Biogerontology
DOI 10.1007/s10522-014-9518-z

INTERVIEW

Aging is something like an opera which can be constructed by many players: an interview with Katsuiku Hirokawa

Tamas Fulop

Received: 21 May 2014 / Accepted: 3 July 2014

I (TF) had the chance to meet Professor Katsuiku Hirokawa (KH) at a meeting in Rome in 1986 where he was talking on the thymus and immunological changes in aging. He made a great impression on me by his work, but also by his humanity and leadership. At the same conference, I presented a talk on the innate immune system, and Dr. Hirokawa then invited me to Tokyo. I went there for the first time in 1988 and had the chance to work with him and his extraordinary team. Everybody liked and respected him for his knowledge, leadership, rigor in science and humanity. He was a mentor for me and helped me to advance on this very difficult and fascinating road called the immune research in aging. Since that time, I have visited him about 20 times, and we have been collaborating in many aspects of the immunological research in aging. He is very modest but his influence in the field of gerontology and especially on the immunology of aging is huge. I hope that through this interview, which was started during our meeting in Tokyo in March 2013 and continued by sending him a list of questions to which he replied in a written form, you will discover the researcher, the medical doctor, the leader and the human being. I hope also that this will give the taste to enter in this field for many young scientists and grow his huge scientific heritage.

TF: How did you come to the study/research on aging?

KH: When I was a student in a senior high school, I had many questions in my mind, just like common young boys. One of the questions was the existence of human beings including myself. I was greatly interested in behavior, body movement, walking and facial expression of people. These bodily expressions were unique to each people and especially different by sex

T. Fulop (✉)
Division of geriatrics, Department of medicine, Research center on aging, Université de Sherbrooke, Faculté de Médecine et des Sciences de la Santé, Sherbrooke, QC, Canada
e-mail: Tamas.Fulop@USherbrooke.ca

Published online: 27 July 2014

and age. At the time of mimicry game, one could tell the name, just looking at the gait which a good player was intending to mimic. Facial expression of students is usually much simpler than that of teachers. Both teachers and students have a lot of problems in daily school life as well as at home. But generally speaking, problems are much numerous and complicated for teachers than for students. The difference can be explained by the life experience which is strongly affected by the time and the surrounding environment after the birth. The time which evenly affects all people can be referred to as aging, however the surrounding environment is different by place and time, and of course by people. Thus, the effect of time and the surrounding environment is different by each individual people, because stimuli are various, and the perception and the sensitivity are different for each people.

It is thus obvious that time/aging and surrounding environment have a great effect on the character formation of people. But at that time when I was a young student, I could not understand why and how aging and surrounding environment influenced the character formation including behavior, body movement, walking and facial expression.

In medical school, I read philosophical books of Descartes, Sartre, Heidegger, Japers, Husserl and Klages. Japanese medical students learned anatomical nomenclature in Latin and Japanese. Most of them also know the short famous sentence by Descartes, "cogito, ergo sum".

So, after I graduated medical school, I first tried to study clinical psychiatry. But after visiting psychiatric ward, face to face counseling with patients was not easy job in psychiatry. I considered other clinics such as internal medicine and surgery. Basically, clinical job is needless to say patient-oriented and physicians are requested to have service mindset. Basic sciences such as biochemistry and physiology were interesting for me. But clinical contribution was usually limited in these sciences. Thus, I finally decided to study pathology which appeared to be between basic science and clinical field. One of study materials in pathology was autopsy cases ranging from newborn to elderly people. First, I was greatly interested in ontogenic development of human body. I observed and studied a number of sub-serial sections of human embryos at different stages under microscope. Development of organs in embryo and fetus showed harmonious, systematic and beautiful morphologic changes and was quite impressive to me. I also observed human thymus of various ages from fetus to elderly people, as I was greatly impressed by Miller's report regarding immunological function of thymus in Lancet (1961). I found that the human thymus was a robust organ at the newborn stage and quickly became atrophic with advance of age. Actually this thymic involution was a big question throughout my life.

TF: What aspect of this research impressed you the most?

KH: When I was a young student, lymphocytes was regarded as non-functioning end cells. People did not know that lymphocytes could proliferate by proper stimulation until early 1960s. Miller's report was the first paper to show that lymphocytes and thymus played a major role in the function of the immune system. Until that time, major players of immune system were antiserum and macrophages. In 1901, Nobel Prize was given to von Behring who made antiserum to diphtheria with a Japanese scientist, Dr. Kitasato. As to cellular immunity macrophages were already known to play a role in the defense against infection, as reported by Mechnikov who received the Nobel Prize in 1908.

I started my research in early 1970s, when the thymus and lymphocytes just joined players of the immune system, although most of their functions were still unknown. In addition, tools for research works were limited at that time. But incidentally, in the late 1960s, rabbit polyclonal antibodies to human IgG, IgA and IgM were obtained from one of my friends. All these antibodies at that time were not easily available and very expensive. Using these antibodies, I examined human frozen fetuses, which were occasionally obtained at the time of abortion. I found immunoglobulin positive cells in thymus and in soft tissue around kidney area, which is now known as AOG region (aorta–gonad–mesonephros). The findings told me that the immune system was working early in the ontogenic stage. The results were published in a paper entitled "Immuno-cytochemical study on the development and differentiation of immune system of human fetus" (Acta Pathological Japonica 19:151, 1969).

Then, I examined human thymus by electron microscopic method and became greatly interested in the thymic stromal (epithelial) cells, embracing lymphocytes. I published a paper in 1969 on human thymus entitled "Electron microscopic observation of

human thymus of fetus and newborn" (Acta Pathological Japonica 19:1, 1969). I was impressed that thymic stromal (epithelial) cells closely related with thymic lymphocytes and the morphological findings strongly suggested an important role of thymic epithelial cells in the differentiation of lymphocytes in the thymic environment.

After graduation of post-medical course, I could fortunately move to the laboratory of professor Makinodan in Baltimore. Professor Makinodan gave me a chance to study the age-related change of thymic function using a mouse model. In this animal experiment, I learned thymectomy in young mice, whole body irradiation, and bone marrow transplantation for production of TXB mice, which had very small amount of T cells. Then, I transplanted thymic tissues under kidney capsule and observed how much T cells could recover in the spleen of TXB mice. In this system I could clearly observed that the ability of thymic tissue to produce T cells declined with advance of age of thymic tissue. This finding was reported In Journal of Immunology in1974. The paper was entitled "Thymic involution: effect on T cell differentiation". So, I can say that I started the study of aging from the immunological viewpoint in the laboratory of professor Makinodan. After coming back to Tokyo, I confirmed this finding and extended the experiments using nude mice with transplantation of thymus of various ages (Clin. Immunol. Immunopathol. 24:251, 1982).

TF: Did you have any influence by a master?

KH: Yes. As a matter of fact, professor Makinodan showed me the direction for my future research work; i.e., aging study in general. He showed me that aging was common to all living animals including worms and insects, and that the study of aging would open the door leading to solutions of many riddles in biology and medicine. He especially taught me that aging must be studied from various aspects including immunology, pathology, biochemistry, neurology and molecular biology. In addition to scientific (biological) field, the social field should be considered. As a pathologist, I have many respectable senior masters, but professor Makinodan is the most influential master throughout my life.

Further, professor Makinodan taught me how to start and promote research works. In his laboratory, the major subject was the study of the immunological aspects of aging. He knew that I was engaged in morphological study of the human thymus and advised me that thymus is the most appropriate subject for me. He told me that the most important function of the thymus was the ability to produce T cells, although the precise mechanism of T cell differentiation was not known at that time. But, it was possible to assess the ability of thymus to promote T cell differentiation in in vivo animal experiments. He did not care the techniques that we had in the laboratory. He told me that if I did not have the technique, I could go anywhere in the states to learn it, if necessary. So he showed me just the direction of goal of experiment and said to me "just do it".

TF: Do you think that the humanity of professor Makinodan was also influential? What was so particular in your encounter with professor Makinodan that you consider him as your master? What did you concretely learn?

KH: Needless to say, professor Makinodan is a great and visionary scientist. Science is a human activity being dependent not only on intellectual activity but also on a strong mental action. This is especially essential for the aging research. For instance, we examined the age-related change of IL-2 production of lymphocytes in vitro. It was a relatively simple study, but he asked me what the effect of IL-2 was when it increased in serum, especially in the aged. At that time, there were not so many people who considered the effect of cytokine on the nervous system. Nowadays, it is well known that many of cytokines originally produced by lymphocytes work also in the nervous system. The interaction between the immune system and nervous system is one of hot topics.

TF: What is your greatest achievement?

KH: The answer is made by a self-serving way. I don't know whether my research work I have done so far is great or not. I know that my research studies were neither glittering nor cutting-edge, but just steady. But, I greatly enjoyed doing the research work during my whole career.

The first achievement was the demonstration of the age related changes of thymic function to promote T cell differentiation by using a mouse model. Actually, this is the topic by which I started the aging research and continued for nearly 30 years extending it to

various related matters. At that time, from late 1960s to early 1970s, T cell receptor was not yet determined and there was still a strong opinion that T cell proliferation was promoted by thymic hormones such as thymosin or thymopoietin. In my experiment, I found that thymic epithelial cells greatly influenced the proliferation and differentiation of lymphocytes in the thymus. I could detect a thymic hormone (thymosin) in thymic epithelial cells, but could not obtain any evidence that the thymosin have something to do with the proliferation and differentiation of thymic lymphocytes. So, I just reported that the ability of the thymic tissue to promote T cell proliferation declined with the advance of age, although the mechanism of T cell proliferation was not known. Actually, precise mechanism of T cell growth and selection was found 10–20 years later, using transgenic or knockout mice.

TF: Do you think that your research helped to change and progress the understanding of T cell functioning?

KH: In 1960s and 1970s, there were not so many people who were interested in the aging of immune system and needless to say even less in the aging of thymic function. Even among researchers on aging, aging of immune system was not so attractive and remained like that. Probably, the immune system is too much complicated for understanding. In the field of Gerontology, oxidative stress is more charming as a cause of aging. In addition, limited cellular division with aging appeared to be conclusive to explain aging phenomena. However, when considering human diseases, we have to think about the immune system, because major human diseases such as cancer, infection and atherosclerosis are all closely related with aging of the immune system. Furthermore, it is possible to restore the immunological function to some extent.

To this aging of immune system, my researches on T cell aging and age-related thymic functions are closely related and may have contributed to its understanding.

Thereafter, I continued my research by making monoclonal antibodies to identify thymic cortical and medullary epithelial cells and analyzed their functions. Many thymic epithelial cell clones were established and they were classified into two types, cortical and medullary. We found that their morphology and functions were different between them. One of them is the antigen presenting function which was different between them. We reported a paper entitled "Medullary, but not cortical thymic epithelial cells present soluble antigen to helper T cells" in J. Exp. Med. (1992).

TF: How do you think that this could impact on further research?

KH: It is now well known that cortical thymic epithelial cells (c-TEC) and medullary thymic epithelial cells (m-TEC) are functionally different cell groups and play major role in positive and negative selection of T cells. This immunological function of the thymus works in the early stage of life, but nobody knows how this essential function changes with the progression of thymic involution. Most of recent immunological evidences have been obtained by using genetically altered mice such as transgenic, knock-out or know-in mice. But we have to consider that genetic functions can be altered by epigenetic modification. And this epigenetic modification increases with advance of age.

The second of my most important achievement is the quantitative assessment of human immune system that has been mainly performed after my retirement from a medical school. The research using animal model is very useful to confirm ideas and mechanisms of basic phenomena, but from a clinical point of view, the findings obtained in animal experiments can't be directly applied to human cases. For clinical purpose, we need human data. Since individual variation is very common in human, we need to examine a large number of people. In other words, symptoms and signs are different by individuals, although they are suffering from the same disease. So the same thing can be said about immune parameters assessed by using peripheral blood. One immune parameter assessed in lymphocytes greatly varies even in healthy peoples of the same sex and at the same age. So first of all, it is important to establish data base using a number of healthy peoples ranging in age from young to old, as described below.

TF: So do you recommend spending more research on immune aging in humans?

KH: Yes we need to do so, because the immune system is different between animals and humans. For the treatment of human diseases, we need information on immune system. It is quite obvious that the age-

related change of immune function closely related with occurrence and aggravation of various human diseases. When assessing human immunity, we confront a number of immunological parameters; the number of lymphocytes and their subpopulation, expression of surface markers of them, and their function of proliferative activity and cytokine production. Each parameter shows individual variation and this variation increases with age and by diseases. Thus, diagnosis and treatment of diseases in human should be done at individual level considering individual variations. For actual clinical practice we need complex system analysis. At any event, in order to combat human diseases we need to know and understand the aging of human immune system, more precisely.

Clinical doctors know that the immune system plays an important role in the occurrence of diseases and the maintenance of health, but it is still not easy to evaluate the level of the immunological functions in an individual patient. The reason is that the immune system is composed of many kinds of cells with different functions. We cannot wait for the solution by the complex system analysis. So for the time being at least, I tried to evaluate many immunological parameters, combine them and provide the level of immune function as a numeral for better understanding.

For this purpose, I examined various immune parameters in nearly 500 healthy people ranging in age from 20 to 80 years. Based upon the data base produced in this way, immune parameters could be given 3 scores. Data over 40 % in the cumulative frequency were given score 3, those between 20 and 40 % were given the score 2 and those below 20 % were given the score 1. In this scoring method, the immune parameters assessed were mathematically standardized and could be treated statistically. For instance, when 8 immune parameters are assessed, the perfect score is $3 \times 8 = 24$ and the lowest score is 8. So, the immunological score (IS) of people ranges between 24 and 8. Thus, we can define the level of the immune function or immunological score as a numeral between 24/24 and 8/24.

Then we further classified immunological score into five grades. Grade V: High zone (IS24), Grade IV: Safe zone (IS23-21), Grade III: Observation zone (IS20-17), Grade II: Warning zone (IS16-13), Grade I: Critical zone (IS12-8). In addition, we also made different scales called immunological age and lymphocyte age. By using these scales, the level of immunological status is easily understandable as "age" even for those who are not familiar with immunology.

We have already obtained more than 5,000 data from patients and healthy people. The first report based on human immunological data was reported for gender differences in immunological aging which was published in "Immunity & Ageing" in May, 2013.

TF: Can you please extend on 1. What parameters are assessed and why? 2. What can be the usefulness of such a score for the individual? 3. What can you do with such a score? 4. Do you think that this can be implemented in the everyday practice or much more need to be discovered before that?

KH: Immune system is so complicated and there are many parameters reflecting whole body immunological activity. Among many parameters, I have focused on those that are susceptible to aging process. Immunological data obtained are at levels of different quality or unit. They must be mathematically standardized by using the scoring method mentioned above. After scoring one can see the immunological level of the whole body as a numeral and so, it is easy to understand that whether it is low, moderate or high. After this process, the level of immune function can be understandable for even those who are not familiar with immunity. Thus, the immunological score is useful for daily clinical practice. As already mentioned, it is preferable to restore the decreased immune functions of the elderly or diseased people. In this case, the immunological score is useful to estimate the level of immunological restoration. As the level of immunological status is mostly parallel to the progress of a disease, the assessment of immunological score tells us the aggravation of the disease. Even in healthy people, immunological score shows circadian and seasonal change and an abrupt decrease can occur when exposed to stressful environment. Those who have inappropriate lifestyle habit generally have lower level of immunological score, but the situation can be improved by improvement of lifestyle.

TF: What do you think how your research contributed to unravel the mystery of aging? What is aging for you? Do you think that we are further than we were some 30 years ago in the understanding of aging?

KH: People are born with determined genes. After birth, various environmental stresses attack people,

causing chemical or structural changes of molecules composing our body, including genes. One of the powerful environmental stresses is oxidative stress including free radicals and ROS. Among a large amount of knowledge and information about aging, I would say that oxidative stress is one of the important cause of aging. The expression of genes can be altered by two ways. One is mutation and the other is epigenetic modification. Oxidative stress is one major cause for genetic mutation. Genetic mutation accumulates with age. Epigenetic modification of genes is another major event occurring and increasing with age.

In the last 30 years, our knowledge about aging is greatly advanced. But at the same time, the mystery of aging constantly increases in number. We could understand many aspect of aging, but many questions arise vastly upon newly obtained knowledge.

Now aging is not only the target of my research, but also the fact that I am now personally confronted with this phenomenon. Occasionally, I feel dizziness and pain on the back and legs. Actually, during the past 30 years, the number of the elderly people greatly increased. Many elderly people including me are complaining of various symptoms.

We have been expected to ameliorate the symptoms related to aging of the elderly people. For that purpose the immune system is very important. The immune system is related to the occurrence of cancer, infection and progress of atherosclerosis. In addition, the function of immune system is closely related with neuro-endocrine systems. This neuro-endocrine-immune collaboration influences the occurrence of numerous and variable indefinite complaints such as fatigability, general malaise, and pains at many sites, and so on in the elderly people. The most important point is that the immune function is partly reversible and it is possible to assess how much the level of the immune system can be reversed.

TF: Why do you think that we still have more than 200 theories of Aging? What is the most important obstacle to unravel the cause of aging?

KH: We don't need to unravel the cause of aging. What we need to know is the process of aging and it is quite obvious that the process of aging is different from individual to individual. That is a reason why we have more than 200 theories of Aging.

TF: Do you think that you were able to create a school of thinking? Where are your students now?

KH: Pathology is an area which always needs thinking in front of various specimens and phenomena. We are observing cells, tissues and organs together with clinical data, finally integrating all findings and data for the final diagnosis. I am taking the similar process in aging study. So even just observing a molecule or a cell, I am always thinking how this molecule or cell is working in an individual. Many young people studied aging with me following the same way of thinking. Unfortunately, not many students are now working in aging research, because positions are not easily available for aging study. I myself was and am still working as a pathologist, not as a gerontologist. I hope my students are studying and performing clinical job in the same way as they were working with me.

TF: What do you think would help to reverse this situation?

KH: As already mentioned, we need to have a viewpoint from the aging perspective for any type of researches. That will not be enough but maximum that we can do for the time being. Perspective or viewpoint is important for research work. For instance, a paper just describing data without describing starting point is not attractive. If a paper describes why authors start a research, it is interesting even if they cannot obtain positive data. When reading any papers that describe no aspect of aging, I am always adding a viewpoint of aging.

TF: What do you think why it is so difficult to attract young people in the field of research on aging? What we need to be more attractive?

KH: I myself, many years ago, gave up attracting young people to the field of research on aging. Most of young people go to areas where data are automatically obtained without much thinking. Fortunately, there is nothing which is not related with aging. Many researches, clinical or basic, are more or less related with aging. We need to show young researchers that they have to add the additional viewpoint of aging in their research whatever it may be.

Probably, a kind of school or seminar is useful. In the school young people can discuss together with seniors in aging research, spending many hours and days. So we need money for attractive aging school for

young people. In a seminar, we read some papers not dealing with aging. In any papers, we can find niche for aging viewpoint. We don't need to teach, but present a lot of questions or mystery in the research on aging, discuss with young people about the questions.

TF: When you were vice-dean do you think that you were able to implement such ideas? Do you think that we teach gerontology/geriatrics correctly at the undergraduate or postgraduate levels? Do you think that just reflect the thinking of the Society towards aging?

KH: Unfortunately, I could not do enough for advancement of aging research. I was in charge of lectures of pathology and immunology and taught important aspect of aging in pathology and immunology. But it was difficult to attract attention of established adult researchers to aging research. The reason is money for researches. Most established researchers are obtaining money by their own specific research activity that are usually not related with aging. In other words, in order to promote aging researches *per se*, we need to establish a big funding source. For that purpose, we need to arouse or promote interest of people or politicians to aging. If someone in the aging field get once the Nobel Prize, it will be quite effective to get attention of people to aging research.

TF: What is the future of the aging research?

KH: Aging research should encompass a broad range of biology or medicine. For instance, without knowledge of embryology, one can't smoothly perform aging research. In other words, every research field has both aspects of development and aging. I can say that recognition of aging aspect rises up the quality of research work in any field. So we should campaign that people should possess the aspect of aging in their research. If this campaign is successful, aging research should expand year by year. We should realize that everybody is subject to aging and aging brings about various backgrounds for many diseases. So we should devote more resources to the study of aging to better understand the age-related diseases and the study of the immunology of aging is an excellent pathway.

TF: So clinician should have much better knowledge on the aging process and chronic diseases associated with aging?

KH: Yes, all clinicians should learn aging in addition to special clinical field. Even pediatrician and obstetrician should do so.

TF: What do you think is aging reversible? Is there any future for 'anti-aging medicine'?

KH: Aging process is just ongoing with time. It is of great problem that many bodily functions are declining, including immune function. As to the immune function, it is fluctuating, day and night, monthly and seasonally. Observing many data that were obtained by immunological score mentioned above, the immune function is further fluctuating depending upon the progress of disease. It is clear that the immune function declines in association with the onset of diseases and is reversible with the recovery from the disease. There are many supplements which are easily obtained through many commercial routes. These supplements are effective in reversing immune functions in some people, but not in all people. The efficacy is different by people because of the individual variations. So it is essential to check the efficacy of supplements by evaluating immunological level before and after the intake. The most effective way of immunological rejuvenation is transfusion of activated T cells. Granulocyte can be increased in number by injection of G-CSF. B cells may be increased by bone marrow transplantation. But T cells are most susceptible to aging and decrease in number. Thus transfusion of activated T cells, after expansion *in vitro*, is useful for the reversion of T cell functional alterations with aging.

TF: Do you think that in a complex aging body increasing unilaterally the immune functions could be somehow harmful? Should we see the immune changes as harmful or adaptive?

KH: Any physiological systems in a body are constructed for maintenance of physiological harmony in the beginning. With advance of aging, however, pace or rhythms of many systems gradually mistime and discordance of harmony occurs, causing many kinds of diseases. Same thing can be said about the immune system. We know autoimmune diseases and some of them increase with advance of age such as rheumatoid arthritis and Hashimoto's thyroiditis. The most important point is that various and many kinds of autoantibodies increase in the serum with aging. Some

of autoantibodies may be helpful to remove damaged cells or tissues, but at the same time, this event causes mild inflammatory changes and gives rise to many indefinite complaints in the elderly people.

TF: What would you say that philosophically would change the society attitude to the aging research and ultimately towards elderly?

KH: Attitude of most people to a baby is gentle, expressing beautiful words and prospective smile. While in front of elderly people, people are generally kind with more or less negative behavior and try to avoid. However, people know very well that everybody will age in the near future. So, negative attitude is lying behind the society attitude to aging and aging research. Here we need to change thinking in the field of aging research.

Thus, we need education for aging. Subjects on aging must be included even in the elementary or primary school, because everybody ages and becomes elderly people, if not die earlier. Pupils should learn the development and the aging of the structure and the function of human body and many diseases which are caused by aging of cells, proteins, organs and tissues. I remember that I was asked to prepare a pamphlet to explain that thymic involution starts at very early phase of life. The pamphlet explains to pupils in junior high school what is thymus, what is immune system, how immune function declines with age, many diseases are caused by immunological decline, and nobody can avoid aging and death. Children can speak mother tongue without effort, because they learn language in the early phase of life. Some children know the death when their relatives passed away. So children should have a chance to learn a story of birth, growth, aging and death. We should have curriculum of aging for elementary school. Some pupils, if not all, discern the importance of aging in daily life and necessity of aging research in future.

TF: So, as a final word how would you encourage a young scientist to enter in the field of aging and more specifically to study immunology?

KH: Aging is a window through which people can understand human being. The landscape through a window could be exciting drama and changing by angles. It is not a simple molecule or cells or tissue, but a combination of them. It could be something like an opera which can be constructed by many players. The important point is the landscape which is very enjoyable.

Dear professor Hirokawa thank you very much for this exceptional testimony on your research, thoughts and perspectives on aging. I think we can draw the conclusion that even if you age you kept a young mind able to adapt, to innovate and being open to new ideas. Aging is a complex phenomenon and certainly immunity is playing an essential role in it. It is also clear that most of the researchers are not attracted, but if researchers like you are so inspiring the hope exists that they will be attracted. We hope and wish that for the health of the aging research especially for the immune aging. We wish you good health and strength to continue your research and bring us more interesting results in the field of the immunology and aging.