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THE HISTORY OF HLA AND TRANSPLANTATION

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Abstract

The origins of the humoral theory of transplantation, which states that allografts are rejected by antibodies (and not directly by cells), can be traced back 100 years. This is when the method of detecting antibodies was first introduced. The methods for detection have become increasingly more sophisticated, permitting development of more specific proof of the hypothesis. Evidence has accumulated that antibodies cause: hyper acute rejection, acute rejection, and chronic rejection of grafts. Moreover, rejection of transplants of essentially all solid organs can now be associated with antibodies: kidneys, hearts, lungs, liver, and islets. Currently, new studies show that removal of antibodies leads to enhanced survival of transplants. If eventually, achievement of high graft survival rates result from antibody detection and removal, the humoral theory of transplantation can be considered to be validated.

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Key words: HLA; Transplantation; Tissue typing; Rejection; Antibody

One area of medicine in which HLA was shown to be of great importance was in transplantation. Again at UCLA, we realized that a meaningful compilation of transplant outcomes —to gauge the effect of matching donor-recipient tissue types—could only be accomplished by testing patients from distant transplant centers since only a few transplants were then being performed at each center. Accordingly, from 1964 on, we started a system of mailing blood samples[1] so that transplants as far away as Paris could be analyzed[2]. This was possible because of our use of lymphocytes as targets, lymphocytes being able to survive for several days. And this program let us document the effectiveness of tissue typing before other labs. We tested blood from donors and recipients from eight different centers, and, in 1968, first showed that kidney transplants from HLA-identical sibling donors have the highest graft survival[3].

Then we discovered a great anomaly. As we accumulated more and more cases, it became evident that mismatched transplants were doing well, contrary to everyone's expectation. I saw no alternative but to announce this finding at The Hague International Transplant conference in September, 1970[4]. This brought down a firestorm on our heads since, on "scientific" grounds, this could not be correct. Patients doing well *must* have been well matched. The NIH, which was funding our work at that point, put together a large committee to make a site visit within three months of the Hague meeting. An account of this is given in Thomas Starzl's book *Puzzle People*[5]. Our NIH contract was cancelled in 1971, six months after the Hague meeting. In addition, Dausset called an urgent meeting of all tissue typers for January, in Paris, to gainsay our Hague statement. Results for the labs were pooled in hope that, collectively, they would show that we were wrong. The analysis was never published—presumably because the data did not support the sought-after refutation. Instead, chapters by the more prominent tissue typers were published in Transplant Proceedings volume 3, 1971, with each author suggesting that *something* was wrong with our analysis as emphasized by the lead editorial by Dausset

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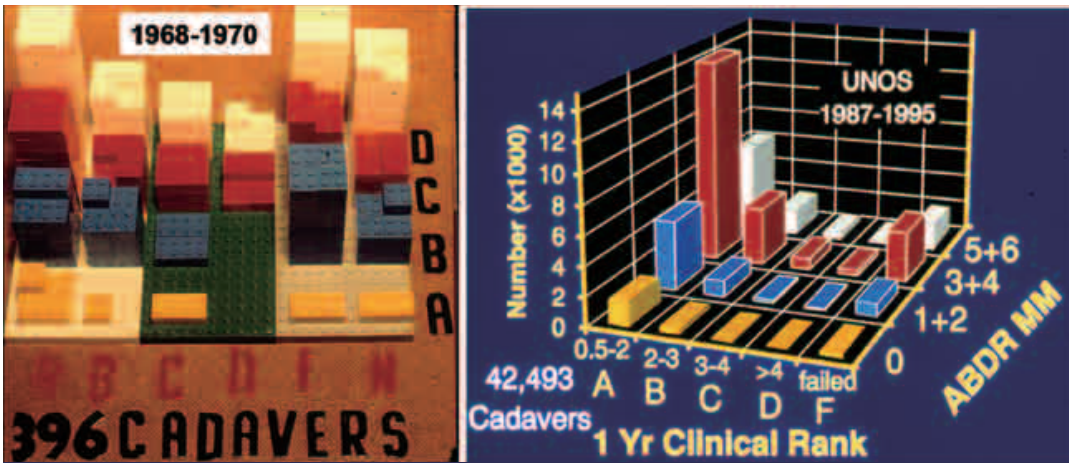

Fig. 1 The correlation between clinical rank in the x axis with the match grade in the y axis is shown here for two periods. The left hand figure was presented at The Hague International Transplant meeting in 1970. It shows the results for kidneys transplanted between 1968 and 1970. Note that although many badly mismatched transplants (shown in white) did poorly, many mismatched grafts had good results as shown by a clinical rank of A. This same tendency of mismatched transplants doing well is noted in transplants performed 17 years later as shown in the right hand graph. There were very few well matched grafts in both eras, and they tended to do well.

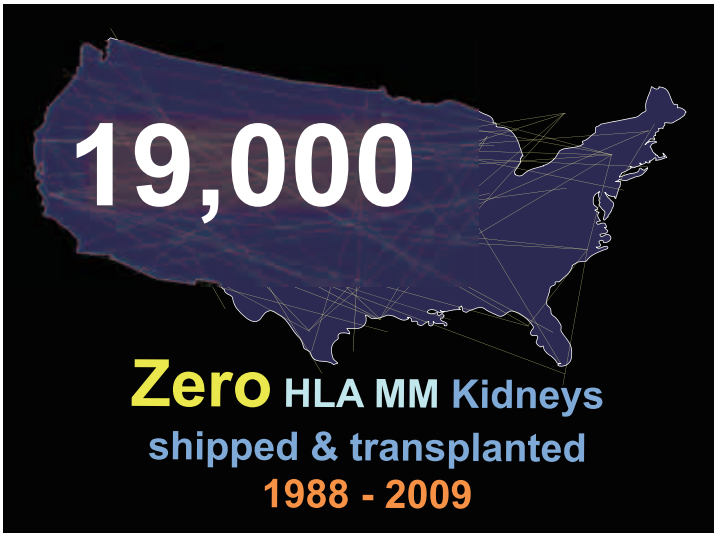

Fig. 2 Because of the extreme polymorphism of the HLA system, it became necessary to ship kidneys throughout the U.S. to obtain matched transplants. To date, as many as 19,000 kidneys have been shipped and transplanted. Whenever a deceased donor is found, the tissue type is matched with the typing of all the waiting transplant patients in the entire country through a central registry in Richmond, Virginia. If some patient is found who matches, the kidney is sent by air freight with simple cold storage.

and Rapaport[6]. On the left hand side of Fig. 3 is the slide I showed at The Hague that caused so much trouble. On the x axis is the clinical rank of the patients, with A being the best; F = failure and N = non-immunological failure. On the y axis is the degree of HLA matching, with D being the worst match. As can be noted, many mismatched patients had a good clinical result. The right hand slide in Fig. 3 shows the results of transplants performed between 1987

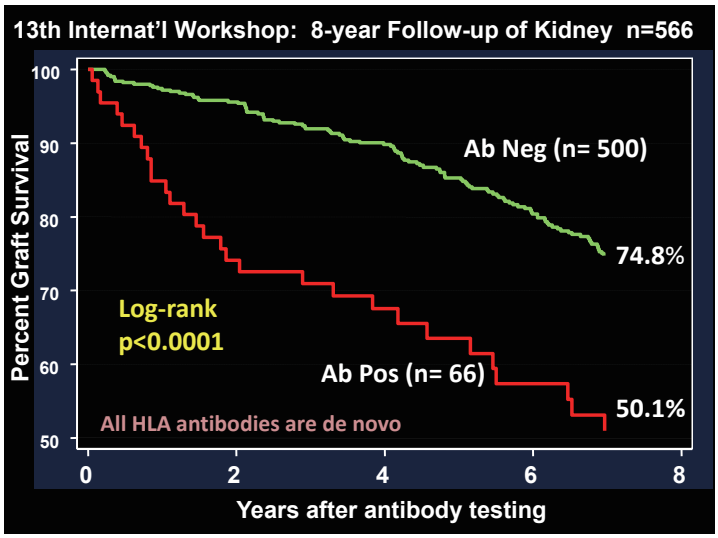

Fig. 3 In 2002, as part of the International Histocompatibility Workshop, kidney transplant patients with well functioning kidneys were tested once, and classified as negative or positive for HLA antibodies. After eight years observation, it can be seen that those who were positive on a single test have continued to lose their grafts compared with patients who were negative.

and 1995 from the UNOS Kidney Transplant Registry. The same type of trend is shown, with mismatched patients doing well, and matched patients doing well, but with relatively small numbers.

The tissue-typing community's upset and turmoil seem understandable. It was "obvious" to everyone with a scientific background that HLA mismatching should result in early failures. Just as, earlier, immunologists who could not obtain more than a few weeks survival of animal skin grafts were shocked that Hume and Murray transplanted kidneys in humans, the same basic scientists concluded that it should be impossible to have mismatched transplants doing well. As noted, the HLA system's extreme polymorphism meant that almost all patients were different from their unrelated deceased donors. But Starzl had no inhibitions about flouting basic "scientific" theory. When he saw that mismatched patients were having good results, he decided that HLA matching should not hold him back from doing transplants. Indeed, he has stated that his major effort in liver transplants was made possible only when he decided to ignore HLA typing. He was the first surgeon to take this path; but others soon followed, and HLA typing started to get a bad name.

In the meantime, bone marrow transplants began, with the first successful operations relying on HLA typing to confirm the tissue match between the patients and their HLA-identical donors[7]. Donnall Thomas started his program that would encompass large numbers of bone marrow transplants, all based on HLA typing, which, for bone marrow transplants, was essential. But finding donors, other than siblings, was a major problem since the odds were against any given unrelated donor being a close enough match to the patient. The marrow donor program, started in 1987 with help from the Navy, was made into a national program to type the HLA of unrelated volunteer donors and create a Registry to find HLA-matched donors for patients. The task becomes herculean as we increase the known number of HLA alleles, currently over 5,000 types. Today, there are more than 9.5 million volunteer donors in the

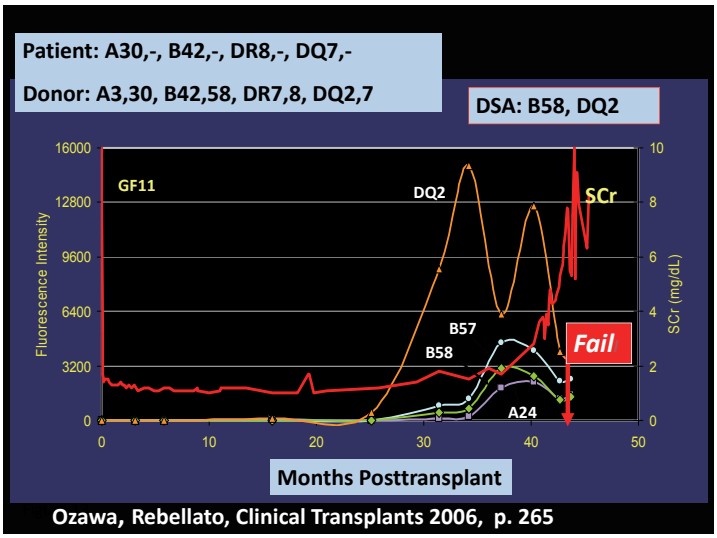

Fig. 4 Thirty-two months after transplantation, this patient produced DSA antibodies to B58 and DQ2, which were mismatched between the donor and recipient. As shown in the red line, serum creatinine increased shortly after the appearance of antibody, and the patient went on to failure. Single antigen beads used to determine the specificity of the antibody were essential in identifying the antibody.

National Marrow Donor Program and 16 million donors registered world-wide.

The role of HLA typing for solid organs has been a completely different story. Despite the incipient "bad name" of HLA matching, when mismatches were made to work—thanks to advances in immunosuppression—clinicians recognized that perfect matches worked best[3]. When we found that mismatched patients were doing well, we started a kidney registry in 1971 to see if factors that influence outcomes could be identified. As a concomitant of that purpose, the UCLA registry became one of the most important resources for tracking the effect of HLA matching[8]. To overcome the obvious difficulty in obtaining good matches, we devised a simple cold storage method to send kidneys long distances to matched recipients[9]. This zero mismatch program was supported by UNOS, and in 1988 the first series of 88 patients who received zero mismatched kidneys was reported and further extended[10]. Since then, more than 21,000 kidneys have been shipped nationally to zero mismatched patients using the UNOS system (Fig. 4), and in all analyses of the effect of HLA matching, patients with well-matched kidneys have always survived at the highest rate[11] while those with the largest number of mismatched antigens have generally fared the worst. Nevertheless, as a class, mismatched kidneys have done almost as well as the zero mismatched. Similarly, numerous analyses done of U.S. data show that the difference between the grades of mismatch has not exceeded a few percentage points. This has impelled UNOS to discontinue allocating priority points for A locus matches in 1995, and B locus matches in 2003. The policy changes have not affected the overall outcome of transplants[12].

Currently, then, HLA matching is essential for bone marrow transplants, but not practical for organ transplants. Only by having a national registry and shipping kidneys to distant placeshas it been possible to obtain well matched kidney transplants in about 14% of deceased-donor grafts in the U.S.

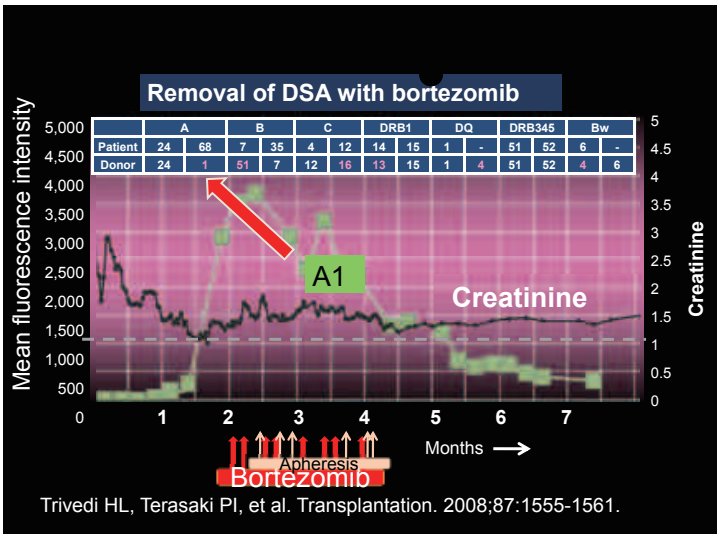

Fig. 5 A patient who developed donor-specific anti-A1 antibodies two months after transplantation was treated with bortezomib and plasmapheresis. The antibody gradually decreased to near negative levels by the 7th month.

HLA antibodies.

HLA antibodies took a trajectory different from that of HLA matching. As noted earlier, only with antibodies was it possible to define the HLA system. However, aside from being used as reagents, antibodies have increased in importance in assessing the result of transplants. Antibodies in the recipient directed against the donor's antigens were found to cause hyperacute rejection of kidneys[13]. Moreover, antibodies present in patients before transplantation could be used to define a state of pre-sensitization, with a higher risk of early failure[14]. It was important to determine the specificity of the antibodies so that donors having those specificities could be avoided. The method used for over 30 years was to test the serum with a panel of pre-typed cells. From the reactions, it was possible, with the aid of computers, to determine the specificity of the antibodies. The problem was that since each cell has A, B, C, and DR antigens, it was difficult to determine which of the antigens had reacted to the antibody—resulting in inaccurate assignments. Only with the advent of recombinant cells has it been possible to coat beads with a single antigen so that each specificity can be detected accurately[15].

In the past decade, HLA antibodies have zoomed in importance to transplantation since they are postulated as the *cause* of graft rejection[16]. Some of the evidence amassed leading to this conclusion is: almost all patients with a rejected kidney were shown to have antibodies[17]; antibodies could be eluted from kidneys that were rejected[18]; patients who developed antibodies after transplantation were shown to have a higher rate of failure than those without antibodies[19] —and this was true for transplants of kidneys[20-23], hearts[24-26], lungs [27, 28], livers[29], and pancreas[30, 31]. Most important, patients with rejected kidneys could be shown to have antibodies detectable *before* rejection of the kidney. All these factors fit the nine criteria, developed by Bradford Hill and Richard Doll, that allowed a logical progression from association to causality[32]. We therefore concluded that HLA antibodies are causally related to rejection of grafts[33]. Recently accumulated evidence shows that following acute antibody-mediated rejection,

antibody reduction led to superior survival compared with that of patients in whom antibody intensity was not reduced[34-36]. This finding was in acute rejection patients who all had histologic reversal of their rejection. Additionally, removing antibodies in kidney and lung transplant patients prior to evidence of allograft dysfunction was also shown to enhance allograft function and improve survival[37]. Improving function by removal of antibodies constitutes final proof that HLA antibodies cause graft rejection, and likely account for the majority of allograft loss.

Summary

HLA, the major histocompatibility locus in man, has had an intriguing history from the time it was first given its name in 1967. In the ensuing 44 years, HLA has emerged as the most polymorphic system known to man, with over 5,000 types identified so far. This variety has made bone marrow transplants from unrelated donors extremely difficult. With most organs —such as hearts, livers and lungs—it was impossible to obtain matches from unrelated deceased donors for transplant recipients. So transplant surgeons were forced to ignore HLA matching, relying instead on ever improving immunosuppression. Even in kidney transplants, use of healthy living unrelated donors has shown that the HLA system can largely be disregarded. However, it is becoming increasingly clear that, although mismatches can be initially ignored, antibodies are eventually formed against the mismatches in a number of patients. The donor-specific antibodies then react against the organs and cause graft loss. Recent findings show that removal of these antibodies results in longer graft survival, the final and persuasive indication that the antibodies had been causing graft loss. The HLA system is thus critical for transplantation, though in ways not initially envisioned.

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