Large-Scale Grants Reshaped My Approach to Research

Shizuo Akira
Professor, Immunology Frontier Research Center, Osaka University

Research Theme Implemented in FY2016:
A comprehensive analysis of innate immunity (Grant-in-Aid for Specially Promoted Research)

Last year, I began receiving a Grant-in-Aid for Scientific Research (Kakenhi) for a project in the category of Specially Promoted Research. This is the second time in my career I am receiving assistance for work in this category. Large-scale projects in this category are eligible for grant funding on a scale of around 100 million yen.

Until going independent, I was putting in every possible effort to the work I was doing at the research labs I was affiliated with, and this work was highly praised. That background enabled me to set up my own lab, and from that point, as my life work, I began exploring themes entirely different from those I had pursued previously.

That said, opening up a new field of research is no easy matter. In 1996, the opportunity to set up my own lab arrived when I left Osaka University and became a professor at Hyogo College of Medicine. Fortunately, at that point in time, I had also just received a large research grant (from the CREST funding project). This allowed me to shift to a style of research I felt was a better fit for projects backed by large-scale research grants.

At that time, researchers had begun putting a lot of energy into making knockout mice, so I began mulling new themes in research that would involve utilizing knockout mice for screening purposes. Studies of mice at the individual level demand not only enormous amounts of research funding, but also special techniques and mouse-breeding equipment. Luckily, Hyogo College of Medicine completed a new facility for the breeding of knockout mice the year after I became a professor there.

As a research theme, I chose to investigate the molecular structure of macrophage differentiation. When stimulated by Interleukin-6 (IL-6), cells in the M1 leukemia cell line stop proliferating and differentiate into macrophages. I was interested in the molecular

mechanisms mediating that process. The MyD series of genes in M1 cells were already known to undergo early expression when stimulated by IL-6. We randomly knocked out these genes one after another and screened them to see if any would inhibit macrophage differentiation. Unfortunately, none of our knockout mice exhibited symptoms attributable to the inhibition of macrophage differentiation. One thing we did notice was that macrophages in MyD88 knockout mice were not stimulated by IL-1. However, because *in-vitro* and cell-culture experiments had already shown that the MyD88 protein mediates IL-1 signal transduction, our observation amounted to nothing more than a demonstration of this relationship in knockout mice.

Interestingly, though, one graduate student in my lab did accidentally discover that MyD88 knockout mice showed no reaction when injected with lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria. This discovery set the stage for our research group to move into a new field involving the identification of toll-like receptors (TLRs) that are pathogen sensors. This was a highly competitive new field that allowed no time to relax, but thanks to generous amounts of research funding, we managed to gain a slight edge over other labs involved in this research abroad.

Accordingly, over a period of about 10 years starting in 1996 and prior to any other research group worldwide, we were able to identify virtually all of the pathogenic ligands recognized by TLRs and also succeeded in elucidating the details of the signal transduction pathway for the TLR family of receptors.

In my first project in the Specially Promoted Research category (FY2008–2012), I drew from the accomplishments made to that point by research on pathogen receptors involved in innate immunity as well as their signal transduction pathways, and strove to develop a comprehensive analysis of innate immunity and shed light on the mechanisms behind the pathogenesis of infectious and inflammatory diseases. More specifically, to investigate the types of physiological phenomena triggered by the transduction of signals from pathogen sensors involved in innate immunity, we looked at genes rapidly induced by the stimulation of macrophages with the pathogenic ligand, lipopolysaccharide, knocked out interesting candidates, and identified their phenotypes.

This work led to our encounter with the genes Zc3h12a and Jmjd3. Through our analysis of knockout mice, Zc3h12a (which we later named “Regnase-1” in light of its function) was shown to be a molecule involved in the destabilization of IL-6mRNA and other specific

forms of mRNA that play a role in the inflammatory process. Jmjd3, by contrast, was shown to be a molecule essential to the differentiation of M2 macrophages following parasitic infection.

These two research findings served as the impetus for my second Kakenhi project in the category of Specially Promoted Research (FY2015–2019), which is engaged in work on two themes: primarily, the identification of the functions of the Regnase-1 and its family of genes and the sets of mRNA they target, and secondly, the identification of mRNA regulation mechanism in inflammatory disease and immune response as well as the macrophage subsets involved in a variety of diseases.

Since going independent some 20 years back, I have continued to engage in research with a focus on the breeding and utilization of knockout mice. From the outset, some doubted my style of research, given that it was less efficient than the forward genetic approach, which involved searching for genes behind the determination of features (phenotypes) that were thought to be inheritable. (I myself at times envied the forward genetic approach.) However, I have been content to stick with my own style because it did not depend on having a hypothetical basis, plus, it allowed for the possibility of unexpected research outcomes.

Recently, the CRISPR/Cas9 system has emerged as a new protocol for the generation of knockout mice. Its adoption affords researchers a way to dramatically speed up the mouse generation routine. I look forward to continuing with research work guided by knockout mice phenotypes.

To wrap up, let me urge that young researchers pursue original research on small themes rather than follow-up research on big themes.