Zoonosis Control through Ecological Studies of Influenza Viruses

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As a principal investigator, I have received uninterrupted support from the Grants-in-Aid for Scientific Research (Kakenhi) program for projects in the categories of General Scientific Research (B), International Scientific Research, Scientific Research (A), and Scientific Research (S). Thanks to Kakenhi, we were able to lay the groundwork for the joint international research we are currently pursuing on the theme of zoonosis control. For that I am deeply grateful. As a crowning achievement of my experience with Kakenhi, I applied for a grant in the category of Specially Promoted Research. Although I had done my best to put together a convincing proposal, my application was rejected without even a chance for an interview. The explanation was that I was expecting to achieve more than seemed possible and that if I planned to pursue team-based research, other sources of funding would be more appropriate. It was at that point I decided to turn to those other sources.

In addition to Kakenhi, I have won research funding from a variety of sources. They include the Ministry of Economy, Trade and Industry's Millennium Project, the Ministry of Education, Culture, Sports, Science and Technology's (MEXT's) Global Centers of Excellence (GCOE) Program, the joint usage/research center program with the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), and the Japan Science and Technology Agency (JST). With that assistance, I have set up the Research Center for Zoonosis Control, pursued programs of education and research with the goal of overcoming zoonoses, and moved ahead with research aimed at assisting in the development of countermeasures against avian influenza, pandemic influenza, and seasonal influenza. These research and education programs have been modeled on influenza virus ecological studies and findings implemented with funding from the series of Kakenhi.

Through the 1960s, it was understood as follows. The seasonal influenza virus underwent changes in antigenicity (antigenic drift) year after year, major changes (antigenic shift) occurred every 10 to 20 years, causing pandemics, and antigenic variations were an outcome of mutations in the genes that code for the spike glycoproteins hemagglutinin (HA) and neuraminidase (NA) in the envelope of the influenza virus particle. After gaining

employment at a vaccine maker in 1969, I cultured and purified A/Aichi/2/68 (H3N2) (Aichi/68), the representative strain of A/Hong Kong/68 (H3N2) (HK/68) (commonly referred to as the Hong Kong flu virus in the news media), which caused an influenza pandemic in 1968. I suspected that this virus was not a mere genetic variant strain of the Asian influenza virus (H2N2) that had been observed up to that point in time.

In 1973, Robert Webster and Graeme Laver published a paper in a leading virology journal noting that the peptide maps of the HA2 subunit of HA in HK/68 and the corresponding subunits in horse and duck influenza viruses were almost identical. Upon reading that paper, I began mulling the possibility that animal viruses were involved in the appearance of pandemic influenza viruses. As someone who has studied veterinary medicine, I then decided that it would be my mission to elucidate the mechanisms behind the appearance of pandemic influenza viruses. In 1976, I left behind my company job to assume a position at Hokkaido University and launched an ecological study of animal influenza viruses.

On October 1, 1977, I isolated the strain A/duck/Hokkaido/5/77 (H3N2) (Dk/5/77) from the intestinal tract of a pintail duck that a hunter had shot in Moseushicho, a town located in Hokkaido's Ishikari River basin. Analysis revealed that the HA of the Dk/5/77 virus was closely related to the HA of A/Aichi/2/68 (H3N2), a pandemic influenza virus that appeared in 1968, and that the NA of Dk/5/77 bore a striking resemblance to the NA of A/Singapore/57 (H2N2), a strain that appeared in 1957. These data points suggested that at least the HA gene of this virus in wild ducks was the origin of that of the HK/68 virus.

Experimental infection studies established that this virus replicates in the columnar epithelial cells forming crypts in the colon of ducks, and that the surfaces of these cells have sialic acid-α2,3-galactose receptors that bind with avian influenza viruses. Influenza virus strains were readily isolated from migratory ducks that fly into Hokkaido from Siberia in the autumn. However, no influenza viruses whatsoever were isolated from ducks on their way to Siberia in the spring. In light of these findings, I felt that virological surveillance of duck nesting lakes in Siberia, Alaska, and Canada were needed.

Because MEXT had created the new Kakenhi category for International Scientific Research, I decided to submit an application for a project aimed at elucidating the ecology of influenza viruses and their mechanism of persistence in the natural world. The virological surveillance and studies in Alaska disclosed several findings, as follows. Migratory ducks are the natural reservoir host for influenza viruses. The viruses are detected in water samples from lakes

where ducks nest during the summer. Influenza viruses detected in fecal samples of virus-infected ducks retain their infectivity and are transmitted by water ingestion to other waterfowl species that share the same nesting areas with ducks. Furthermore, the viruses are preserved in those lake waters over winter in a frozen state to the following summer. Studies in Siberia yielded identical findings and demonstrated that the gene pools for avian and mammalian influenza viruses in Eurasia originate from influenza viruses found in Siberian migratory ducks. These surveillance projects in Alaska and Siberia each lasted four years, for a total of eight years combined, and were funded entirely with Kakenhi for International Scientific Research. On the research findings from studies in the two locations, only one paper each was submitted for publication in scientific journals. Original undertakings in basic research of this kind are possible only with Kakenhi funding, and are beyond the scope of project-type research. Kakenhi has become the only source of funding that allows researchers to pursue grand dreams in their proposals for research.

Areas of specialization seem to be further subdivided with every scientific advance. This pattern of segmentation has also extended to the Kakenhi program. It is not possible to provide research in every scholarly field with equal levels of support. Integrated approaches are more important than further segmentation. My own impression is that the Kakenhi program should strive to drastically expand its funding base and employ application reviewers that command the ability to effectively evaluate applications even on themes that are outside their own areas of expertise.