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This Week's Citation Classic

Mittal K K, Mickey M R, Singal D P & Terawki P I. Serotyping for homotransplantation. 18. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation* 6:913-27, 1968. [Departments of Surgery and Biomathematics, University of California, Los Angeles, CA]

The paper presents a microtest method for human leukocyte antigen (HLA) typing and tissue matching of human donors and recipients of organ or tissue transplants. The method was made simple and highly reproducible through critical evaluation of each step of the test procedure. [The SCI® indicates that this paper has been cited over 625 times since 1968.]

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"In order to comment on the wide use of the human leukocyte typing method reported in this paper, it is important to mention some of the prior history of the HLA field. Soon after the discovery of the first alloantigen on leukocytes in 1958, a number of investigators attempted to resolve the degree of polymorphism and the mode of inheritance of these antigens. However, they encountered great technical difficulties with the reproducibility of the then current leukoagglutination methods.

"In 1964, Dr. Paul Terasaki made a major contribution to this newly emerging field by introducing a microcytotoxic assay for detection of these antigens.[1] Among several advantages, this method eliminated the risk of those false positive reactions which resulted from spontaneous agglutination of leukocytes in the leukoagglutination methods.

"About this time the investigators in this field initiated joint leukocyte-typing workshops. One such workshop was held in Torino, Italy, in June 1967. An interesting feature of the workshop data was that in certain of the eleven families tested, it appeared as if an offspring had reacted positively with a typing serum, while both parents of that offspring had produced negative reactions with the same serum.

"I first met Terasaki when I was at the California Institute of Technology. Because of my interest in genetics, I was fascinated by the anomalies in the workshop data. Hoping naively that there might exist some 'hybrid' antigens (similar to those in rabbits) which are expressed only after appropriate alleles come together in an offspring, I came to Terasaki's laboratory to explore the phenomenon further.

"By performing rigidly controlled family studies, it did not take us long to discover that what we had suspected to be 'hybrid' antigens were in fact a consequence of variations in the methodology, and in the reactivity of cells from different donors. The need for a highly reproducible tissue typing method thus became quite apparent. A year's intensive effort at evaluating and refining every single step of the lymphocytotoxic test produced this paper.

"A number of modifications of this test have since been attempted; however, the great simplicity and high reproducibility of this method have resisted any significant alterations in the procedure. Its use has assisted in the identification of over 50 alloantigenic determinants of the HLA system. HLA typing is now being applied increasingly not only to organ or tissue transplantation, but also to platelet transfusions, and the prognosis and/or diagnosis of certain diseases. This recently increasing cljnical application of this test has finally led to its standardization[2] by the Food and Drug Administration of the US Department of Health, Education and Welfare."

1. **Terasaki P I & McClelland J D.** Microdroplet-assay of human serum cytotoxins. *Nature* (London) **204**:998-1000, 1964.
2. **Mittal K K.** Standardization of the HLA typing method and reagents. *Transplantation* **25**:275-79. 1978.