Autologous typing: a tedious but orthodox approach for defining human tumor antigens with clarity

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When Toshitada Takahashi was in Nagoya, Japan, in 1972, one year after he left New York, he received a phone call from Lloyd Old, asking him to return to the Sloan-Kettering Institute (SKI). Dr. Old wished to organize a human tumor immunology group. By the early 1970s, tumor-associated antigens such as carcinoembryonic antigen (CEA) and α-fetoprotein had been defined by analysis of heteroimmune sera, and the association of Epstein-Barr virus (EBV) with Burkitt's lymphoma and nasopharyngeal carcinoma had been demonstrated. However, the most important unanswered question was whether tumor cells express antigens that are able to induce host immune responses, ultimately resulting in tumor regression.

Around that time, attempts were made by various investigators to define such antigens, predominantly by analyses of the reactions obtained by allogeneic combination of cultured tumor cells and sera and/or lymphocytes from patients. At SKI, the members of the cell-mediated immunity subgroup under Herbert F. Oettgen, Michael A. Bean, and Yoshihisa Kodera were already very actively working on melanoma patients. So, the newly organized virology subgroup of Gaetano Giraldo and our serology subgroup joined together to form the human tumor immunology group headed by Dr. Old, who was just promoted to vice president of Memorial Sloan-Kettering Cancer Center (MSKCC) and associate director of SKI. When Dr. Takahashi met Dr. Old for the first time in his office to initiate a group of human cancer serology in 1973, Dr. Old told him that there was not yet a textbook for human tumor immunology, and that they would be exploring a new research field.

There was a concern that analyses based on the allogeneic combination of cultured tumor cells and sera and/or lymphocytes from patients may result in detection of alloantigens, such as blood group antigens and histocompatibility antigens, rather than tumor antigens. Consequently, we decided to apply our approach of autologous typing with serological techniques; in other words, we restricted our analysis to the study of autologous reactions (reaction between sera and tumor cells from the same patient). We primarily chose analyses of serology rather than cell-mediated immunity because of clarity of specificity (1). We also had much experience, in the 1960s, in serology for murine immunogenetic research. For serological techniques, we used rosette assays, such as the immune adherence (IA) assay to detect complement-dependent antibody, predominantly IgG, and mixed hemadsorption assay (MHA) for IgG detection; both techniques are very sensitive and convenient ways to detect surface antigens on cells grown in monolayers, and both are superior to complement-dependent cytotoxicity microassays. The most difficult part of autologous typing was deriving cultures of target tumor cells from solid tumors, but we were very lucky to have Lois A. Resnick working as a technical assistant in our laboratory, a very hard worker with 'magic hands' for cell culture. Thanks to the careful arrangement by Dr. Oettgen, we were able to obtain and cultivate various types of tumors, and found that the highest success rate was with melanoma (~25-30%), with slightly lower success with renal cancer and glioblastoma (~10-20%). The success rates for other tumors were much lower, and we therefore chose these three tumor types for further study.

We conducted autologous typing of sera from 75 melanoma patients. Four patients were found to have antibodies identifying individually distinct tumor antigens (class I), and five patients had antibodies detecting shared tumor antigens (class II). Among class I, the AU antigen, defined by Thomas E. Carey (2), was further studied, since antibody titers residing in the IgG fraction were relatively high, up to 1/256 by MHA; however, characterization of the AU antigen by the conventional radioimmunoprecipitation technique was not possible. Instead, we used antibody inhibition assays to follow antigen solubilization and characterization. AU antigen is easily solubilized by papain and has a molecular weight in the range of 20,000-50,000; unfortunately, the gene encoding for this antigen was not isolated (3).

Among class II, the AH antigen was most extensively analyzed. This antigen was defined by Hiroshi Shiku from Nagoya, who joined as a member of the serology group (4, 5), while working with Eiichi Nakayama on Lyt-phenotyping of T cell functions. AH antibody was present in the IgM fraction of the sera of a melanoma patient who had remained alive for six years after resection of recurrent melanoma. This antigen has been found on 70% of melanomas and almost all glioblastomas, but not on normal cultured cells. Biochemical characterization of AH antigen was attempted, but it proved very difficult because of our limited knowledge and experience of membrane chemistry. We asked Kenneth O. Lloyd, from the Texas Tech University School of Medicine in Lubbock, to join as a member of the human tumor immunology group; he kindly accepted our offer in 1976. In 1982, Takeo Watanabe and Kenneth Lloyd demonstrated that the AH antigen molecule resides on a glycolipid molecule, GD2 (6). It is noteworthy in this context that analysis of mouse monoclonal antibodies against melanoma also demonstrated that GD2 and other gangliosides such as GD3 and GM2 are tumor-associated antigens (7). The order of immunogenicity of gangliosides in humans appears to be first GD2, then GM2, and last GD3, whereas in mice, GD3 is the
most immunogenic. Later, Philip Livingston and Herbert Oettgen carried out melanoma vaccine programs using gangliosides, such as GM2, as immunizing antigens (8). Massive efforts were made by Hiroshi Yamaguchi and Koichi Furukawa (7, 9) to produce human monoclonal antibodies from immunized and non-immunized patients; two such antibodies were identified: one detecting GM3, and the other detecting GD3. Both antibodies exhibited selective reactivity with tumor cells of neuroectodermal origin, particularly melanomas. As for the biochemical characterization of unique antigens, the first report by Drs. Furukawa and Lloyd appeared in 1989; that study demonstrated that a unique FD antigen epitope is carried on the common melanoma glycoprotein gp95/p97 (also known as melano transferrin) (10).

In addition to melanoma, autologous typing of glioblastoma and renal cancer was conducted by Michael Pfreundschuh (11) and Ryuzo Ueda (12), respectively. In the case of glioblastoma, sera from two patients (from a total of 30 tested) recognized class I antigens, while four recognized class II antigens. Interestingly, one of the class II antigens was found to be serologically related to the AH antigen of melanoma, i.e., GD2 antigen. In Ueda’s study of 28 patients with renal cancer, sera from one patient detected a class I antigen, and sera from three recognized class II antigens. Autologous typing of cryopreserved leukemia cells was also conducted by Thomas J. Garrett (13); sera from one patient detected a common acute lymphocytic leukemia (ALL) antigen. A similar study was conducted later in Japan by Kazuyuki Naito (14), who also reported a common ALL antigen.

Thus, these autologous typing studies clearly indicated the presence of tumor antigens that are able to induce antibody responses in patients. It is noteworthy that this approach laid the foundation for a novel serological approach, SEREX (serological identification of antigens by recombinant expression cloning), by Dr. Pfreundschuh and his colleagues, Ugur Sahin and Özlem Türeci, in 1995 (15). SEREX enables definition of tumor antigens from any tumor type. Autologous typing was also applied to T cell-mediated immunity by Alexander Knuth (16), leading to the establishment of killer T cell lines that allowed the isolation of tumor-specific antigen genes. More detailed stories about how autologous typing helped lay the groundwork for new methods to identify tumor antigens recognized by antibodies and T cells will be told later in this issue by Drs. Pfreundschuh and Knuth, respectively.

References

Afterword
Presented by Hiroshi Shiku at the service in remembrance of Dr. Lloyd Old on March 19, 2012, at Memorial Sloan-Kettering Cancer Center, New York, NY, USA.

Even several months after Dr. Old has passed away, it is hard for us to believe that he will never join us in person for meetings and fruitful discussions with his encouragement as we have been used to over many decades. However, we can still strongly feel the impact of his thoughts and ideas on our everyday decisions and our way of doing and organizing science and research.

Let me share with you some very personal memories I have of Dr. Old, our mentor in science and beyond. My memory of Dr. Old goes back to the 1970s, when I spent 7 years with him in the laboratory of Dr. Oettgen at the Sloan-Kettering Institute for Cancer Research in New York.

At that time, immunogenetic research based on extensive serological analysis of murine lymphocyte surface antigens was performed with Drs. Ted Boyse, Elisabeth Stockert, and others. This research was extended to studies on cellular differentiation markers. Segregation of killer T cells and helper T cells by differentially expressed Ly antigens is one example. This clearly promoted lymphocyte differentiation antigens as markers, as well as essential functional molecules of lymphocytes with diverse functions, which soon became a widely appreciated and indispensable concept in modern immunobiology.

Dr. Old also initiated a group of human cancer serology led by Toshitada Takahashi, whom he particularly assigned to this task. Analysis of cancer cell surface antigens by the use of patient's own cells and serum, an approach called "autologous typing," opened new avenues for the search of cancer-specific antigens and the understanding of anti-cancer immune responses with more clarity, while most other approaches were based on the use of allogeneic systems. The approach soon became a standard in human cancer immunology.

In 1973, Dr. Old was appointed associate director of the Sloan-Kettering Institute; every day he had to consecrate extremely long hours to administrative duties. It was for us a daily event when he visited each fellow bench by bench every evening to discuss the findings of the day. Fellows eagerly waited for their turn to talk with Dr. Old, appreciating his words of encouragement like patients waiting for rounds in the hospital.

More than 50 Japanese fellows studied in his laboratory, and over 30 fellows studying in other laboratories at SKI also had a chance to follow his lessons. He was in very close relationship with innumerable Japanese scientists, a fact that made him very influential on the scientific activity in Japanese cancer immunology. He visited Japan only once to deliver a special lecture at the 42nd Annual Meeting of the Japanese Cancer Association in 1983. His talk gave such a strong impact to all audiences that it is well memorized even now. Dr. Hashimoto, one of Dr. Old's earliest fellows, founded the Japanese Association of Cancer Immunology, where Dr. Old's many pupils gather once a year. It is hard to imagine how the society and its activity will persist without his presence. We plan to have a memorial symposium in his remembrance in early summer in Japan.

Dr. Old was an eminent scientist, the Nestor of contemporary cancer immunology, the father of cancer immunotherapy, and a teacher of thousands of scientists over the world. His interest covered an extremely broad area of cancer research, even beyond immunology. We will miss him, his style, his gentleness, and we will remember him not only as a scientist but also as someone who knew how to set priorities in life even beyond the scope of science.

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