CLINICAL HISTOCOMPATIBILITY TESTING

VOLUME 1

CHARLES B. CARPENTER
WILLIAM V. MILLER

A Transplantation Proceedings Reprint
March 1977 Supplement

CLINICAL HISTOCOMPATIBILITY TESTING

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GRUNE & STRATTON
A Subsidiary of Harcourt Brace Jovanovich, Publishers
New York San Francisco London

Clinical Histocompatibility Testing is the hardcover edition of the March 1977 supplement (Volume IX, Number 1) of the quarterly journal *Transplantation Proceedings*, an official publication of the Transplantation Society.

Grune & Stratton, Inc.
111 Fifth Avenue
New York, New York 10003

Library of Congress Catalog Number 77-74805
International Standard Book Number 0-8089-1022-1

Printed in the United States of America

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Introduction

THE second annual meeting of the American Association for Clinical Histocompatibility Testing (AACHT) was held in April of 1976. Histocompatibility testing is no longer a narrow field involving only those engaged in clinical transplantation, but encompasses a wide range of immunobiologic and clinical interests. The expansion of work in definition of the human major histocompatibility complex (HLA) and its biologic role is reflected in the subject matter presented at the 1976 AACHT meeting. In this publication, the papers of both the invited speakers and those presenting their original investigations are grouped topically and provide a review of current activities in HLA-A,B,C serology, HLA-D typing, B-cell (Ia) antigens, disease associations, clinical transplantation, blood component therapy, and paternity testing.

The AACHT was organized in St. Louis, Mo. in April 1974 and held its first meeting in Birmingham, Ala. in 1975. The organization represents over 350 scientists and technologists involved with clinical histocompatibility testing throughout the United States, Canada, and South America. The organization grew out of both the need for a scientific forum and for a "political action arm" to represent the needs of the organization's members to the federal government. The Association had a loose relationship with the New World Workshop, which held its second meeting just before the Association's scientific session. Summary results of the SD, LD, and B-cell portions of the Workshop of the Americas are presented here. Future workshops will be coordinated by the AACHT, which also plans to publish the proceedings of its scientific meetings on a regular basis.

CHARLES B. CARPENTER, M.D.
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Editors

Presidential Address

W. E. Braun

IF I MAY, I would like to establish a precedent—and hopefully not simultaneously destroy it—by offering a few allegorical observations on our histocompatibility association, AACHT, its origins, its present state, and its future. We have now reached the ripe age of 2 years in a country that is 100 times that old. We grew from pioneer stock that was as diverse as that of the settlers of this country. Coming to the little island of AACHT have been blood bankers, computer experts, geneticists, hematologists, immunologists, internists, mathematicians, nephrologists, pathologists, technologists, and transplant surgeons. And the immigration list grows more rapidly now because word has spread from the cartographers and geologists of genes, that is, gene mappers and biochemists, that the tiny tranquil island of AACHT not only may be the gateway to that mysterious and fabled land called Immune Response, but also has on it fountains of sera that can restore youth to aging research grants. Just as many of the early settlers of America came because of persecution, many of the early settlers of AACHT came because of what seems most appropriately called presensitization. But despite the high level of presensitization that may have existed in some of our pioneer pool, they successfully retained grafts of donor information and good will so generously given—showing, incidentally, that here also nonspecific presensitization does not adversely affect graft survival.

The flourishing of the land of AACHT is held also to be proof that intellectual xenografting, which took place among its inhabitants, can be successfully performed.

Before reaching the shores of AACHT, early adventurers were given shelter on a smaller island known by the peculiar name of Tray Workers' Workshop. It was governed by a fiery but fatherly figure known as Captain Kayhoe, who now has gone to his reward with the Red Cross.

Let me move on from this allegory of the past to an allegory of the present. The inhabitants of the lovely little land of AACHT, who have worked so diligently, so proudly, and with such a deep personal imprint on everything they do—and they have done a great deal—now find that their unique product is being bought up by what seems to be a single large industry, known as the Health Care Industry (Fig. 1). It is a curious industry. Its executives, on the one hand, extol the virtues of individualized medical care and scientific freedom and are extremely defensive about depersonalization of either the recipient or the provider of medical care. Yet, on the other hand, they create a factory-like atmosphere for the providing of health care and encase it in a cold corporate-like image. Its size and its bureaucracy tend to frustrate the very purposes for which it was created, namely, to deliver sound medical care not only with efficiency but also with understanding.

Another problem is that the products of the Health Care Industry are becoming of more uncertain quality. Already the quality of one of this industry's products, known simply as "good science," has suffered from heavy contamination with pollutants, such as political science and even science fiction.

A puzzling fact for the inhabitants of AACHT is that in order for them to work for the Health Care Industry, unlike the others involved with that industry, they must enter the factory through numerous gates of accreditation, exit, and reenter through still another gate in order to satisfy the curiosity of the plant's guards, who never have seen AACHT-kins before (Fig.

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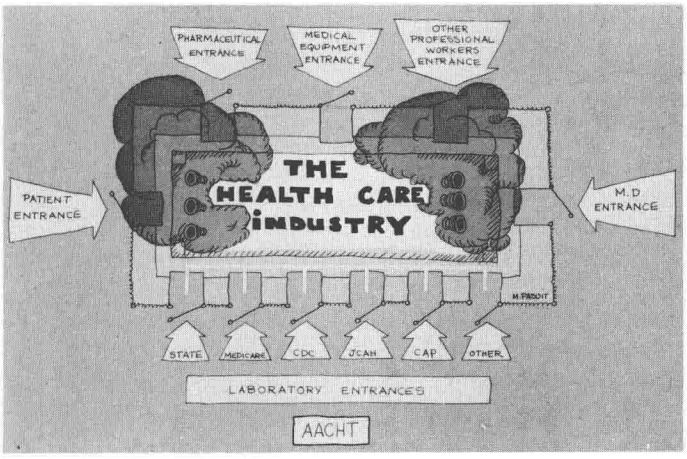

Fig. 1. Here is the conglomerate to end all conglomerates—the Health Care Industry. At the bottom of the figure are the numerous accreditation gates through which AACHT-kins must enter the health care factory and the redundant paths they must travel into that industry.

1). Some say that the guards, who are frequently at odds with one another, do not even know what the qualities of a real AACHT-kin are. Yet each guard dutifully affixes his stamp of approval to AACHT-kins, who soon will come to appear like those in Figure 2.

Finally, a future allegory. While there will be some who come to AACHT-land to pan only for quick riches in the swift streams of disease association, most will take up permanent residence and truly work the land. They will freely form a "more perfect union" through associations that provide at all levels the raw data, the new technology, the sophisticated research and development, and the intelligent management systems necessary for AACHT-kins to fulfill their scientific and moral destiny. The huge Health Care Industry, which will probably stand until it falls of its own weight, will be immensely improved by newly installed catalytic conscience converters that will help to eliminate at least some of the polutants of political science and by another kind of national immunization program to ward off an epidemic of that dreaded carrier of science fiction known as the Patchwork Mouse.[1] Bright new leaders arising in the Health Care Industry, as in AACHT-land, who have learned to speak each other's language and all their tribal dialects, shall survive their political and scientific odyssey with "the will to strive, to seek, to find, and not to yield."[2]

Fig. 2. An AACHT-kin—fully approved.

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WORKSHOP SUMMARIES

SD New World Workshop Report

C. M. Zmijewski and P. I. Terasaki

THE SD PORTION of the Workshop of the Americas was carried out in two phases. The first of these was held in late 1975, and the results have already been published.[1] The second, which was just completed, had three major goals: (A) to type as many cells as possible with the standard sera selected as a result of the first cycle in order to establish a national reference panel; (B) to attempt to gain additional data on American Blacks; and (C) to further define antisera that are readily available to American investigators.

A total of 75 laboratories from the United States, Canada, and South America participated in the workshop. Cells from 2822 individuals were typed with 90 reference sera plus an additional 140 new sera. A breakdown of the cells is given in Table 1. As a result of the two cycles of the workshop, we now have a national reference panel of 4151 cells. Steps are presently underway to establish a file of these cells so that future experiments to explore the fine detail and "splits" of some of the HLA antigens can be designed using selected cells from this national panel. In addition, 112 of these cells can be considered as being exceptionally characterized, since they were tested in both phases of the American Workshop as well as the VI International Workshop.

Also included in this experiment were 539 cells from American Blacks, and the organizers are certain that the fine analysis of these data will offer a clearer understanding of the HLA system in this population. Although the HLA system has been extensively studied in certain populations of African Blacks, similar studies have not been carried out on American Blacks.

In order to further define our antisera, the results obtained by the 75 participating laboratories were collated and subjected to Boolean analysis by Dr. M. Ray Mickey.* At this writing, only preliminary conclusions can be made, since definitive ones will require a careful study of the analysis. In a preliminary way we can say, however, that some antigens can be easily defined using sera at our disposal. Each of these can be defined with at least two sera that show a correlation among themselves and the respective standard antigen designation of 0.8 or greater; such antigens are: HLA-A1, A2, A3, A9, Aw24, A10, A11, B7, B8, B12, B13, B14, Bw15, Bw17, B18, and B27. Other antigens will pose some problems in definition with the sera used in these two cycles; these are: HLA-Aw26, Aw25, A28, A29, Aw23, Aw32, Aw33, Aw34, Aw30, Aw31, Aw36, B5, Bw38, Bw39, Bw21, Bw22, Bw42, Bw35, Bw37, Bw40, and Bw41. Included with those that are difficult to identify are the third locus antigens: Cw1, Cw2, Cw3, Cw4, and Cw5. This could be due, at least in part, to the fact that these antigens are still somewhat difficult to define even at the international level using large batteries of antisera, and so poor correlation with the relatively few sera tested in this workshop could be due to a lack of agreement on the standard reference antigen. It should be pointed out that these re-

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*Computing was supported by National Institutes of Health, Bureau of Biologics, Food and Drug Administration Special Research Resources Grant RR-3.

Table 1. Panel Members Included in the Two Cycles of the HLA Workshop of the Americas

Second Cycle (1976): 75 Laboratories
Total cells including exchange—2822
1870—Caucasian, 539—Black
First Cycle—334
247—Aarhus, 112—All (Aarhus, 1st and 2nd Cycles)
First Cycle (1975): 30 Laboratories
Total cells including exchange—1329
Total western hemisphere typed cells—4151

sults are only preliminary. A number of investigators are currently reexamining the data of both workshops, and it is expected that a further analysis with definite conclusions and recommendations will be forthcoming.

The vast majority of the antisera used in the workshop were originally identified and characterized using the standard NIH technique[2] that uses 1 μliter of antisera plus 1 μliter of cell suspension incubated for 30 min at room temperature followed by the addition of 5 μliter of undiluted rabbit complement and a further 60-min room temperature incubation. Approximately 10 of the 75 participating laboratories elected to use the Amos technique, which differs from the NIH standard in that the initial incubation of cells with antibody is followed by a wash and the addition of specifically diluted complement. This technique is known to be more sensitive than the standard. The original data had been coded for the type of technique used. Accordingly, the exercise allowed for the delination of so-called technique-independent antisera. The bulk of the data was collected by the NIH method, and the general analysis was made. Data obtained through the use of the Amos technique was analyzed by Dr. Ward, and the identification of sera obtained by this method were compared with the data obtained via the NIH method. Of the approximately 240 antisera used, only 21 of them defining 14 of the HLA antigens could be said to be technique-independent, i.e., they identified the same HLA antigen regardless of the technique employed. This, of course, reemphasizes the point that techniques do make a difference, and a serum identified and characterized by one method must not be used by another technique and expected to yield a result compatible with the original definition.

Finally, it must be emphasized that any workshop is a cooperative effort involving the talents and labors of many individuals. Therefore, the cooperation of all the laboratories that participated in this experiment should be acknowledged and especially that of the members of the organizing committee: Dr. Fotino, Dr. Fuller, Dr. Duquesnoy, Dr. Ward, Dr. Yunis, Dr. Terasaki, Dr. Thomas, Dr. Stastny, and Dr. Miller. The chairman, along with the entire committee, is especially grateful to Dr. M. R. Mickey and his staff, who collated and analyzed the data, and Dr. Richard Viale, who performed a preliminary analysis of the data used for the conference in New Orleans.

All in all, the workshop was a successful exercise and it is certain that further analysis will lead to a great deal more information.

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Report on the Second New World B-Cell Workshop

J. S. Thompson, W. E. Braun, C. B. Carpenter, J. B. Dossetor, B. Dupont, J. A. Falk, S. Ferrone, A. H. Johnson, J. F. Shaw, D. P. Singal, P. I. Terasaki, G. M. Troup, and R. L. Walford

IN CONJUNCTION with the First Americas Workshop plans were initiated for an exchange of serum known to contain antibodies detecting specific antigens on B lymphocytes. Twelve laboratories participated and 120 experimental and/or control sera were tested against 268 enriched peripheral blood B cells (PBB), 103 chronic lymphatic leukemia cells (CLL), and 11 continuous lymphoid cell culture lines. As a control for residual anti-HLA-A,B, and C activity, lymphocytotoxic reactions of these sera were also tested against 98 isolated T-lymphocyte preparations. No restrictions were placed on the participants with respect to methods of cell isolation or specifics of the lymphocytotoxicity procedure, but all were based on dye exclusion as the indicator of cytolysis.

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THE ANTISERA

Thirty-nine sera were found to have some anti-T-cell activity. Of this number, the frequency of reaction with the panel of normal cells was less than 3% in 9. Several of the sera had been extensively absorbed, but the majority were not. In some instances, absorption did not completely remove T-cell reactivity. With respect to anti-B-cell reactivity, 101 (84%) sera were active, 74 (62%) reacted with up to 25% of cells, 39 (33%) with 26%–50% of the cells, 6 (5%) with 51%–75% of cells, and 1 (1%) with 76%–100% of cells. Comparative reactions of a control negative serum placed in two positions unknown to the participants revealed that there was approximately a 2% false positivity inherent in the system. Using this as the baseline, 47 sera were judged to be good B-cell reagents by virtue of specific B-cell reactions occurring with greater than 3% of the panel in the absence of any T-cell cytotoxicity, and 22 reagents were considered to be fair as judged on the criteria of having greater than 3% B-cell cytotoxicity and less than 2% T-cell reactivity. Fourteen other sera were considered to be borderline in quality because their B-cell reactivity was less than 3% and could be accounted for by the 2% error rate noted above. Three sera submitted by Dr. Ferrone were of special interest since they were prepared in rabbits to solubilized cultured lymphoid cell line antigens. They were completely free of T-lymphocyte activity, and they were among the strongest and most frequently reactive sera with either PBB, i.e., 46.2%, 44.7%, and 20.1%, or CLL, i.e., 73.4%, 87.8%, and 39.8%.

TARGET CELLS

On the basis of this analysis, the responses with chronic lymphatic leukemia cells appeared to give somewhat sharper distinction, less false negative and positive tests, and generally qualitatively stronger responses. These conclusions were supported by several lines of evidence. First, 43 (36%) sera exhibited weak reactions 50% or more of the time. Even when factoring the number of weak reactions by 2 (to account for proportions of PBB to CLL), they occurred overwhelmingly with PBB (40/43 weak reactors), suggesting as one possibility that the quality of PBB preparations may account for intermediate reactions.[3] Further support for this is the fact that there were 97 uninterpretable results with PBB and only 17 with CLL. This observation could not be accounted for by an inherent difference in the sensitivity of PBB and CLL cells, e.g., 67% of PBB and 58% of CLL reacted with less than 25% of the sera, whereas 27% of PBB and 42% of CLL reacted with 26%–50%.

The question of reproducibility was reanalyzed with respect to the test cell by comparing the duplicate reactions of a positive sera placed in widely spaced wells. With PBB cells there were 11.6% discordant positive–negative or negative–positive reactions as compared to only 5.6% discordant tests with CLL cells. Concordant positive reactions were similar, i.e., 8.2% PBB versus 9.8% CLL, suggesting that many of the additional discordant responses with PBB were probably false positive. Inspection of the test scores revealed that concordance was generally excellent when the scores were 6 or 8, whereas discordance occurred more commonly when one of the responses was a 4. There were too few cultured cells analyzed in the workshop to allow any conclusions regarding sensitivity and reproducibility, but reactions appeared to be generally higher than with either of the two other B-cell substrates.

SERUM RESPONSES

Correlations and Associations

Although the majority of the participants had tentatively grouped their reagents prior to this workshop,[1–5] the serum assignments of two groups were initially chosen for comparison. Dr. Terasaki submitted six sera thought to detect discreet antigens on PBB belonging to two independently segregating groups.[3] A substantial number of sera included by other collaborators correlated to some degree with these groupings, but only a few exhibited a moderately high degree of similarity, i.e., r greater than 0.65. Dr. Walford included sera detecting 11 of the 13 Merrit group of antigens described with chronic lymphatic leukemia cells.[2] Of interest was the comparison of the reactions of this latter group of sera, defined on the basis of CLL reactivity, with those submitted by Dr. Terasaki and defined on enriched PBB. Several observations may be summarized. First, a high degree of association was noted between Merrit group 13 and Terasaki group 1, as judged by good correlation of the reactions with both CLL and PBB (Table 1). Sera 85 and 86 (Wal-

Table 1. Correlation (r) of Merrit Group 13 (Walford—Serum 99) With Group I (Terasaki—Serum 117)

Serum No.							
32				CLL			
34	.49						
41	.40	.33					
57	.47	.48	.48				
83	.25	.55	.42	.62			
99	.47	.50	.74	.52	.56		
117	.24	.40	.58	.52	.59	.76	
	32	34	41	57	83	99	117
117	.52	.44	.46	.43	.58	.65	
99	.50	.53	.48	.49	.71		
83	.46	.53	.42	.35			
57	.48	.50	.29				
41	.43	.48					
34	.70						
32				PBB			