Microscopy as a powerful strategy for in vivo analysis of host defense mechanisms

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Host defense mechanisms including humoral and cellular immune responses have been actively investigated since the second half of the 20th century. Earlier investigations were carried out in vivo on various immunological phenomena. Many complicated immunological phenomena have been simplified into in vitro studies, resulting in a storehouse of theory and knowledge. Many host defense mechanisms, however, have not completely clarified and totally estimated in vivo yet.

I have been conducting research on the basic events of non-specific and specific host defense mechanisms in alimentary tracts in vivo. The alimentary tract constantly receives the various non-self substances; food antigens, occasional pathogens, and resident bacterial flora, etc. Non-specific host defense against these non-self substances are carried out by various biochemical, histological, and physiological factors. The acute renewal of mucosal epithelial cells is considered a factor of non-specific host defense. In chickens, the life spans of villous epithelial cells are 2-3 days in the small intestine, and 3-4 days in the large intestine. The villous epithelial cells throughout the chicken intestine undergo apoptosis during cellular migration and finally exfoliate into the intestinal lumen as well as in some mammals. Epithelial apoptosis might be induced by CD3+CD8+TCR2+ intraepithelial lymphocytes unlike in mammals. The clarification of the functional significance of epithelial apoptosis is presently progressing with development in knowledge of the specific host defense.

The specific host defense mechanism in alimentary tracts has conspicuous characteristics. The intestinal immune system, which is mainly constituted by gut-associated lymphoid tissue (GALT), reacts to orally-ingested non-self substances to initiate intestinal immune responses. In many cases, this intestinal immune response that finally results in specific secretory immunoglobulins secretion, and affects the other mucosal immune system throughout the body to form the common mucosal immune system. On the other hand,

orally-ingested antigens generally induce systemic immunological tolerance (oral tolerance) against the same antigens. This oral tolerance constitutes a beneficial system in the suppression of food allergies. Oral tolerance further induces systemic bystander suppression, i.e., the suppression of systemic immune responses against the other antigens which simultaneously and systemically invade tissue. These phenomena have been reported in several mammalian species. In avian species, however, the existence of these phenomena had been controversial. However, I have demonstrated both oral tolerance and bystander suppression in chickens.

In chicken intestinal immune response, Harderian gland (HD) also plays an important role. The absorbed antigens from intestines are delivered to the HD and initiate the secretion of antibodies into tear. The antibodies are transported to oral cavity and act as protective antibodies. Antibody-producing cells (plasma cells) which proliferate in the deep stroma, are induced apoptosis by CD3+CD8+TCR2+ lymphocytes and are finally engulfed by macrophages just beneath the primary or secondary duct epithelia in the HD.

The initiation of intestinal immune responses is generally formed by M cells in follicle-associated epithelia of mucosal lymphoid tissues. M is short for Membranous or Microfold . M cells are characterized by their dome-shape, the harboring of numerous intraepithelial lymphocytes, thick microvilli, and numerous small vesicles. M-cells function as transporters of luminal substances from the intestinal lumen to the lamina propria. Non-specifically absorbed luminal substances might be undegraded in M cells, and directly delivered to intraepithelial or lamina proprial antigen-presenting cells (macrophages or dendritic cells). The nature of M cells has been actively investigated in mammals, but their origin, lifespan, and fate are little known. Our findings indicate that the life span of M cells is within 4 days, and M cells are directly differentiated from undifferentiated epithelial cells of intestinal crypts and further differentiate into the general microvillous epithelial cells in chickens. As well, the follicle-associated epithelial cells undergo apoptosis during cellular migration and finally exfoliate into the intestinal lumen. Epithelial apoptosis might be induced by CD3+CD8+TCR2+ intraepithelial lymphocytes as well as in intestinal villi. In these experiments, three-dimensional observation under scanning electron microscope is the most effective, due to the impossibility of analyzing the complicated stream of the epithelial cells on the follicle-associated epithelium by two-dimensional observation.

Throughout my research, microscopy, including light and electron microscopy has always been the most effective strategy for the in vivo analysis of host defense

mechanisms. With the elucidation of the human genome, life sciences leading edges will take us into post-genomic era. Light and electron microscopy are indispensable tools for in vivo exploration. In the post-genomic era, the importance of microscopy will increase because the findings of molecular science must be reflected back to in vivo science.