Steps toward the New Horizons of Immunopathology Continuing from the Reach of My Research Career

MASAHISA KYOGOKU

Emeritus Professor, Tohoku University, Sendai 980-77

I started my academic life in 1954 soon after graduating from the Kyoto University Faculty of Medicine. After spending one year of internship at Kyoto University Hospital, I joined the Department of Pathology at Kyoto University as a graduate student. I started my scientific career with both practical training to become a clinical pathologist and basic studies to establish immunofluorescence techniques. I learned basic procedure of autopsy from professors Shigeyasu Amano and Yasuaki Nishizuka. They taught me a kind of central dogma in doing autopsies which can be summarized that a pathologist should try to show why and how the disease proceeded, after making a definite pathological diagnosis, what was it? In addition, I learned through the basic training of histopathology the fact that the appearance of cells was quite variable depending on their functional statuses, and that not only inflammations but all diseases were processes, not statuses. These notions mean that the final objective of pathology is to seek the cause of disease or pathogenesis, as we have tried to show in my earlier papers (Hamashima et al. 1956; Kyogoku and Hamashima 1959; Kyogoku and Maekawa 1960; Kyogoku 1966; Haebara et al. 1969; Kyogoku et al. 1972, 1977; Hattori et al. 1979). Since then this fundamental attitude has always existed as the basis of my academic life.

As a researcher, I joined the group of Dr. Yoshihiro Hamashima under the auspices of Prof. Kitasu Suzue. At that time Dr. Hamashima was directing a project to establish independently the methods of immunofluorescence. We chemically synthesized aminofluorescein, introduced an isocyanate group into the fluorochrome molecule, and conjugated it with a protein, in most cases immunoglobulins. After five years of innumerable failures, we finally succeeded to make it work (Hamashima et al. 1955, 1959; Mitsui et al. 1959; Kyogoku and Hamashima 1961; Kyogoku 1962). We went through the pains and joys of pioneers and had personal experiences to understand the Japanese proverb, “Failures are mothers of success” (success comes after many failures). All of the
information we acquired through this experience was described in detail and published in 1963 as the *Textbook of Immunohistology* (Hamashima and Kyogoku 1963). This account gained a good reputation, and two following editions were published (Hamashima and Kyogoku 1968, 1973), because it contained fully detailed descriptions of our own techniques, even including the pitfalls into which unexperienced researchers tend to fall. Thus, the first ten years of my research life was a period of basic training for me as an immunopathologist.

From 1961 to 1962 I had the chance to work at the Roswell Park Memorial Institute in Buffalo, N.Y. Under the supervision of Dr. David Pressman I developed techniques of radioisotope-labeled antibody methods, and applied the techniques in combination with my own immunofluorescence techniques to an analysis of the distribution in vivo of anti-hepatoma antibodies. The results of this experiment were published in journals such as *Cancer Research* (Kyogoku et al. 1964) and *Annals of New York Academy of Science* (Pressman and Kyogoku 1962). During this period, I also had a chance to observe Japan from the outside.

In 1963, soon after my return to Japan, I was given a chance to be a panelist at the symposium on the pathogenesis of rheumatic fever held at the 18th annual meeting of the Japan Rheumatism Association. At the symposium I reported the results of my analysis on how the antistreptococcal antibodies attacked heart muscle cells both in vitro and in vivo. Various immunopathological techniques which I had learned in the previous ten years were fully utilized in this presentation. The conclusion of this work was that the Aschoff bodies in rheumatic heart disease were a sequela of the type II allergic reaction (antibody-dependent cytotoxicity) resulting from crossreactivity of antistreptococcal antibodies to heart muscle cells (Kyogoku 1968; Kyogoku et al. 1975, 1976). The article describing the results obtained from this research project appeared in the *Acta Pathologica Japonica* (Kyogoku 1968) and I received an award from the Japan Rheumatism Association for this contribution. It was a milestone in my research career as a rheumatologic pathologist. I further continued this research project after my transfer to Kobe University. Many young researchers joined my new laboratory at Kobe University and helped in doing detailed in vitro experiments. Among them, Dr. Masato Nose was inspired with the importance of cell surface sugar chains during the course of our analysis on immunocytolysis of cultured heart muscle cells (Kyogoku et al. 1975; Koide et al. 1977; Nose and Kyogoku 1979; Nose et al. 1981). He still seems to be pursuing the same idea after having collaborated with Prof. Hans Wigzell at the Karolinska Institute to study the role of carbohydrate chains of immunoglobulins (Nose and Wigzell 1983; Ohuchi et al. 1984, 1986; Nose et al. 1987a, 1988, 1990a, b; Axberg et al. 1988; Heyman et al. 1988; Nose and Leanderson 1989; Matsuda et al. 1990; Mizuochi et al. 1990; Nose and Heyman 1990; Takano et al. 1990a, b; Wiersma et al. 1990).

As I mentioned above, I moved to Kobe in 1971 being assigned as Chairman
and Professor of the Department of Pathology, and had a chance to become a section chief of a nationwide project team under the auspice of Prof. Yuichi Shiokawa. The project team was established to perform pathological analyses of human collagen diseases, especially the polyarteritis or necrotizing arteritis, and malignant rheumatoid arthritis which is complicated with arthritis. We pathologically characterized malignant rheumatoid arthritis and classified the disease into five major groups: 1) systemic arteritis form, 2) peripheral arteritis form, 3) pneumonitis (interstitial pneumonia) form, 4) systemic infection form, and 5) amyloidosis form (Kyogoku 1975, 1977a; Sawai et al. 1981). The first group, the systemic form, is lethal, but the patients of the second or peripheral arteritis form survive longer despite a transient ischemic crisis of the limbs. Probably because of the guidance for diagnosis and treatment proposed by this working group, the number of lethal cases among the systemic form markedly decreased in the following years.

This project was taken over by Tohoku University when I was asked to accept the position of Chairman and Professor of Pathology in Sendai in 1978. Through the analyses of human arteritis we found the fact that apparently similar pathological lesions were caused by different etiopathogeneses. For example, arteritis in systemic lupus erythematosus (SLE) is characterized by the deposition of IgG immune complexes, those in progressive systemic sclerosis (PSS) by IgM immune complexes, and those in polyarteritis nodosa (PN) by fibrin deposits, but all of the arterial lesions look apparently similar to each other (Kyogoku 1977a, 1985, 1987c, 1988, 1989b; Sawai et al. 1981, 1987). In 1988 we published the Atlas of Vasculitides (Kyogoku) with contributions from the committee members, which was the first example of its kind in Japan.

In the same period Dr. Tomisaku Kawasaki, the famous discoverer of the Kawasaki disease, asked me to join his research group to explore the disease’s still unknown etiopathogenesis. Through the course of studies on this disease, Drs. Junichi Fujiyama, Mitsuyasu Kato, and myself became interested in the characteristically thickened intima of the coronary aneurysms. After extensive histopathological examination of autopsy cases of Kawasaki disease, we found that the coronary arteries in this disease were injured by infiltration of granulocytes and monocytes mostly coming from the adventitial side. However, in more severe cases vascular walls were also attacked from the intimal side. When the injury was not so severe, muscle cells of the tunica media were stimulated and started to migrate into the intima. Muscle cells proliferated in the intima and transformed to a synthetic form to secrete extracellular matrices including proteoglycans, collagen, and elastin. Consequently, very rapid intimal thickening occurred even after endothelization of the intimal lining (Kyogoku 1987b; Kato et al. 1989a). Several animal experiments performed by applying LPS on exposed femoral arteries of rabbit infants (Fujiyama et al. 1986; Kato et al. 1989b), and in vitro studies of cultured aortic smooth muscle cells (Kyogoku
1987b; Kato et al. 1989a, b; Kato and Kyogoku 1990) revealed that stimulated medial smooth muscle cells changed into a movable form, and under the influence of the platelet-derived growth factor (PDGF) and similar factors they change the phenotype from the contractile form to matrix-synthesizing form. Afterwards, other factors paracrinoid from non-proliferating endothelial or smooth muscle cells, and influence from contact with surrounding extracellular matrices such as heparan sulfate seemed to regulate the proliferation, and the muscle cells returned to their contractile form (Kyogoku 1987b; Kato et al. 1989a, b; Kato and Kyogoku 1990).

The results obtained from these studies were published in a book titled Kawasaki Disease (Kyogoku 1987b) and Annals of New York Academy of Science (Kato and Kyogoku 1990). Dr. Mitsuyasu Kato who was a major investigator in this project is now in Uppsala, Sweden, at the laboratory of Drs. Keiko Funa and Carl-Henrik Heldin to isolate a growth factor and its receptors involved in the phenotypic transformation of smooth muscle cells (Smits et al. 1991).

After my arrival at Tohoku University, we started a new research project on immunopathological analyses of rheumatoid synovitis with a special stress on the mechanisms of bone and cartilage destruction. Results obtained so far are as follows: 1) The initial histopathologic change at the beginning of rheumatoid synovitis is neovascularization of the synovial tissue. 2) Normal constituents of the synovial lining are the A cells of histiocytic nature that have microvilli and are full of lysosomes, and the B cells that are fibroblastic in nature being full of rough endoplasmic reticulum and metalloproteinases. Both cell types proliferate and are functionally activated at an early stage of the synovitis. However, maximal levels of proliferation seem to be limited to about 10 layers. 3) After the proliferation of the A and B cells, at least two additional types of cells, originally located in the perivascular zone of the underlying fibrous tissue, proliferate, and seem to move into the covering lining layer and ultimately replace the lining cells. We have proposed to call these cell groups DM and DF because they are dendritic (D) in shape, and are either macrophage-like (M) or fibroblast-like (F) in their ultrastructural and functional characteristics and immunohistologic phenotypes. Thus, DM cells contain lysosomes, and are Leu M1 (CD15) and Leu M3 (CD14) positive. On the other hand, DF cells contain metalloproteinases and express a mesenchymal cell marker (FU3). Morphologically both DM and DF cells are elongated in shape and possess microvilli. They both express MHC class II antigens DP, DQ, and DR strongly on their surfaces (Itoh et al. 1992). Therefore, it is quite difficult to morphologically distinguish DM cells from DF cells even by using an electron microscope. The amounts of proteolytic enzymes in D cells are smaller than in A and B cells. However, the total number of D cells are much larger than those of A and B cells. In addition, since D cells do not express decay-accelerating factor (DAF), an inhibitor of complement activation (Itoh et al. 1991a), inflammatory reactions can easily occur and perpetuate at the sites
where D cells are covering the synovial surfaces. D cells, through the aid of their abundant MHC class II molecules, can easily contact with activated CD4+ T cells, and are stimulated to release many different kind of cytokines such as IL-1α and β, IL-6, and TNFα. They also secrete superoxides. Thus, in the synovial tissue in rheumatoid arthritis, D cells are quite destructive to cartilage and bone tissues. Drs. Takashi Sawai, Kazuhiro Murakami, Shiro Mori, and Junpei Itoh were the major contributors to this project (Kyogoku 1985, 1989a, b, c, 1993a; Mori et al. 1988; Sawai et al. 1990; Itoh et al. 1991a, 1992).

The final research projects I was involved in were two studies on animal models of immunological diseases. One of these projects was a study on SL/Ni strain of mice. In 1974, when I was at Kobe University, Dr. Yasuaki Nishizuka of the Aichi Cancer Center asked me to join the research project to analyze the etiopathogenesis of systemic necrotizing arteritis and glomerulonephritis that were incidentally found in a substrain of SL mice. The SL strain was famous in Japan for its spontaneous development of lymphomas. We decided to call the arteritis-prone substrain SL/Ni. Drs. Rimpei Shimomura, Hirofumi Shirane, Masaji Kawashima, and Masaaki Miyazawa clarified that in this strain of mice an endogenous ecotropic retrovirus was expressed in arterial smooth muscle cells. In addition, circulating antibodies against retroviral gp70 envelope antigen, along with complement, induced immunocytolysis of the muscle cells, followed by insudation of serum immune complexes. Resultant necrotizing arthritis occurred in 30-40% of the mice (Kyogoku 1977b, 1980; Nose et al. 1980; Kyogoku et al. 1981, 1987, 1989a, b; Miyazawa et al. 1987).

The results of the virological and in vitro immunological analyses appeared in the *J. Exp. Med.* (Miyazawa et al. 1987). Since then, Dr. Miyazawa, the first author of this article, has been involved in the studies of immune responses against virus infections such as Friend virus-induced leukemia, AIDS, and murine lupus (Miyazawa et al. 1988a, b, 1990, 1992a, b, c, d, e; You et al. 1989; Chesebro et al. 1990; Ishihara et al. 1991, 1992; Mori et al. 1991, 1992; Robertson et al. 1991; Sugamata et al. 1992; Iwashiro et al. 1993). He spent more than 3 years under the supervision of Dr. Bruce Chesebro at the NIH Rocky Mountain Laboratories, and has organized this international symposium as the general secretary.

The other project on animal models was a study on MRL/Mp-lpr/lpr (MRL/lpr) and C3H/HeJ-gld/gld (C3H/gld) mice. Drs. Masato Nose, Hiroyuki Kanno, Satoru Takahashi, and Masao Ono found the following: 1) The *lpr* gene is just an accelerating factor that works on several background genes. The background genes are actually responsible for the development of glomerulonephritis, arteritis, and arthritis. These genes are probably located separately on different chromosomes (Kyogoku et al. 1987; Nose et al. 1987b, 1989a, b; Kanno et al. 1992; Nose 1993). 2) For the development of glomerulonephritis (GN) in MRL/lpr mice, deposition of IgG3 cryoglobulin is critical (Takahashi et al. 1991, 1992). Although many different clones of B cells are involved in the development of GN, each pathogenic
clone of B cells induces a single histopathologic type of the disease, such as membranoproliferative GN or wire-loop lesions (Itoh et al. 1993). Moreover, somatic mutation of immunoglobulin molecules seemed to be unnecessary for their nephritogenicity (Takahashi et al. 1993). 3) Granulomatous arteritis is another characteristic of MRL/lpr mice. The attacker to arterial walls is activated macrophages which are stimulated by lymphokines. They phagocytize large amounts of IgG immune complexes and make a large number of intracellular dense bodies without digesting them (Nose et al. 1987b; Kanno et al. 1993). Polyarthritis is also induced by angry macrophages with dense intracellular deposits, but the genetic factors determining susceptibility to arthritis seemed to be different from those determining the susceptibility to arteritis (Kyogoku 1985; Nose et al. 1989b; Itoh et al. 1991b; Nose 1993).

This project is now producing many publications, some of which have appeared in J. Immunol., Amer. J. Pathol., and in a monograph, New Horizons in Animal Models for Autoimmune Diseases, published by Academic Press (Kyogoku and Wigzell 1987). Dr. Takahashi, one of the members of this project, is now in Geneva under the supervision of Prof. Shozo Izui.

The information obtained from animal models of immunological diseases tells us that diseases of humans and animals develop basically under the same biological rules. This is the reason why diseases of an animal can be used as models of human diseases. However, there exists a definite species barrier between humans and animals which cannot be overcome, as easily understandable from the differences in size, life span, total number of cells and genes composing the body. Therefore, one should be very cautious to extrapolate the information obtained from animal models to the analysis of human diseases. From animal models, one can take only a few variables into the more complicated equations of human beings (Kyogoku 1985, 1987a, b, 1993b; Kyogoku et al. 1989a).

Finally, the following are our principles in how to approach human diseases (Kyogoku 1993b):

1) The key to unlocking the mystery of a disease must be hidden in the site of diseased tissue. Therefore, complete pathobiological analyses of the tissue specimen should be performed to give, first of all, a correct pathological diagnosis through objective observation (what is it?).

2) Then, thorough and logical analysis of the disease process from beginning to end should be performed (why and how did it happen?).

3) In vitro studies including tissue culture can sometimes follow. Generally speaking, in vitro studies are better to explore the native characteristics of cells and the influence of their microenvironment on them. However, one should notice that there is no guarantee that the cells will behave in the same manner in vivo.

4) Look for a suitable animal model to get more information about the human disease in question. After thorough investigation of the animal
model, even up to the molecular analyses of gene abnormalities, return the newly obtained information to the understanding of human diseases to solve their etiopathogenesis. In doing so, do not forget the genetic barrier between humans and animal models.

Coming closer to the end of this review of my history as a researcher, I should add something about the future. For my younger colleagues, this volume must be the starting point for their new approaches. For myself, at present I am interested in fibrosis and sclerosis of various organs, which are probably on the same line with the studies on smooth muscle cells. Organ fibrosis belongs to a group of intractable diseases that will become more common with the increasing population of elderly people in Japan, and thus will be more important in the 21st century.

As I reflect back on the 38 years of my career as an immunopathologist, I would like to express my appreciation to all the teachers, supervisors, friends, and coworkers who have given me so much help and support. If my career has been successful, I owe it entirely to them. To all the people who have made this symposium and this publication possible, and who have gathered in Sendai to attend this meeting from all over the world, I would like to express my sincere thanks. When I was appointed to the position of Associate Professor at Kyoto University in 1966, Prof. Suzue, who was the chairman of the department, gave me a Chinese poem. I have adapted it to express my feelings as I approach my retirement:

"I have come down the river on a boat with my colleagues, with the help of people nearby. I have made a step forward being confident of my way, making a narrow path through a jungle of immunological diseases."

I will continue to go forward, but from now on I will be taking the sidewalks instead of the busy streets, taking my time to enjoy the sights, picking tiny flowers named fibrosis along the way, and watching my own personal scientific garden grow and blossom.

References

International Kawasaki Disease Symposium, edited by T. Kawasaki. Japan Heart
Foundation, Tokyo, pp. 156–158.

the factors for the endothelial proliferation in repair of arteritis. J. Pharmacobiodyn.,
12, s-103.

25) Koide, N., Nose, M., & Muramatsu, T. (1977) Recognition of IgG by Fc receptor and
838–844.


Circ. J., 30, 1593.

28) Kyogoku, M. (1968) Experimental studies on the interaction between anti-A group


30) Kyogoku, M. (1977a) Pathological studies of malignant rheumatoid arthritis in
Japan. In: Vascular Lesions of Collagen Diseases and Related Conditions, edited by

nodosus. In: Vascular Lesions of Collagen Diseases and Related Conditions, edited by

281–294.


34) Kyogoku, M. (1987a) Present status of research on animal models of autoimmune
diseases in Japan. In: New Horizons in Animal Models for Autoimmune Disease,

suggestions about its etiopathogenesis. In: Kawasaki Disease, edited by S. T.

Mixed Connective Tissue Disease and Anti-Nuclear Antibodies, edited by R.

Tokyo.

38) Kyogoku, M. (1989a) Microenvironmental factors make connective tissue cells either
destructive or productive. Dermatologica, 179, Suppl. 1, 140.


40) Kyogoku, M. (1990c) Pathology of rheumatoid synovitis. Known and unknown in

1998.


44) Kyogoku, M. & Maekawa, Z. (1960) An autopsy case of bilateral cortical necrosis of


60) Miyazawa, M., Nishio, J. & Chesebro, B. (1988b) Genetic control of T cell responsiveness to the Friend murine leukemia virus envelope antigen: Identification of
class II loci of the H-2 as immune response genes.  

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