Cell surface antigens: invaluable landmarks reflecting the nature of cells

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Although Lloyd J. Old was involved in various studies of the interactions between cancer and the immune system, it seems to us that his ideas often originated from the studies of serological identification of mouse lymphocyte antigens. The findings from these studies allow us to distinguish cells of different lineages and differentiation stages, and also to distinguish leukemia cells from normal lymphocytes (1). It is amazing that essentially a single serological technique, i.e., the Trypan blue exclusion test (presumably introduced in Peter Gorée's laboratory by Edward A. Boyse), was used to define a series of these antigens. This technique was used in conjunction with absorption analysis to elucidate the specificity of antisera, based on a vast knowledge of mouse immunogenetics. In the laboratory, Elisabeth Stockert (who was a technical assistant at that time) was the great master of these serological techniques, carrying out her own projects of cell surface antigens, as will be described below, while managing the day-to-day business of the laboratory. Similarly, Elizabeth A. Carswell and Gayla Geering investigated tumor necrosis factor (TNF) and retrovirus-associated intracellular antigens, respectively, while working as technical assistants.

When Toshitada Takahashi left Nagoya, Japan, for New York in 1968, the TL and Lyt (Ly) series of antigens of thymocytes and peripheral T cells had already been identified by Drs. Old, Boyse, and Stockert (1–3), and congenic strains of mice for each antigen system had been established. Contemporaneously, Katsuaki Itakura was preparing to initiate genetic linkage studies of these differentiation antigens. In the late 1960s, the concept of T cells and B cells was becoming more and more widespread, and after the First International Immunology Congress in Washington, D.C., in 1971, this concept became widely accepted all over the world. The Thy-1 (Θ, CD90) and Lyt series of antigens are considered to be markers for thymocytes and peripheral T cells, whereas TL is a marker for thymocytes. Thus, Takahashi's project was focused on B cells. When he started his study on cell surface antigen analysis of plasma cell leukemias (PCL), Yoshiyuki Hashimoto kindly taught him how to carry out day-to-day experiments. At that time, Dr. Hashimoto was a visiting investigator from the Biochemistry Institute in Tokyo, Japan, working on cell-mediated immunity against TL-positive leukemias. It is noteworthy that, as the founder of the Japanese Association of Cancer Immunology, Dr. Hashimoto greatly contributed to the progress in tumor immunology in Japan. Dr. Takahashi defined a novel PC.1 alloantigen that is expressed on PCL cells and mature antibody-forming cells (IgG type hemolytic plaque-forming cell, IgG-PFC), but not on peripheral B cells (4). Furthermore, he demonstrated reciprocal expression of surface immunoglobulin, which is expressed on peripheral B cells, but on neither IgG-PFC nor PCL cells. We had to wait until 1973 to see the first report of a B cell alloantigen by Hidetoshi Sato (5), although it is named Lyb-2 (CD72). This protein was later shown to be a ligand for Lyt-1 (CD5) and appears to be present on all B lymphocytes except for plasma cells.

In 1973, Hiroshi Shiku, also from Nagoya, went to the Sloan-Kettering Institute for Cancer Research (SKI) and started working on cell-mediated immunity with Herbert F. Oettgen and Michael A. Bean. Although the chromium release assay had already been established by Cerottini et al. and was widely used to target suspension cells, this assay was not always suitable for monolayer cell cultures derived from solid tumors. In order to detect killing activity against attached target cells, Dr. Bean developed a new micro assay using [3H]proline for labeling, instead of chromium 51; this assay was used by Dr. Shiku for his phenotype analysis of T cell subpopulations. A very exciting result was obtained in 1975, namely, that the killer T cell population is relatively rich in Lyt-2/3 (CD8) surface antigens, but relatively poor in Lyt-1 antigen, suggesting that T cells with different functions could be distinguished on the basis of their Lyt phenotypes (6). Subsequently, Eiichi Nakayama clearly demonstrated selective blocking of cytotoxic cells by Lyt-2 and Lyt-3 antisera in vitro in the absence of complement, suggesting that Lyt-2/3 determinants on the surface of cytotoxic T cells have a close spatial relationship to the T cell receptor (7, 8). Furthermore, it was shown later by Drs. Nakayama and Akiko Uenaka in Japan that in vivo administration of Lyt-2/3 antibodies is able to block the cytotoxicity of killer T cells responsible for tumor rejection (9). Thus, Lyt-2/3 was demonstrated to be not merely the marker of killer T cells, but a molecule that is important to their key function. As for the phenotype of effector cells (Th1) and regulatory T cells (Treg), Shimon Sakaguchi and his colleagues demonstrated in 1982 that both populations express Lyt-1, but not Lyt-2/3, i.e., the CD4 phenotype (10, 11); they accomplished this by analysis of an organ-localized autoimmune disease model that develops in mice thymectomized on day 3 after birth. It is now well known that CD8 and CD4 molecules are involved, respectively, in the interactions between T cell receptor and target antigen peptides presented on major histocompatibility complex (MHC) class I and class II molecules. This research laid the groundwork for identifying the cell surface markers that allow cells to be experimentally and diagnostically separated and distinguished.
revolutionizing immunology and medicine as it is practiced today.

Unlike the Lyt series of antigens, the TL antigen system has unique characteristics, e.g., TL expression is restricted to thymocytes in certain (TL+) strains of the mouse, indicating a character of differentiation antigens, whereas TL appears in a proportion of T cell leukemias (lymphomas) developed even in TL- strains that normally never express TL during fetal or adult life; these can be regarded as leukemia-specific antigens. Yuichi Obata, who worked with Dr. Old for 12 years, succeeded in 1985 in cloning genomic TL genes as members of MHC genes of chromosome 17 (12). These may be regarded as the first set of cloned genes encoding tumor antigen; the P815 mast cell tumor antigen gene, P1A, was reported by Boon's group in 1991. In 1985, Dr. Obata then returned to Japan to be a staff member at the Aichi Cancer Center Research Institute in Nagoya, where he continued his TL project. One of the interesting findings obtained thereafter is that when a chemical carcinogen (NBU) was administered to C57BL/6 and C3H strains, T cell leukemia development was slower than in T3B-TL gene-transduced counterpart strains expressing TL ubiquitously as self antigens, suggesting that anti-TL immunity may play a protective role in immune surveillance (13). Another important finding is that immunization with TL-positive skin from T3B-TL transgenic mice produced cytotoxic T cells exhibiting TL- (but not H-2-) restriction (14). This indicated that TL antigen is a transplantation antigen, although in the 1970s it was considered to be a serologically defined antigen.

In the late 1960s and early 1970s, cancer research focused on viral carcinogenesis, since the Epstein-Barr virus (EBV) had been discovered as a candidate for human tumor virus, while retroviruses such as murine leukemia virus (MuLV) and murine mammary tumor virus (MTV) had been shown to be associated with development of murine leukemia and mammary tumor, respectively. In Dr. Old's laboratory, cell surface and intracellular antigens of MuLV and MTV were extensively studied, and many antigenic systems were defined. Particularly, detailed analyses of the relationship between gp70 of MuLV classes and cell surface antigens were conducted by Drs. Stockert and Obata (15-18). Four antigens were defined as markers for each of the MuLV classes: GIX and G(RADAI) distinguished two types of ecotropic MuLV; G(ERLD) identified all xenotropic MuLV; and G(AKSL2) served as a marker for dual-tropic viruses in high-leukemia strains such as AKR mice. GIX antigen has characteristics of a differentiation antigen and is expressed on thymocytes in low-leukemia strains such as 129 mice; a linkage study conducted by Hisami Ikeda demonstrated that the antigen expression is controlled by linkage group 8 on chromosome 4 (19). During the course of these studies, Kouhei Kawashima obtained an interesting finding: thymocytes exhibit amplified expression of gp70-associated antigens during the late preleukemic period in the AKR strain, which is accompanied by the emergence of MuLVs with dual-tropic (but not ecotropic) properties (20, 21), leading to the belief that dual-tropic MuLVs are the proximal vectors of leukemogenesis in the AKR strain.

In addition to the studies of leukemia, an attempt was also made to detect individual antigens of chemically induced mouse sarcomas by use of the complement-dependent cytoxicity microassay. The presence of such antigens was demonstrated in the 1960s by in vivo transplantation experiments, but the nature of the antigens was not well understood. In 1977, Albert B. DeLeo produced antisera that detected antigens with an exceedingly restricted distribution on two BALB/c methylcholanthrene-induced sarcomas, Meth A and CMS4 (22). During the course of these studies, he defined a transformation-related intracellular antigen of sarcomas and leukemias with a molecular weight of 53 kilodaltons (23); this antigen is now known to be the product of the p53 tumor suppressor gene. Two genes encoding for individual transplantation antigens detected by cytotoxic T cells were subsequently isolated in Japan from different tumors: RAMP (exon extension mutation) from Meth A, by Dr. Nakayama in 2003 (24), and ERK2 (point mutation) from another sarcoma, CMS5, by Hiroaki Ikeda et al. in 1997 (25). To date, the relationship between these gene products and the serologically defined antigens has not been elucidated.

Here, we have only introduced a part of the mouse cell surface antigen studies initiated at SKI, and briefly discussed their further development in Japan. It is noteworthy that not only genes encoding these antigens, but also fertilized eggs of the pertinent congenic and transgenic mouse strains are maintained even now at the BioResource Center of Riken Tsukuba Institute (director, Dr. Obata), and are available to researchers interested in this field.

References


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