The study of innate immunity in Japan: a historical perspective

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Abstract

Innate immunity is key for host defense in a vast range of organisms, from invertebrates to vertebrates. A number of Japanese scientists have made significant contributions to the clarification of innate immune mechanisms and have applied this knowledge to treatments for various diseases including cancer, allergy and autoimmune disorders. Here, we present an overview of the development of innate immunology by highlighting several Japanese contributors.

Introduction

The immune response is traditionally and conveniently categorized into two systems—innate immunity and adaptive immunity—although these systems interact and overlap extensively. The concept of innate immunity has its origin at the end of 19th century, following experiments on the larvae of starfish by a Russian microbiologist and zoologist, Mechnikov (1).

Adaptive immunity is based on recognition of antigen by diverse repertories of receptors. Antibodies are produced by B cells and their diversity is established by the somatic gene rearrangement system (2), which also works in generating the repertoire of TCRs, and enables two important features of the immune system in advanced vertebrates—adaptation and memory. Innate immunity plays critical roles in host defense in all plant and animal species and cooperates with adaptive immunity in the more-advanced organisms. To a large extent, the development of immunology has been driven by a desire to clarify the functions of adaptive immune cells rather than innate immune cells; however, it has become increasingly clear that innate immunity is more sophisticated than was once thought and crucially influences adaptive immunity.

It is obvious that immunologists all over the world have illuminated the mechanisms involved in innate immunity, but here we focus on the contribution of several Japanese immunologists to the topic.

Phagocytes: from Mechnikov’s time to the 21st century

The cells that Mechnikov identified in starfish can incorporate and digest invading micro-organisms. Cells that do this are called phagocytes and the engulfment phenomenon is called phagocytosis; this is just one of a wide range of mechanisms used in innate immunity.

Innate immune cells such as phagocytic macrophages can migrate toward lesions and are critically involved in establishing inflammation. In the 1960s, Hideo Hayashi at Kumamoto University characterized several factors that can attract various kinds of leucocytes. For example, leukoegreсин was identified as a chemotactic factor for polymorphonuclear leucocytes (3). These soluble factors are nowadays known as chemokines (4).

Phagocytes such as macrophages engulf not only microorganisms (non-self) but also self-derived apoptotic cells. In the 1990s and the early years of this century, Shigekazu Nagata (5), while working at Osaka Bioscience Institute and Osaka University, questioned how apoptotic cells are recognized by phagocytes and revealed the mechanism. He identified several molecules of phagocytes that recognize an ‘eat-me’ signal, which is expressed on the surface of dying cells. He further clarified how engulfed apoptotic cells, and especially self-derived DNA, are processed within phagocytes. The disturbance of this process can result in autoimmune disorders such as arthritis.

An important function of innate immunity in vertebrates is antigen presentation to T cells. This antigen-presenting ability is critical for linking innate and adaptive immunity. Antigen presentation depends on adherent non-lymphoid lineage cells and phagocytic activity. Therefore, macrophages had been...
considered to be major players as antigen-presenting cells (APCs). The pioneering work of Steinman and Cohn (6) at the Rockefeller University in New York showed APC activity in non-adherent and non-phagocytic cells, which are now called dendritic cells (DCs). Kayo Inaba and Shigeru Muramatsu (7) at Kyoto University identified non-macrophage cells as critical cell components for in vitro antibody response. Then Kayo Inaba, through fruitful collaboration with Steinman's group, contributed to clarifying DC functions. She revealed that DCs can be generated by an in vitro culture from bone marrow precursors.

Using innate immunity to combat cancer

Around the same time that Mechnikov described phagocytes, an American surgeon called Coley developed immunotherapy against cancer (8). He observed that a cancer patient who suffered from severe infection with *Streptococcus pyogenes* recovered from the cancer. On the basis of this experience, Coley succeeded in shrinking tumors by injecting a brew of *S. pyogenes* directly into tumors. This success led to the generation of the Coley vaccine that is composed of dead *S. pyogenes* and dead *Serratia marcescens* bacteria. It is now well recognized that production of tumor necrosis factor during the infection contributes to the curative effects on cancers.

Yuichi Yamamura is another pioneer in developing a therapeutic maneuver, based on innate immunity, for cancers (Fig. 1). He was mainly involved in clarifying the mechanisms by which *Mycobacterium tuberculosis* or related substances cause disease and in applying knowledge about mycobacteria to help treat cancers. In the 1950s, the pulmonary cavity in tuberculosis patients was considered to be generated by live mycobacteria, like a house being eaten away by termites. While working as a clinician at the Toneyama Hospital in Osaka, Yamamura demonstrated that dead mycobacteria or lipoprotein extracts from mycobacteria can induce cavities similar to those in patients with tuberculosis and that this cavity formation depends on T-cell-mediated immunity (9).

These findings can be now interpreted as follows: microorganism-derived molecular components, which include some Toll-like receptor (TLR) ligands (see below), can induce the cavity formation by stimulating innate immune cells to activate T cell-mediated adaptive immune responses. At Kyushu and Osaka Universities, Yamamura has further extended his studies by finding a potent macrophage-activating ability in the cell wall extracts of a mycobacterium, *Nocardia rubra*, and applying them for certain cancers. He also made a great contribution to establishing the Japanese Society for Immunology, which started in 1970, and organizing Fifth International Congress of Immunology in Kyoto in 1983.

Tohru Tokunaga et al. (10) at the National Institute of Infectious Diseases in Tokyo first found that immunostimulatory activity of the extracts from a *Mycobacterium bovis* strain, bacillus Calmette–Guérin (BCG), can be ascribed to the bacterial genomic DNA (Fig. 2). DNA purified from BCG inhibited tumor growth, augmented NK cell activity and induced type I IFNs from murine spleen cells and human peripheral blood lymphocytes. Then Tohru Tokunaga et al. clarified that the activity required the palindromic sequences including the 5'-CG-3' motif (11). They further found that the activity can be detected widely in DNAs from microorganisms and invertebrates, but not in DNAs from vertebrates or plants and suggested that this differential activity...
depends on the frequency of 5’-CG-3’ motifs (12). This work was performed before Krieg’s group reported that bacterial DNA containing the CpG motif can directly activate B cells (13). Thus, Tokunaga has illuminated the concept of immunostimulatory DNA, which now shows a great promise as an anti-allergic or anti-cancer vaccine.

**Evolving innate immunity: what Toll has told us**

Invertebrates do not have adaptive immunity, but this does not mean that host defense systems are undeveloped in invertebrates. Invertebrates have instead acquired an advanced type of host defense by evolving innate immunity, and Japanese investigators were heavily involved in delineating the innate immune responses in various invertebrates.

The horseshoe crab is a marine arthropod found most commonly in East Asia and North America. This arthropod, which has been called a living fossil, is exceptionally useful for biochemical analysis on proteins in the hemolymph because several hundreds of milliliters of hemolymph can be taken from one horseshoe crab without threatening its life. The hemolymph contains a single species of hemocytes that is extremely sensitive to bacterial endotoxin, LPS. In the 1990s, Sadaaki Iwanaga at Kyushu University clarified the molecular mechanisms of hemolymph coagulation cascades in the horseshoe crab (14). These cascades sense microorganism-derived components including LPS and (1→3)-β-D-glucan and are essential for immobilization of microbial pathogens, which are subsequently killed by anti-microbial peptides.

The limulus test is a sensitive LPS detection system widely used in experiments and clinical medicine and is based on the work of Iwanaga. It is to be noted that the components of the cascades exhibit homology to proteins that act upstream of Toll. Toll is a *Drosophila* transmembrane receptor that plays critical roles not only in insect development but also in anti-microbial immune responses (15). Invading micro-organisms are recognized by soluble factors in the hemolymph and protease cascades are subsequently activated. Then a host protein, Spaetzle, is cleaved and activates Toll-mediated signaling that leads to production of anti-microbial peptides. Iwanaga’s studies provided a framework for many cascade reactions in invertebrate innate immunity, which contributed later on the investigation of the *Drosophila* Toll system. Shunji Natori (16) at Tokyo University has isolated several other proteins that act in insect immunity, including anti-microbial peptides from the flesh fly, *Sarcophaga peregrina*, and, before the discovery of Toll acting on innate immunity and development, demonstrated that some insect immunity proteins have dual functions in defense and development.

In the 1990s, Janeway (17) at Yale University stated that effective immune responses require certain APC-activating signals in addition to protein antigens. Those substances are immune adjuvants and he called them the immunologist’s ‘dirty little secret’ in that article. Immune adjuvants are required for effective antigen uptake as well as for activating APCs.

LPS is a representative of the immune adjuvants and has been extensively investigated for decades; however, the critical signal-transducing receptors for LPS were not identified until the 1990s. In 1998, the responsible mutation for LPS refractoriness in mice was found to be located in the gene locus encoding Tlr4, which is structurally related to *Drosophila* Toll (18). This ignited the interest of lots of immunologists and microbiologists. Mammalian TLRs, which are now known to consist of ~10 members, have their specific roles in sensing infection by micro-organisms through the detection of microbial components such as lipids or nucleic acids. Since the end of the 20th century, Shizuo Akira, while working at Kyorin University, has clarified most of their functions and signaling mechanisms mainly through generation and characterization of knockout mice (Fig. 3, reviewed in this issue). These studies have also elucidated the molecular mechanisms behind adjuvants’ ability to activate immunity. For example, Akira’s great achievements include identification of the receptor for the immunostimulatory DNA which carries the unmethylated CpG motif (19).

**Conclusions**

Thus, a number of Japanese immunologists have contributed to the understanding of innate immunity. It is difficult and beyond our capacity to refer to all contributions and the achievements listed here are only the tip of the iceberg. We know that we have much more to learn but we hope that Japanese scientists will continue to play an important role, together with our colleagues worldwide, in making these discoveries.

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Abbreviations

APC antigen-presenting cell  
BCG bacillus Calmette-Guérin  
DC dendritic cell  
TLR Toll-like receptor  
TNF tumor necrosis factor

References