Potential and Problems of Alternative Research
-Alternatives, Biosafety and Ethics in Laboratories with Human Cells-

Selected Presentations from the Symposium in the 13th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments (November 13-14, 1999, Tokyo)

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Framework is Indispensable to Promote the Use of Human Material in Pharmacogenetics

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Public demands for the fair use of human tissues in research and development have been evident. The recent report from the Ministry of Health and Welfare (MHW, Kurokawa report) publicly announced the guideline on the use of human tissues obtained at surgical operations in mainly pharmacological studies. The report was the first official one in the area in Japan.

On the other hand, the growing information on the human genome in combination with the power of bioinformatics assists in developing novel strategies in pharmacological studies and industries. The use of genomic information and human material should be beneficial for the progress of pharmacogenetics. However, stable growth of the area requires 3 essential components, being, 1) establishment of an ethical and legal environment supporting the activities in Japanese society, 2) scientific studies supporting pharmacogenetics, and 3) the publicly supported repository (banking system) that provides us human material from the public. In this symposium leading scientists raised and discussed these issues.

In the scientific section, Dr. T. Kamataki presented alternative expression systems of human drug metabolizing enzymes, Dr. M. Hosokawa talked about the use of human cell lines in pharmacological studies, and Dr. S. Ozawa summarized the polymorphisms of human drug metabolizing enzymes.

In the ethical and legal area, there are two major problems; 1)handling of human material and 2)security of personal information especially genome information. Dr. H. Takebe talked on sampling and handling of human material and information in genome sciences. Dr. T. Matsumura summarized the efforts of the Committee on Ethical Affairs, the Japanese Tissue Culture Association in constructing ethical standards of handling human tissues and cells. Dr. R. Hirai, also a member of the committee, presented biosafety issues.

On the subject of public human material resources; Dr. H. Mizusawa of the Japanese Research Resource Bank, MHW, presented the cell bank system and its quality control issues. Dr. S. Suzuki, Human Animal Bridge, reported on its tissue banking system set up in cooperation with National Disease Research Interchange, USA. In addition, they also demonstrated their trials on the use of surgical liver specimens in pharmacological studies in collaboration with Dr. Yasuhara, Showa University, Medical School. The report revealed that the critical issue in obtaining reasonable specimens is the on-site quality control of the liver

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tissue.

Though at the meeting the use of human liver specimens from volunteer donors was a minor topic, its contribution to pharmacogenetics has been expected and thought to be indispensable. Because Japanese society has not been prepared in ethical issues, we should try to develop the public consent on the use of human materials in research and development.

From the end of the last year, the Japanese government took initiative in constructing and releasing guidelines on the handling of human material and associated information in biomedical research. We hope these guidelines will be well discussed and responsibly taken by the society. We hope the guidelines will lead the promotion of biomedical research under the public support. We researchers ought to take this chance to reinforce appeal the importance of human material in medical sciences and to cultivate our own motivation for the ethical issues.

In addition, the Organ Transplantation Act will be under review from October 2000. Because the Japanese population may have its own characteristics in drug metabolism, the verification systems for drug safety for the Japanese people would be indispensable. Academic and commercial domains should demonstrate the importance of the use of human liver specimens in research and development, especially in pharmacology and pharmacogenetics. The Kurokawa report announced the necessity of modification in the Organ Transplantation Act, opening up the possible use of liver specimens from brain-dead people in pharmacology. However if we researchers do not speak up now, the lawmakers may not realize the indispensable nature of human materials in protecting the health of Japanese people.

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Ethical Considerations on the Use of Human Tissues and Cells in Non-clinical Science, Technology and Practice

— From the Discussion at the Japanese Tissue Culture Association Committee for Ethical Issues —

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Background information

Increasing opportunities for researchers, technologists and practitioners of non-clinical professions to be exposed to tissues and cells of human origin have been giving rise to new situations in which mutual understanding between donor participants and users is essential in legal, ethical and safety issues.

Over the past ten years, attentions have been paid to these issues by the Japanese Tissue Culture Association Committee for Ethical Issues (JTCACEI) which consists currently of JTCA members; Tohru Hasumura M.D., Hiroki Hata M.D., Reiko Hirai Ph.D., Tohru Masui Ph.D., Keiki Sato, Ph.D., Motoko Shibanuma Ph.D., Noriho Tanaka Ph.D., Makoto Umeda M.D., non-JTCA member; Shin Utsugi Professor of Law, and chairperson; Toshiharu Matsumura, Ph.D.

Particular attention has been paid in JTCACEI to the fact that the availability of human biomaterial from domestic Japanese sources is very limited in both quantitative and qualitative terms in comparison with that in the United States and other western countries, while that related activities in science and technology as well as business are becoming more and more international, and therefore that Japan as a country faces a moral crisis in such a way that they rely much more on the supply of human biomaterials from donors in other countries than from those in its own country.

JTCACEI reports

For JTCACEI, it is an urgent neccessity to inform this situation to JTCA members as well as to the public, and to stimulate open discussion about the use of human biomaterials nationwide.

In 1995, it publicized an interim report on the consideration of the use of human biomaterials, and in 1999 it finalized the report, both of which are available on website (http://www soc. nacsis. ac.jp/jtca).

The essential tasks accomplished by the committee in these reports are summarized as follows:

1. Historical review on the development of science and technology based on human cells and tissues.

This includes the story of HeLa cells as the first established cell line of human origin and its immediate contribution in the detection of polio virus, the development of vaccines using cultured diploid human cells, and other historical notes showing the exponential expansion of knowledge and practical applications based

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on human biomaterials. It will not be necessary to go into detail of this section for the readers of this article.

2. Historical review on the development of regulations and resources for human biomaterials.

This includes mention of the disasters of the distribution of unsafe drugs before the initiation of human subject study, and the unethical experiments done on human subjects in the world war II, leading to the Declaration of Helsinki. Along with the ethical considerations, mention is made of the legal framework as represented by the establishment of the Unified Anatomical Gift Act in the United States and the Human Tissue Act in the United Kingdom, and the initiation of several cell and tissue banks such as CIMR and NDRI which form the basis of modern development of human biomaterial-based science and technologies. A particular situation in Japan exists in which human biomaterial resources on a national scale as well as legal frameworks have been very poorly developed in the past 50 years.

3. Basic points to consider as noted by the committee:

a. Difference from non-human materials: Although separated from the body, human tissues and cells are still bound to their donors in various ways including the wishes of donors or the families of donors. In many cases, detailed procedures for handling materials have not been legally specified. Therefore, it is important not only to consider the fundamental laws and ethics, but to pay attention to local opinions and customs as well as those of other countries.

b. Those in scientific and technological professions should recognize donor participants as equal partners.

c. It should be recognized that people have diverse beliefs concerning the relationship between the physical and mental aspects of the human body.

d. Injuring the human body can be criminal. Therefore, taking tissue and cell samples should best be done following specific laws to exempt special situations if such laws exist. It should however be recognized that there is currently an almost complete absence of such laws in non-clinical settings in Japan.

e. In circumstances where the law does not regulate, people have the freedom to act upon their own responsibility. Such action should proceed carefully, being accompanied by efforts to assists society to accept this action.

f. Safe handling: Human tissues and cells are best treated with the precaution that they may be contaminated with transmissible elements.

4. Recommendations by JTCACEI to those who handle human biomaterials:

a. Practice of reviewing: Handling human tissues and cells without a prior review should be avoided. Consultation and advice of those who are in independent positions, preferably forming an institutional review board, are essential.

b. Keeping records: Record keeping should be carefully practiced. If any problems arise, precise records will allow the quickest possible resolution.

c. Distinction between keeping secrecy and publicizing information : Privacy of donors and secrecy regarding intellectual properties should be protected. However, this protection should be carefully balanced with the right of the public to be informed of the use of human tissues and cells.

d. Adherence to the law, and establishing and publicizing self-regulatory rules in individual organizations : International recommendations such as the Helsinki Declaration should be respected, and the domestic laws adhered to. As to matters not specifically described in the law, self regulatory rules should be established in each institution, and these rules publicized. Such regulatory rules should not only be compatible with the existing domestic laws, but in harmony with social beliefs of the local region.

(1) Minimally handled tissues and cells, and cultured cell lines obtained during the course of scientific research should be provided to

users without monetary compensation, except for expenses such as handling fees. Exceptions may be applied to those with intellectual property right.

(2) While asking for free provision of tissues, cells and cell lines to legitimate users, those who receive such tissues, cells, and cell lines with industrial and/or commercial intention should establish their own rules for sharing a part of their profit, once obtained, with the donor or the providing entity in socially responsible indirect ways.

(3) It should not be prohibited for inventors or institutions to obtain intellectual property right on tissue, cells and cell lines which they possess.

e. It is recommended that laboratories engaged in human biomaterial research to be equipped with physical containment more strict than for an ordinary hospital laboratory, since people working there are often less trained than in hospitals for handling human biomaterials.

f. To ensure that human biomaterials are handled ethically and safely, it is recommended that public cell and tissue banks take part in all aspects of transaction involving donor participants and users.

Open discussion

In November 1999, after the publication of the committee report, an open symposium was held in Shimbashi, Tokyo, inviting a number of non-JTCA members as discussants with the intention of deciding whether the proposals made by JTCACEI were publicly acceptable. Among the discussors were Koichi Bai, Professor Emeritus of Tokyo Metropolitan University Law School, Mr. Toshihiko Kanzaki, Human Science Foundation, Kenzo Kato, MD, National Institute of Infectious Diseases, Yutaka Mizushima, MD, Professor and Member of Parliament, Kikuo Nomoto MD, Professor of Kyushu University and the President of Japanese Association of Transplantation, Ms. Tomoko Sakoda, Senior Reporter, NHK, and Mr. Chris Stuart, British Embassy Department of Science and Technology in Tokyo.

Although some lines of support were given in the symposium to the framework proposed in the JTCACEI report, several crucial questions were raised as follows:

1. How are self-regulatory rules ensured as being faithfully practiced? Do people in general rely on others regarding scientific and technological matters as much as has been indicated ?

2. It appears from the report that they (JTCACEI) admit profit from handling human biomaterials. Do they think that it can ever be acceptable by the Japanese Community at large?

3. The report stands on the point that handling human tissues for non-clinical purposes should be clearly distinguished from handling for clinical use. However for ordinary people in Japan, it may be very difficult to imagine that what is done in the hospital can be different from clinical purpose guidelines.

4. They are suggesting the use of human biomaterials for research and development in the broad field, and thus recommend the participation of public cell and tissue banks. However, the motivation of Japanese people at large is very oriented foward to those whom they know individually, rather than to the public as a whole. In fact, there are a great number of organ transplantations being done between living family members, while donors to the organ transplantation network are limited. People generally do not want to give their tissues and cells to those they do not themselves know.

Although it is apparent that not all people in Japan have adopted this attitude, there are still a large number for whom careful attention should be given along these lines.

Subsequently, JTCACEI divided its activities into two parts, one to discuss with ethicists and lawyers, the fundamental issues raised in the above meetings, and the other to remodel the committee itself so as to be composed of

those who actually are really interested in establishing public tissue banks for research and development. In September 2000, a workshop for this latter direction was held by the committee in Okayama, and at which current activities of several groups giving effort to develop public tissue banks were reported. The successful initiation of a tonsil tissue bank for research use in the National Childrens' Hospital was presented by Dr. Shin Enosawa, giving a firm hope that public resources of human biomaterials can be established with the support of donor participants (Enosawa et al., Tissue Culture Res. Comm. in press). Mr. Satoshi Suzuki, of the Human Animal Bridge, non-profit organization importing human liver from the National Disease Research Interchange to be used as gifts, and Dr. Ichio II, Asahi Techno Grass Company, importing primary cultured human cells from Biowhittaker Co. on a commercial basis, revealed their activities with particular attention to their ethical, legal and safety practices. To the author's knowledge, this was the first meeting in Japan in which those who handle human biomaterial in commercial companies were invited to join in an academic meeting and spoke in public.

On the other hand, an intensive discussion meeting was held in October 2000, as a joint effort of the JTCACEI and Jurist, a Japanese legal journal, in which insight into public opinion, the role of laws, governmental guidelines and self regulatory roles, and the participation of commercial companies, were discussed. The discussion paper appeared in February 2001 issue of Jurist.

During the past two years, a number of governmental committees were set up, and worked hard to provide reports in various fields in which human biomaterials are utilized. They will be appreciated for their efforts in maintaining ethical standards particularly in the government-supported research projects using human biomaterials. It is apparent, however, that still more continuous efforts should be paid to developing the fundamental laws, governmental guidelines, and self regulatory rules by which those who are involved in handling human biomaterials, both from the donor side and the user side, are protected.

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Bioethics of Human DNA Sampling

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Genetic information has been obtained through family analysis and with genetic markers such as enzyme activities. Recent advances in gene technology have given us a new methodology for human gene analysis, using DNA samples obtained from patients and clients, or from the general public. DNA can be extracted from human materials like blood, biopsy specimen, cultured cells and even from stored samples and specimens.

Bioethical aspects should be considered in collecting DNA samples. In Western countries, the topic has been considered and discussed in recent years, and the First International Conference on DNA Sampling was held in 1996 in Montreal. Guidelines and principles for collecting DNA samples were proposed by the World Health Organization (WHO) and the Human Genome Organization (HUGO). The author participated in the discussion in HUGO and the followings are proposals for adopting the principles to human gene analysis in Japan.

1. Standard informed consent must be obtained before collecting any DNA samples. Doctors should explain in detail of the purpose(s) of the test(s), possible outcome, what can be done on the basis of the results which may not be favorable for the clients, etc, before obtaining informed consent. When the purpose of the test is for research only, the clients should understand that there would be no direct merit from the test. The right not to know, particularly for the diagnosis of medical conditions without effective treatments, should be respected.

2. The donors of the samples should agree in advance whether the samples will be discarded after the tests, or can be stored and used for future research.

3. The donors should be asked in advance whether they would wish to be told of any unexpected accidental findings from their samples.

4. Samples are preferably to be coded, or to be anonymous. This would make the results of the tests impossible to be directly given to the donors. If agreed to be decoded and for specific information to be gien to the donors, mistakes in coding and decoding should be carefully monitored.

5. The archived materials which have been stored anonymously can be used for further research without renewed informed consent, unless otherwise agreed.

6. Special care must be taken for the transfer or leak of genetic information to third parties, who may use the information for insurance, employment, etc. We should note that such precaution has not been seriously discussed in

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Japan.

7. Genetic information of a person should be shared with the blood relatives of the person. In the international guidelines, blood relatives are entitled to be told of the genetic information of the person. Only in the U.S.A. (due to private health insurance) and in Japan has this principle not been supported so far.

The above mentioned principles may sound unfamiliar or troublesome to the medical communities in Japan. However, we should realize that these guidelines have been approved in international organizations and would become standard principles. Such standard has been already or would be required when researchers give or obtain human samples through cell banks or among researchers internationally. Some journals already require certificates of informed consent for the use of human samples. We should have our own guidelines which have the same basic principles as the international guidelines, with some modification. Such modification is likely to be needed, as understanding of human heredity is considerably different in Japan in comparison with the Western countries.

The issues outlined here may be the same in many other countries, particularly in Asia. The most important issue should be the conscience of doctors and researchers who are expected to have bioethical aspects in mind.

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Biosafety in Laboratories Working with Human Materials

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Abstract

The Committee of Ethical Affairs, Japanese Tissue Culture Association, proposes that the safety level in non-medical work places utilizing human materials should correspond to Biosafety Level 2 (BSL-2) or higher of the WHO standard. The materials should be tested for pathogenic microorganisms that require special attention such as HBV, HCV, HIV, etc., before experiments. Each institution should have its own biosafety standard. It is very important to keep records of both donors and users as referable forms in order to minimize a risk of unknown transmittable pathogens.

Biosafety in working with human cells and tissues is almost equivalent to that required in working with pathogenic microorganisms that infect or may infect human cells and tissues. When a material is clearly infected with a specific pathogen, the material should be handled at a biosafety level based on the virulence of the pathogen. In general, laboratory tests for pathogenic microorganisms that require special attention (HBV, HCV, HIV, etc.) should be performed before experiments to confirm the materials to be free from such pathogens. The materials should be regarded as "infected" until test results have been obtained. Even after obtaining negative test results, human materials should be handled under the assumption that they are infected with indigenous viruses, bacteria or mycoplasma that may be potentially virulent in the laboratory. All materials should be decontaminated properly by autoclaving or disinfection before discarding.

In contrast to medical facilities, the procedures and containment for experimental or investigational studies can be produced in advance so that laboratory staff are physically separated from pathogen-contaminated human cells and tissues. The Committee of Ethical Affairs, Japanese Tissue Culture Association, proposes that the safety level in non-medical work places utilizing human materials should correspond to Biosafety Level 2 (BSL-2) of the WHO standard (1) or higher. Procedures and practices at BSL-3 are recommended in cases where the infection of pathogenic microorganisms, which require special attention, is confirmed or suspected. The recently revised version of CDC & NIH BMBL (4th Ed.)(2) has an appendix, titled Working with Human and Other Primate Cells and Tissues, which describes basically a similar recommendation to the above.

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At present, we do not have legally recognized safety guidelines for experimental studies with pathogenic microorganisms such as those for radioactive isotopes or recombinant DNA studies in Japan. Therefore, each institution should have its own biosafety standard, provide good experimental equipment, determine procedures for safe operations and prepare preventive measures against accidents (education, training, infection tests, vaccination and storage of serum). The biosafety standard at each institution should fulfill the WHO guidelines. The Safety Standard for Pathogenic Microorganisms, the National Institute of Infectious Diseases (3), is a good example of Japanese biosafety standards for experimental laboratories. It is reasonable to determine the safety standard on the basis of each institution because criteria of biosafety change over time. However, we hope that a general biosafety standard for laboratories working with pathogenic microorganisms will be established in Japan.

The above discussion is limited to issues of safety concerning already-known pathogenic microorganisms. In laboratories, however, we have to be prepared for unknown pathogens because even the latest method of science and technology cannot detect all the pathogens present in human cells and tissues. In addition to procedures and containment at BSL-2 or higher, it is very important to keep records of both donors (clinical records or health conditions) and users (how human cells and tissues are used in laboratories) as referable forms, especially in case of accidental pathogen transmission.

References

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