling for interferon induction in human monocytes. *Sci Signal* 10:10.
17 Foxman EF, et al. (2015) Temperature-dependent innate defense against the common cold virus limits viral replication at warm temperature in mouse airway cells. *Proc Natl Acad Sci USA* 112:827–832.
18 Yockey LJ, et al. (2016) Vaginal exposure to Zika virus during pregnancy leads to fetal brain infection. *Cell* 166:1247–1256.e4.
19 Yockey LJ, et al. (2018) Type I interferons instigate fetal demise after Zika virus infection. *Sci Immunol* 3:3.