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Review

Jon van Rood: The pioneer and his personal view on the early developments of HLA and immunogenetics

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ABSTRACT

A single observation in a patient with an unusual transfusion reaction led to a life-long fascination with immunogenetics, and a strong wish to improve the care for patients needing a transplantation.

In 2017, Jon van Rood, one of the pioneers in the field of HLA and immunogenetics of transplantation, passed away. Several obituaries have appeared describing some of the highlights of his career. However, the details of the early developments leading among others to the routine use of HLA as an important parameter for donor selection in organ- and hematopoietic stem cell transplantation are largely unknown to the community. After his retirement as Chair of the Department of Immunohaematology and Blood Transfusion (IHB) in 1991, Jon van Rood wrote regularly in the "Crosstalk", the departmental journal, and gave his personal view on the history of the discovery and implications of HLA. These autobiographic descriptions were originally written in Dutch and have been translated, while texts from other sources and the relevant references have been added to illustrate the historical perspective. This special issue of Transplant Immunology combines the autobiographic part, Jon's own version of the history, with other facts of his scientific life and the impact of his findings on the field of clinical transplantation. Hopefully, this knowledge of the history will be of benefit for future developments in transplantation immunology.

1. Introduction

The role of histocompatibility testing in clinical transplantation is well recognized as the best results, both in renal- and hematopoietic stem cell transplantation, are obtained with fully HLA-matched donors [1,2]. The discovery of the HLA antigens as the most important transplantation antigens and their role in the alloimmune response has been crucial for the developments leading to the routine application of clinical transplantation. One of the pioneers in this field was Jon van Rood, a medical doctor from Leiden, The Netherlands. Jon has played a pivotal role in the early developments both in the field of the immunogenetics of HLA and its application in clinical transplantation. He was, among others, the founder of the international organ exchange organization Eurotransplant [3]. Until the very end he was very active in research, enabling the selection of the optimal donor for a patient in need for a transplant.

In 2017, Jon passed away at the age of 91 (Fig. 1). After his retirement as head of the Department of Immunohematology and Blood Transfusion at the Leiden University Medical Center in 1991, he started to write his personal impressions on the early days of HLA in the departmental journal called "Crosstalk".

In this review, which is of course dedicated to Jon, these personal reflections have been combined with more background information on the person Jon van Rood, his motivation and his research. As the authors could not verify all personal reflections, they share them with the readers without taking the final responsibility for all the statements made. Although his research also involved the role of HLA in infection and autoimmune diseases, the focus of the current manuscript is the impact of his findings for the field of allogeneic transplantation and blood transfusion.

2. Van Rood and his family

Jon van Rood was born in the spring of 1926, and he grew up in Wassenaar, a quiet green suburb between The Hague and Leiden. His father was an engineer, his mother a musician, his stepfather Bob Bruyn a portrait painter.

His grandfather from the van Rood side owned a tapestry factory in the middle of the country. His wife died in 1885 during delivery of their third child Albert (van Rood's father). Regrettably, the tapestry factory burned down, leading to the bankruptcy of Jon's grandfather, who died soon thereafter from a heart attack.

Because of this, Albert Hendrik van Rood (1885–1947) had to seek his own position in life. After the HBS (high school), he went to Delft, where a rich cousin enabled him to study civil engineering and architecture at the Technische Hogeschool in Delft. He was involved in some

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Fig. 1. Prof dr. Jon J. van Rood, 1926–2017.

Fig. 2. Painting of the young Jon van Rood by his stepfather Bob Bruyn.

prestigious Dutch architectural projects and became the director of construction at the Koninklijke Nederlandse Hoogovens & Staalfabrieken (Royal Dutch Steel) in Velsen. He would stay with the Hoogovens for the rest of his life. He married three times: in 1907, at the age of 22, with Helena Burgers from Pretoria, South Africa, who had studied medicine. They had four children together of which only one daughter, Jon's favorite half-sister Katinka/Toos, stayed in The Netherlands. She was a well-known sculptor and instructor of for instance Prinses Beatrix, the later queen. Albert and Helena divorced in 1920.

In 1924, Albert married “freule” Anna Maria (Rientje) Röell (1895–1961); together they had two children, Jon and his sister Marijn. Rientje had a daughter Dea of her previous marriage. After marrying Albert, Rientje had especially in the weekends to take care of many children from her's and Albert's previous and current marriages. She provided a happy atmosphere with music for Jon and his (half)-siblings.

In his early childhood, Jon had frequent ear infections and had many hospital stays. At one time, one feared for his life, and a painting was made of him by a painter named Bob Bruyn (Fig. 2). His mother Rientje fell head-over-heels in love with Bruyn, divorced Albert in 1931 when Jon was 5 years old, and married Bruyn, by Jon called “Daddy”, and they had a long and happy life together. Daddy was a violist and painter and Jon grew up among many artistically-inclined people. After his parents divorced, Jon saw his own father formally every fortnight (if he was not ill) and when the war came, father and son were completely separated.

When the Germans invaded The Netherlands, the employer of Jon's father Albert, the Steel company Hoogovens, ordered him to go England, because he knew too much about the working processes of the iron factory. During the war, Albert van Rood worked for Shell in London and, after the war, returned to Hoogovens as leading civil engineer. He died in 1947; he had one child with his third wife, Adriana van Rijswijk, Jon's half-brother Frans.

The otitis media that Jon suffered from as a toddler led to lifelong deafness in one ear; his hospital stay introduced him to medicine. When he had to choose a study, he decided -despite his father's hope that he would become an engineer- to study medicine.

Being 14-years old at the start of the Second World War, Jon's high school education got disturbed. He actually escaped three times from being deported to work in Germany. The first time, he was in a group of youngsters who were picked up after dancing classes and sent by train to concentration camp Vught, still wearing dancing cloths and shoes. After a nervous week, all youngsters were sent home. The second time, he and his friend Paul Hugenholz managed to escape in a crowd. The third time was in the family house in Woubrugge. He and a friend escaped through the fields to hide in a pigsty where search dogs would not be able to smell human presence due to the stench of the pigs. It worked.

After passing his high school exam in 1944, the winter of 1944/1945 was bitterly cold. Because the Market Garden attack of the Allied forces at Arnhem had failed, there were shortages of food, heating and light, while Jon prepared for the study of Medicine.

Directly after the war when he had just turned 19, van Rood became a medical student in Leiden, where he was going to spend almost his entire life. Van Rood described this in “HLA and I" [4] as follows:

“After the war, the facilities of the University left much to be desired, although they must have been splendid in comparison to those found by Georg Klein in Budapest in 1945. We had our lectures in cinema theaters; the backlog of students was so great that the lecture halls could not contain all of them. Student social life was thriving and I made some friends for a lifetime. To compensate for the massive ex cathedra lectures, numerous debating groups were started under the guidance of senior faculty members. I joined a group “Morula” with Professor Piet Gaillard, a histologist, as mentor. “Although the emphasis was on preclinical studies, my first encounter with transplantation occurred when Gaillard told us of a patient who had had a successful parathyroid allotransplant”. Gaillard's scientific interest was bone formation, and he had developed sophisticated bone and parathyroid culture techniques. When, during the war, a girl who had undergone a partial thyroidectomy became hypoparathyroidal, it was realized that her parathyroid glands had been removed as well. What to do? There was no parathyroid hormone available. The girl had life-threatening seizures caused by hypocalcemia. Gaillard and the surgeon Kooreman, decided to transplant some of the parathyroid cell clusters which Gaillard had cultured for several weeks [5]. An allogeneic transplantation avant la lettre! The girl recovered in a few days and went home. A year later, she was run over by a tram in front of the hospital, where she died. At autopsy, normal parathyroid tissue was found! During the discussion I asked why transplants normally were rejected. Gaillard said he did not know and that it had probably something to do with the nucleus [not bad!], but mainly with his excellent tissue culture technique [even better!]. This was in 1946, the year in which, unknown to

me at the time, Medawar published his classic paper on skin transplant rejection and the ability of leukocytes to elicit a second set rejection [6]. This publication to a large extend formed the basis of my scientific life."

3. A new world

In 1950, prior to starting clinical rotations in Leiden, Jon van Rood visited a girlfriend in New York. Van Rood's mother had arranged that he was introduced to Dr. Robert Loeb, professor of internal medicine at the Presbyterian Hospital in New York. Dr. Loeb accepted him as a clinical clerk. In "HLA and I" [4] van Rood describes his experience as follows:

"Being a clinical clerk sounds wonderful but was in fact quite tough. We started at 7 AM, had teaching rounds each morning, classes in the afternoon, and library sessions in the evening. Dr. Loeb, who had written with Dr. Cecil the classic textbook on Medicine, was a superb teacher and clinical scientist and had collected around him physicians of equal status. In all probability, this period programmed me to become an (academic) internist. It was also here that for the first time in my "medical career" I felt the responsibility for individual patients, such as Mary, a young girl with IDDM, J.R. with a hepatoma, G.L. with heart failure, and Billy, a small-time gangster who, if he got in a squeeze, put red ink in his urine so that he would be admitted to the safe haven of the hospital. My first encounter with blood transfusion was John, an alcoholic who donated blood (for which he was paid) to obtain the cash to buy whisky. When he became so anemic that he collapsed on the street, he was brought into the hospital where he was given blood transfusions.

After my return to the Netherlands, I started my internships. During my attending the obstetric wards, I was for the second time, and in a dramatic way, confronted with blood transfusion medicine. A patient suffered post-partum bleeding. We tried to stop the bleeding, however despite 2 units of blood, she died. I had hoped to get a consultation from the department of internal medicine, but because of my low position as an intern and probably also because the professor of obstetrics, Holmer, was not on the best terms with his colleague in the department of internal medicine (Professor J. Mulder) this did not happen. At the autopsy, I raised the point of the missed consultation again. The next morning, I was called by Professor Holmer, a rather authoritarian man, and was told that because I criticized the decisions made in his department, I obviously had nothing to learn and could go home. It would have meant the end of my medical career. I did not like the idea, but neither did I like backing out of what was my sincere medical opinion. We had what is called a frank discussion, and I was allowed to continue my studies. The sequelae of pregnancy and the role of blood transfusions have remained of central interest to me and have dominated my medical and scientific life.

After graduation and having worked for 6 months replacing a general practitioner in the small villages Ter Aar and Aarlanderveen, I started my residency in internal medicine at the University Hospital, Leiden (Head: Professor J. Mulder). The regular duties of a resident included responsibility for a ward with 16 patients, four admissions a day, and being on call. A weekend shift started on Friday morning and ended Monday evening. Moreover, the youngest resident would also supervise the Blood Bank."

4. At the beginning, the Blood Bank

"On the 1st of November 1952, wearing a white coat and my stethoscope in the pocket, I went in search of room 7, where the Blood Bank was located (Fig. 3). The newly appointed Prof. Dr. Jaap Mulder, just arrived from Groningen where together with the pharmacist Huizinga he had learned the advantages of a blood bank, took the initiative after his appointment in Leiden to start a blood bank in the Academic Hospital. It was quite different from what we know now: it had one

Fig. 3. The laboratory of the Blood Bank.

room, two beds, a centrifuge that worked on hand power, and which had originally been intended for urinalysis, a refrigerator that could have come from a butcher, and a microscope that could only be used when enough light reached the mirror. There were two adjunct technicians, Aad van Leeuwen and Suus Kloots, who kept the business going, officially under the supervision of the youngest resident in internal medicine. During the first year, 1400 bottles of blood were collected. We did not know plastic at the time. Because the youngest resident in internal medicine did this work of love only as long as he was the youngest resident, and as the influx of new residents was great, the ladies had the space to themselves. In 1952, the number of blood collections was 4200, but even with such numbers, accidents can happen. In case a bottle of blood had been mistaken, and the patient had developed a hemolytic ABO incompatible transfusion reaction, the youngest resident was fired on the spot.

Not so much later, I came in as the youngest resident. With some effort I found the door, entered and saw two attractive young women, bent over a paper, and not wishing to notice me. It is at that moment that my well-known cough was born. After I tried this twice, the ladies turned around a bit disturbed, looked at me and said without looking at me: "O, that must be the new doctor", and without greeting me, went back to their paper. It was very clear "who" and "what" was most important here. I could do little else than, after they had told me that they would help me when they had time, to sit at the table, where I noticed Dr. Patrick Mollison's book (1951): "Bloodtransfusion in Clinical Medicine" (Blackwell Publishing). I started to read and was soon fascinated by a subject that I had always found quite boring, i.e. the ABO blood groups. Mollison turned it into an exciting story. The morning did finish on a positive note. When Aad and Suus had finished, they gave me a cup of coffee and we started to discuss "blood banking".

I learned that the hospital's blood bank was a local chapter of the Dutch Red Cross, which kept the donor administration. Examinations were hardly performed and I would notice within a few months, that some of the donors might not be really healthy. A sedimentation rate and hemoglobin levels were determined, but otherwise the only examination was: "Are you feeling ok?" With consent of the head of the local Red Cross I started to practice blood banking, as described by Mollison.

In the same year, 1952, the thoracic surgeon, Prof. Dr. Gerard Brom, had been appointed. Brom was the first in The Netherlands to perform open heart surgery using an artificial heart-lung machine. These first oxygenating machines consisted of a kind of swimming pool that had to be "primed" with sometimes up to 20–25 bottles of blood. For a blood bank with a turnover of 4000 bottles/year, such an operation each week made a real difference. It was soon clear that our donor base would be insufficient. At that time donors gave blood once every 6 months, and we would soon be short of blood. I made a nice graph illustrating the point and went to Mulder, the head of Internal

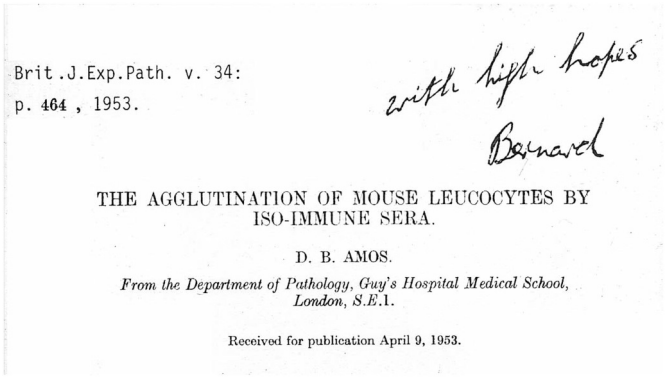
Medicine. “Professor, I think that because we are now doing open heart surgery we will have a shortage of blood within 3 months”. Mulder was busy, gave me an irritated look, and said: “That will solve itself, don't bother me with that”. I have to admit that it gave me quite some satisfaction when my prediction became reality. Again, I went (with my graph) to Mulder, and reminded him of our prior conversation. His reaction was typical: “Van Rood, you have a good head on your shoulders. I was wrong and you were right; you have ‘carte blanche’ to put things in order. In case of a fight, come to me, and if you make mistakes, I will scold you, but you can count on my support.”

As a first-year resident, I had to tell the professor of cardio-thoracic surgery that he temporarily could not operate. And that was how I became the Head of the Blood Bank for the rest of my career. The only solution to the lack of blood was getting more donors. However, in 1953, a war between the Allies against the communists was ongoing in Korea, and blood transfusions and even the Red Cross were associated with fighting, which was impopular so soon after World War II. In spite of this, we could double the number of donors. We visited factories and other large companies. At one of these meetings, a foreman stood up, a big guy, a bit similar to the “Docker”, and he yelled into the audience: “Men, don't do this: if you give blood, your sack will burst”. I was rather surprised by this, and only later realized that he had been referring to an announcement in a national newspaper called the Telegraaf, which reported that the first plastic bags of blood had been tested for errors and that some had shown bursts. I have often been amazed how little people knew about blood transfusion at the time, outside medicine. I should not have been so surprised, as I did not like it either when I started. With the support of Mulder, an increasing donor participation and good negotiations with the different department heads, the Blood Bank services quickly improved.

In the fifties, only syphilis testing on blood donors was mandatory. Although transmission of hepatitis was known, the micro-organism was unknown. We started to identify donors who could transmit hepatitis. The first liver function tests (the Hanger test, named after a doctor in the Presbyterian Hospital in New York, and the Thymol flaketest) were introduced, and we determined whether a positive blood donor caused jaundice in the recipient. We first performed a retrospective study. We contacted patients half a year after a blood transfusion of a suspected donor. I remember a crying widow of a man whom we had called, but who had died a few months after his surgery. One does not forget these things.

I liked being in charge, setting up the donor and transfusion administration, organizing the medical check-up of donors (we found two cases of open tuberculosis, a case of chronic lymphatic leukemia, and many other serious pathologies), and above all, improving the laboratory equipment and techniques. For the latter, the Central Laboratory of the Red Cross in Amsterdam under the guidance of Jochem van Loghem Jr. was our gold standard. I spent several months there under the tutelage of Mia van der Hart, his head technician. We introduced the Coombs test. This test uses anti-human gamma globulin, bridging erythrocytes coated by non-agglutinating IgG antibodies against rhesus and other blood group antigens for the pretransfusion cross-match. The latter was of utmost importance as an audit organized by Van Loghem showed that almost half of the hospitals in Holland at that time (1953) were not able to do a decent cross-match or reliable blood group typing.”

At that time, 14 different blood groups had been identified on red blood cells. Transplantation antigens had been identified in mice, named H-1,H-2 etc. by Gorer [7]. In the 1950s, it was generally known that immune responses play a role in skin graft rejection. Medawar had demonstrated in 1946 that a first skin graft in rabbits is rejected after 10 days, but that a second skin graft from the same donor gets rejected faster than the first. This sensitivity could also be induced by an injection with donor peripheral blood leukocytes prior to the first skin graft, showing that leukocytes share transplantation antigens with the skin graft. The search to investigate the presence of anti-leukocyte antibodies in patients who had received blood transfusions was not very successful.


Brit.J.Exp.Path. v. 34:
p. 464 , 1953.

with high hopes
Bernard

THE AGGLUTINATION OF MOUSE LEUCOCYTES BY ISO-IMMUNE SERA.

D. B. AMOS.

From the Department of Pathology, Guy's Hospital Medical School, London, S.E.1.

Received for publication April 9, 1953.

Fig. 4. Personal message of Bernard Amos on his paper on leuko-agglutinins in the mouse.

In 1953 (Fig. 4) Bernard Amos had showed that the murine transplantation antigens could be identified using leuko-agglutinating sera [8]. In humans, leukocyte-agglutinating (auto)antibodies had been observed as well [9] [10]. In 1954, Jean Dausset described the presence of anti-leukocyte antibodies following blood transfusions [11]. These antibodies were directed against a genetically determined antigenic system as leucocytes of monozygotic twins showed similar reaction patterns [12]. A great interest in these antibodies developed after it was shown that such antibodies could cause transfusion reactions [13,14]. However, in contrast to erythrocyte groups, no leukocyte groups had been identified due to a lack of reproducibility of the test.

5. A new family

In January 1957, Jon married Sacha Baronesse van Tuyll van Serooskerken. Sacha had finished high school and wanted to do something useful. Her mother suggested she could become a doctor's assistant and in 1956 she came to work in the Blood Bank in Leiden to learn to take blood. It was here that she met Jon. At their marriage party, it was mentioned that Jon was promoted to head assistant, a rank that gave a salary raise to 186 guilders a month (nowadays 84 euro). An aunt (married to the ambassador in Rome) remarked that she spent this amount of money each month on stamps alone! Their first child, Yanda, was born in 1959, their son Peter was born in 1962, followed by second daughter Tinka in 1964.

6. Setting up research in Leiden

The Blood Bank was part of the Department of Internal Medicine. Jon became head of the Blood Bank in 1957. Many departments were independent kingdoms, occupied by big egos, making collaborations problematic. No scientific efforts were required from residents, and just a few received a PhD degree. The general attitude among residents was to become an internist and then find a profitable hospital position. When van Rood indicated that he wanted to stay in Academia, many people wondered about this weird wish. Research was not a popular subject. Van Rood wanted to continue in hematology, and to be involved in patient care as well as in the lab. Luckily, under the leadership in Internal Medicine in Leiden at that time, research that benefitted patients was quickly integrated in patient care. The Blood Bank department, also because of the need for larger number of donors, grew and became localized in a new building, a wooden barrack. This new space helped to add research to the routine of the Blood Bank. Van Rood's first successful project involved the role of auto- and allo-antibodies in platelet survival (as described in “HLA and I"):

“We worked mainly as consultants, but we did have “our own”

patients of which we took care if they were hospitalized. Among us were several active clinical scientists, including Koen Hemker, Willy Hijmans, Henk Leeksma and Fredi Loeliger, who produced beautiful curves depicting the kinetics of coagulation factors and who wrote quite important papers. I must confess that I was jealous – more of the graphs than the papers.

After an unsuccessful attempt to screen for hepatitis (unpublished), we concentrated on the survival time of platelets and erythrocytes, in relation to the presence of auto- and alloantibodies [15]. In the beginning, we used DF32P to trace cells; 51Cr became available much later. That work formed the basis for the thesis of George Eernisse and later Leo Bosch, and not only confirmed that erythrocytes survive for 120 days and platelets for 10 days, but also showed the influence of auto- and alloantibodies on survival [16]. ".

7. Transfusion reactions? We never see those here!

"Patients who frequently needed a blood transfusion came to the Blood Bank for transfusions. The reason for this was-, as already mentioned, the lack of interest in blood transfusions associated with a lack of experience with the transfusion-related complications. Patients who receive transfusions regularly can develop non-hemolytic transfusion reactions, caused by anti-leukocyte antibodies. We now know this, but in the fifties, we did not, and assumed that dirty needles and re-used improperly-sterilized bottles caused pyrogenic transfusion reactions. These resemble the non-hemolytic transfusion reactions caused by leukocyte antibodies.

Mr. R. visited us once a month to get a transfusion. During the war, Mr. R. had driven a lorry with a so-called wood gas generator. Burning wood would create a toxic gas, and this probably had caused his aplastic anemia. The monthly blood transfusions became a monthly disaster. A few hours after every visit, his temperature would rise, Mr. R. started to sweat effusively, complained of pain in his back and in his legs, and a flu-like feeling, and we stood by, powerless. A couple of drugs helped a little, but there was no effective, let stand preventive, treatment. Following the transfusion, it took over a day before he had recovered a bit. Not only did Mr. R. develop transfusion reactions, but other departments reported these as well. We started the Blood Bank consultation service. The doctors who worked at the Blood Bank, including of course myself, went to see the patient. Indeed, some improperly-sterilized needles still contained blood clots, and many of the transfusion reactions were indeed caused by pyrogens because of insufficient cleaning of needles. But not in Mr. R's case.

In The Netherlands, the field of hematology was emerging, and blood transfusions clearly belonged to hematology. During one of the hematology meetings, I addressed Huizinga, the pharmacist who, together with Mulder, had started one of the first Blood Banks in The Netherlands, and I told him about our problems with transfusion reactions. Huizinga, a proper "Groninger" (which means: coming from a city in the north of The Netherlands), and not timid, made it clear that he thought that the apothecary in Leiden did not do a good job, and did not sterilize properly. Looking at the situation with the transfusion needles, I partly had to agree with him, but I strongly defended our apothecary, who in my opinion did a fantastic job. Huizinga ended the discussion with a disdainful: "Transfusion reactions. We never see those here (read: in Groningen)!"

In 1954, Dausset had published that blood transfusions might cause leukocyte antibodies [11]. At the time, leukocyte agglutinins were considered auto-immune antibodies. Dausset was not so certain himself, as in the same year, he published a paper in which he described that in patients with neutropenia (read: aplastic anemia), the leukocyte antibodies were indeed auto-antibodies [17,18]. We checked the serum of Mr. R. and indeed found leucocyte antibodies in his serum, with the help of the Central Laboratory of the Blood Transfusion service (CLB). In 1956, the CLB, together with Dr. Bok from Dordrecht, had published a paper in a Dutch medical journal on a patient who closely resembled Mr. R. He too had leucocyte antibodies, as well as transfusion reactions. However, the most important observation was that when they gave blood without leucocytes, the transfusion reactions did not occur [13,14]. Of course, we wanted to help Mr. R and remove the leucocytes from his transfusions. We more or less knew what to do, as one had to centrifuge the blood and suck out the buffy coat. But the search for a suitable centrifuge in the hospital and a suction (liver biopsy) needle was a real effort. However, the nurse in charge of liver biopsies, was very clear and refused to give these expensive hollow needles to me. Therefore, we stole a needle and when Mr. R received his first transfusion without leukocytes we were all surrounding his bed. This is one of my most satisfying moments of me as a doctor, when this man, first looking anxious and then increasingly happy, said after 45 min: "Doctor, I do not notice anything". And indeed, there was no transfusion reaction. The protocol that Van Loghem and his staff had developed also worked in Leiden. This was a real "godsent" for many patients. We started to have a new look at all non-hemolytic transfusion reactions in a systematic way and could classify patients with a transfusion reaction into those that certainly had leucocyte antibodies as the cause, those where they possibly played a role, and those where we could not find another cause than the previously-mentioned pyrogens. The latter group became smaller and smaller, and we really felt that we were getting control [19].

In 1958, Dr. Betty Gloor, an American married to a Swiss geneticist, was the Blood Bank doctor in charge. In early April, she was called to see a woman who had experienced a very serious non-hemolytic transfusion reaction. It was Mrs. H., who after the delivery of twins had severe blood loss needing a blood transfusion. She had responded to the transfusion with the familiar chills, transpiration, high fever, nausea, fainting etc. We found in her blood strong leucocyte antibodies. So, the question came up: when had Mrs. H. received her blood transfusions as we assumed that that should have been the cause of the antibodies. We checked the archives but could not find anything. Betty Gloor and I went back to the patient, who was sitting in bed quite happily with her twins, and I asked her where she had received her previous blood transfusions. Mrs. H. looked at me with frightened eyes and said: "Listen doctor: this is the first blood transfusion I have had in my life, and I have never been so ill, so please do not speak to me about blood transfusions!"

The twins were the results of her seventh pregnancy (Fig. 5). In those days, we already knew that rhesus antibodies could be formed during pregnancy, and at that moment the famous light lit up: could it be that not only blood transfusions, but also pregnancies can induce the formation of leucocyte antibodies? We had a freezer (something very special in those days!), and had collected sera from women with rhesus or other erythrocyte antibodies in pregnancy. Aad van Leeuwen and Ali Schippers, found that sera from 4 of the 30 women tested indeed

Fig. 5. The serum of this mother with twins led to the conclusion that pregnancy can induce HLA antibodies.

contained strong leucocyte antibodies. This was a real “discovery”. With the help of Willy Hijmans, then immunologist at the department of rheumatology, who had just returned from America and therefore knew everything, we wrote our first real paper, which shortly after was published in Nature [20].

We started a study to check all samples that we could get from the Women Clinic for the presence of leucocyte antibodies. About 10% of women possessed strong leucocyte agglutinating antibodies, of which we could later show that they reacted with the leucocytes of their husbands and with all or some of their children. In the same year a similar observation was published by Rose Payne and colleagues in California [21].

A whole new world opened up because these antibodies not only were the perfect reagents to identify leucocyte groups, they also gave us a chance to understand rare adverse transfusion events. Such as patient K. who suffered from hemophilia. Dr. Fredi Loeliger (later professor of coagulation disorders in Leiden) prescribed fresh plasma transfusions to raise the factor VIII level when a new hemorrhage loomed. It must have been in 1960 or 1961 when I was called after patient K. had received the plasma: he had turned as white as a sheet, complained of chest pain, was out of breath, and started to pee blood. When I reached his bed, he was hardly breathing and looking like the patients seen for obduction at the Department of Pathologic Anatomy. At that moment, K. opened his eyes and said with a typical local Katwijk accent: “not good, doctor”. It was to us a riddle what had caused this transfusion reaction. K. had not developed any leucocyte antibodies himself, and we could not find any aberrations. We checked his blood counts after the transfusion and saw to our surprise that all neutrophils had disappeared from the blood. Again, a light lit up. Who had been the plasma donor? Mrs. B., by chance also from Katwijk, was the mother of 9 children. She was asked to return, donated another blood sample, and yes, she had strong leukocyte antibodies, that reacted with the leucocytes of patient K., who in the mean time had recovered. A transfusion reaction caused by passive infusion of those leucocyte antibodies. “.

8. The Blood Bank and the clinic

“In the late fifties, we went into the world with our data on leucocyte or HLA antibodies, but their clinical relevance was still not recognized. Studying anemia and thrombocytopenia was considered much more interesting. It was the time that techniques applying isotopes were introduced in the clinic, first by Dries Querido and his collaborators. After passing an exam that gave permission to work with isotopes, I joined the work of Henk Leeksma, an internist and chef de clinique of Querido's department. Leeksma had access to di-isopropyl-fluorophosphonaat (DFP), labeled with radioactive fosfor (DF32P). DF32P binds to platelets and erythrocytes, and enabled determination of the survival time of these cells.

Mollison had shown how important it was to perform survival studies in case of hemolytic transfusion reactions without an explained cause. Mollison used the differential agglutination technique described by Asby, in which the patient's erythrocytes were agglutinated and the free donor erythrocytes were counted. Aad van Leeuwen did a couple of erythrocyte survival studies with the Asby technique and compared the results with those using DF32P. The DF32P technique allowed more precise measurements of the survival time. Hematologists at that time had much less interest in malignancies than nowadays. Patients with leukemia or Hodgkin could hardly be helped, and most patients died within 6 to 18 months after diagnosis in contrast to cases with anemia. It was around this time that J.V. Dacie in the UK wrote his famous books about hemolytic anemia, which we studied carefully (1954, J. & A. Churchill Ltd, London). The difference between acquired hemolytic anemias and congenital hemolytic anemias, especially congenital spherocytosis, received a lot of our energy. When I say our, I should specifically mention George Eernisse, who later wrote his thesis on this subject.

Unfortunately, the half-live of DF32P isotope was short, one had to work fast once the label had come in. This not only meant that patients that you wished to test had to get their labeled platelets at the same time, but post-infusion samples had to be processed and counted immediately. Of course, our plans were too ambitious and we included so many patients, that we had to continuee work non-stop for 2–3 days. This work was not only done by George, but especially by Aad van Leeuwen and Ali Schippers. We worked around the clock, my wife Sacha came in on a regular basis with food and drinks.

Because it was not known what the normal platelet survival time was, we studied different types of thrombopenia, and showed that the survival time of the platelets of a patient with aplastic thrombopenia could be normal, while it was only a few hours in a patient with an auto-immune disease.

In the 1960s, DF32P was replaced by 51Cr. In spite of the fact that Chromium eluted from cells, leading to a red cell half-life of 30 days, while it was in reality 60 days, there were also advantages. 51Cr is a gamma radiator and this allowed us to see whether the erythrocytes were destroyed in the spleen (something that was especially seen in congenital spherocytosis), or in the liver or intravascularly. This opened up a completely new world of insight into the kinetics of erythrocytes and platelets in different diseases [16].

In the meantime, I had become an internist. We were collecting patients with acquired hemolytic anemia and it was fascinating to make the diagnosis by a positive Coombs test and to prove with survival studies that erythrocytes had a shortened survival and to localize the site of destruction. Our satisfaction when splenectomy cured a patient was great. Once a week, the hematological cases were discussed. The first resident was Leo Bosch, who did his PhD on platelet survival [19]. He was the first of a large number of Hematology PhD students.

9. Using pregnancy sera for the detection of HLA antigens

After the discovery that pregnancy could induce leucocyte antibodies, Aad van Leeuwen and I set up a systematic study to check all samples from the Department of Obstetrics for the presence of leucocyte antibodies. This work led to the identification of Leukocyte groups, comparable to the blood groups seen on red blood cells. One serum that contained such an antibody was analyzed further [22] by cross-absorption experiments (with 9 positive and 5 negative leucocyte donors). This suggested that only one antibody, called anti-IV, was present in the serum. The frequency of positive results with the white cells of 100 random donors, using the agglutination technique, was about 50%. In this abstract, we suggested to investigate the relationship between leucocyte antigens and tissue antigens by absorption experiments.

For some still unknown reasons, pregnancy-induced agglutinins remain stable for many years after delivery and are much better suited for immunogenetic studies than agglutinins induced by blood transfusion which tend to decrease and loose specificity.

10. 4A and 4B

“The transfusion reaction of Mrs. H. took place in April 1958. We saw an opportunity to submit an abstract for a meeting in Rome in the autumn. In the months in between, we found that not only the serum of Mrs. H., but those of several others agglutinated the leucocytes of some donors and not of others. We called the serum of Mrs. H. nr. 1, and identified several other “nice” sera that gave clear positive and negative reactions, in contrast to “dirty” sera, that did not provide clear positive or negative reaction. We identified two other women with beautiful antibodies, sera 2 and 3, and these sera were used for the first family studies.

We went to Rome in a great spirit, and met many people we knew. Van Loghem and his “crew” stayed in the same hotel as we. This was my first real big presentation, and I was quite nervous. I had given the text for the conference publication to van Loghem to see whether this was

ok. In the evening, van Loghem returned the text with several stains, and he told me he had read it in the bath and thought it was fine. The presentation itself went well.

The same afternoon, a plenary session was held, in which Jean Dausset gave a presentation about leucocyte agglutinins. In those days, conferences were at least bilingual: English and French were both spoken. He described his serum MAC in French. This serum came from a woman with Hodgkin's disease, who had received many blood transfusions and had developed leucocyte antibodies that reacted with about 50% of individuals. Dausset used a slightly different agglutination technique than ours. He had selected some patients who needed a blood transfusion, and when they were negative with the MAC serum, he gave them a MAC-positive blood transfusion, and a number of patients produced antibodies, compatible with anti-Mac specificity. He almost received a standing ovation. At that time, Dausset was already a well-known immunohematologist, and had written a book about immunohematology. In this book he remarked that he had not found antibodies among a hundred sera from women who had been pregnant! It was about the same time the rumor had it a couple of technicians in the laboratory of Dausset performed “sink diagnostics”: if they were not in the mood for testing, they threw a couple of things together, pretended to look at them, dumped them in the toilet and filled out the results at random. The cleaning lady of the lab, Madame Legrand, did point this out to him. The technicians were fired and Madame Legrand became the head of the laboratory. Although I admired what Dausset had achieved, I felt sorry for us. Our findings in pregnancy sera and our family studies (a primary) faded in comparison to the story told by Dausset in a plenary session.

The next morning, we met Dausset and Colombani on the steps leading to the congress building and they were arguing about the capability of platelets to absorb leuco-agglutinins. When Dausset heard that we had found they could, he turned to Colombani and said: “What did I say! Are you now convinced?”

Many similar anecdotes reflect how little we knew and how uncertain we were about our findings. This was certainly related to the poor reproducibility of our techniques: 70% for the defibrinated (Dausset) agglutination technique that most people were using, and 80% if one used, as we did, EDTA as an anticoagulant, a technique introduced by Wasastjerna [23] and popularized by Van Loghem and Engelfriet (Fig. 6).

Back home from Rome, we continued to search for informative sera, with clear positive/negative results. Building up a panel was much easier than nowadays: no medical ethical committees or department chairs that needed to be informed. You just asked your friends and acquaintances, mostly people working in the hospital, to give blood, and most people reacted positive. In this way, a standard panel was developed. In 1959, we analyzed the data, and noticed a pattern like Dausset's anti-MAC [24]. A number of sera from different women showed a similar agglutination pattern, while differences were seen as well. The group 4 system was as solid as the MAC system [25].

Reproducibility of the leucocyte agglutination reaction using leucocytes from defibrinated and EDTA blood

		Defibrinated blood, Results of first test			EDTA blood, Results of first test		
		Neg.	Dub.	Pos.	Neg.	Dub.	Pos.
Results of second test	Neg.	10	11	6	41	2	3
	Dub.	9	8	9	5	2	6
	Pos.	2	7	38	0	2	39
	Total	21	26	53	46	6	48

Fig. 6. Data showing the poor reproducibility of the early leuco-agglutination assays.

However, the discrepancies remained bothering to me. In red blood group serology, a positive reaction was always a clear positive reaction, a negative reaction clearly a negative one and there was no room for errors. I wondered whether the leukocyte agglutination test, which clearly was less solid than the erythrocyte agglutination test, might cause false-negative tests. To test for that, we absorbed serum 36, with doubtful anti-4a activity, but which reacted negatively with cells that were positive with other anti-4a sera. Subsequently, absorbed serum 36 was retested with another 4a-positive cell with which serum 36 had previously given a positive reaction. By this approach many of the discrepancies could be explained. Although the leucocytes were not agglutinated, they were able to absorb the anti-4a antibody from the serum. We called this new phenomenon agglutination-negative, absorption positive, ANAP. I have always loved acronyms. Well, that was another invention, because this happened frequently. With this approach, we found a number of sera which on a panel of 60 cells provided a more or less identical pattern of plusses and minuses. We baptized the “group” that was recognized: Group Four.

In September 1959, the Central Laboratory for Blood Transfusion Service organized a big party because of their 25-year anniversary. Many famous people from abroad were present: Race and Sanger, Dausset, Greenwalt, Hugh Fudenberg and many others, and we were allowed to give a presentation there. Based on these findings, van Loghem suggested to perform a large study with combined sera from Leiden and Amsterdam. How to organize this? Donor counseling took place in the evening, where the staff in Leiden took a blood sample from 150 donors, and transported them to Amsterdam. At 10 PM, a number of technicians tested dozens of sera against the 150 cell samples. Two hours later, the results could be read, and at 3.30 AM, we had a large sheet full of plusses, minuses, question marks and other comments. The data analysis encountered so many problems, that the lead technician of the CLB, Mia van der Hart, did not wish to spend any more time on this lousy test.

Despite this Amsterdam adventure, we had developed a taste for this large scale approach, because it seemed a good way for further unravelling of the leucocyte groups. We bled systematically our friends and acquaintances, and soon we could test a hundred individuals with 60 of our most beautiful sera, which gave only clear positive and negative reactions. At least, they did so during the screening. But when we used them against the large panel, quite a number of sera gave results that were hard to interpret. It was nasty work that was mainly performed by Aad van Leeuwen and Ali Schippers. When most of the work had been performed, we realized we had no clue how to solve this in a systematic way.

The solution came during a course on medical statistics by De Jonge, that I took at that time. I had not done my homework properly and was sitting in the back row, hoping not to be called upon, when de Jonge explained the 2×2 table and Fisher's test. While listening, I realized that when two sera, even when they were not identical (this was what bothered us so much) would significantly agglutinate the same samples, this would mean “something”. That “something” could be that both at least identified a similar antigen. Back to the panel. If you wish to compare 60 sera, that means you have to calculate 1800 chi-squares by hand (lacking electronic devices). The available computers were developed for complex calculations in physics and astronomy and were not really suitable for simple mass calculations.

We subsequently approached IBM. A very decent gentleman in a grey suite visited us, looked at our data and declared that this of course was not a problem for IBM. A week later he came with one of their experts. The expert was dressed more simply, but understood exactly what we wanted and said: “yes, this is something for our 1008”. The 1008 was indeed IBMs new machine but the only one in The Netherlands had been sold to the government's personnel administration department in The Hague. We were given the chance to analyze our data. The results were sorted on decreasing Chi squares. It was directly clear that not only the definition of 4A was very obvious,

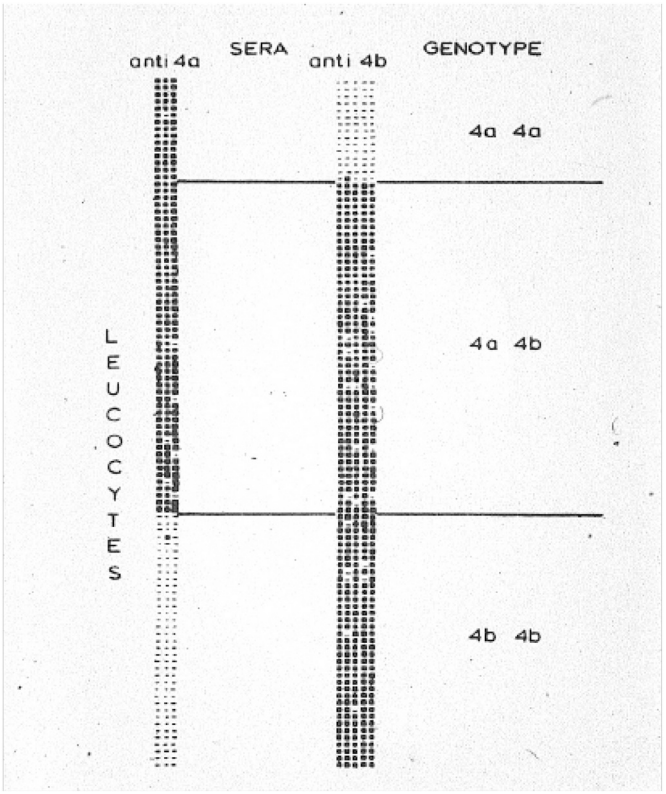


Fig. 7. Antibody reactivities leading to the detection the di-allelic 4a, 4b system, nowadays known as Bw4 and Bw6.

another system was equally obvious, and we called this, advised by van Loghem, 4B.

After obtaining access to the most extensive computer facility available at the time in The Netherlands, specific patterns were indeed identified and named sequentially, groups 1 to 4. However, 1–3 were not reproducible and only group 4 remained as diallelic system, 4a and 4b, now known as HLA-Bw4 and -Bw6. (Fig. 7). This work was presented for the first time in Vienna in 1961 during the meeting of the European Society of Hematology. The data were put together in my thesis: "Leucocyte Grouping, a Method and its Application," a title suggested by my friend and colleague Fredi Loeliger. In Holland, a thesis is a real printed book, and we proudly sent it to all the workers in the field (Fig. 8). For my thesis and defense at Leiden University, I received the highest honors, a cum laude, from my promotor, Professor Mulder. A year later, the data were published in the Journal of Clinical

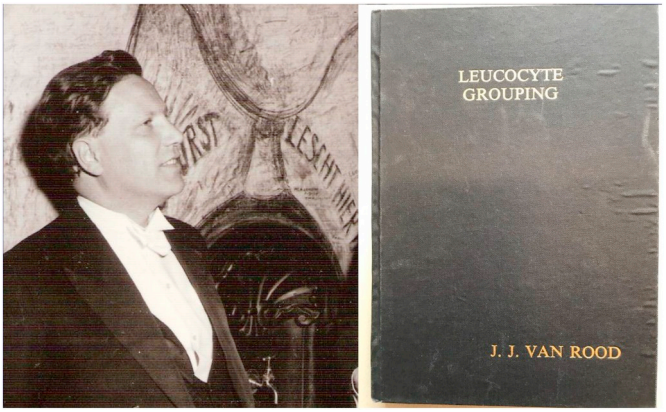


Fig. 8. Jon van Rood after the defense of his thesis entitled "Leucocyte grouping".

Investigation [25]. Also, others Rose Payne and Jean Dausset were publishing similar findings around this time [26–30]."

11. Go west young man

"I defended my thesis on July 9, 1962. We celebrated this on a party boat on the Kaag and the Brasem lakes, viewing the sunset. We discussed the good things in life, the future and other profound items such as a sabbatical in the US. I concluded that I had no wish to go to New York. The work in Leiden was really going well, I had figured out how we could really demonstrate that matching for leucocyte groups would be important for skin and other transplants, so what was I going to do there?

However, important faculty members, especially Querido and Mulder, considered it essential. And indeed, this sabbatical changed my "scientific" life.

12. New York

Sacha and I were lucky as we both had family in the USA. They placed an advertisement: "Young Dutch doctor with wife and two children looking for apartment." A cousin explained: "it is important to formulate carefully. The New Yorkers like the Dutch, you are married and you have children and thus not a homosexual (that was not accepted at the time in the USA), and on top of that you are a doctor and that is a real winner". We found a terrific apartment, with a view on the Hudson river, close to Washington Bridge.

Jeanette Thorbecke, then already at NYU, arranged that I could spend a year with Frank Adler and Marvin Fishman, then at the Public Health Research Institute of the City of New York.

Sacha was in the last phase of her pregnancy of Peter, and would come later after the delivery. I left in August, and first went as invited speaker to a meeting in Mexico. On the way to Mexico City, I visited Rose Payne in Los Angeles. It was the first time we met. She was married to a cook who prepared a delicious meal. After dinner, her husband lost one of his contact lenses, and we spent the rest of the evening on our bellies on the floor, looking for the lens. Luckily, we found it. This creates a bond.

After the congress, I went to New York, where with the help of family members, I decorated the apartment. I flew back to Leiden to be present at Peter's birth, which went smoothly.

The main thrust in the institute of Adler and Fishman was in nucleic acid research. Adler came originally from Vienna and was one of the, even at that time, rare persons who oversaw the whole field of immunology. He spent about 1 h every day taking me through Kabat and Meyer's textbook, chapter by chapter. His laboratory was overlooking the East river. I was sitting in a room where Freund had concocted his famous adjuvant. Marvin Fishman worked with Adler and was New York born, highly intelligent and very nice. He trained me in many basic techniques, including working with rats. My lab experience then (and up till now) was minimal and it took me a lot of effort to get the techniques going. I was so slow, that I found myself working even on Christmas Eve, implanting some more chambers in rats. The rats were properly housed in plastic cages with a metal door on top. After a while, I heard a strange metal on metal sound. When I turned around, I saw that several rats had been able to open their metal doors, and had climbed out, and were now watching me. A weird experience on Christmas Eve!! The fact that I was so slow had also an advantage. Previously, they had only shown the development of IgM antibodies, but because I was so behind in collecting and testing samples, I discovered the wave of IgG following the IgM.

Because the lab of Adler and Fishman was quite isolated, we went to lunch each Tuesday at New York University, where Baruch Benacerraf and many researchers were working. Zoltan Ovary, an Hungarian refugee, chaired the lunch seminars, that were attended by Jeanette Thorbecke, Jonathan Urr, Ellen Vitetta, Jerry Lawrence and many

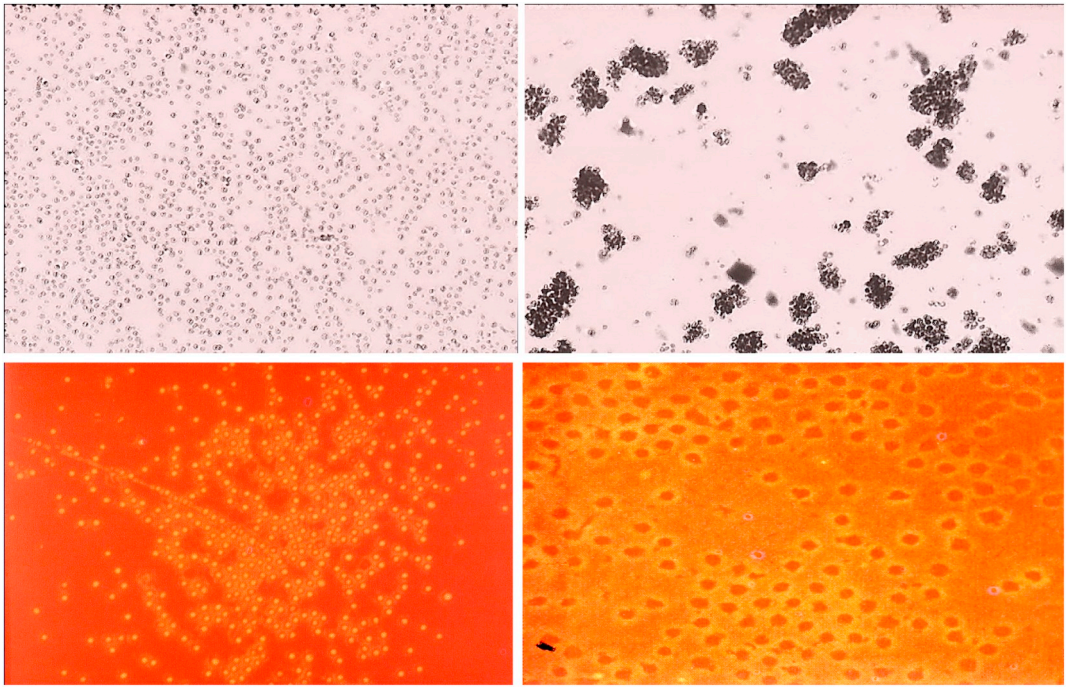


Fig. 9. The agglutination assay (upper panel) was replaced by the micro-cytotoxicity assay (lower panel. Negative reactions at the left side, positive reactions at the right.

others. On request of Benacerraf, I lectured to the students on transfusion medicine.

This was the time that the work of Watson and Crick induced further discoveries. Following the recognition of the DNA structure, the role of RNA came into focus, and subsequently reverse transcriptase. All of these things were discovered around the same time. Although the link with immunology was still lacking, many of us attended Saturday morning lectures at the Presbyterian Hospital to hear famous scientists. There I met Felix Rapaport, who had developed a technique to do experimental skin transplants [31]. The secret was not to do a full thickness graft, but to shave the skin off and add the donor tissue on top of it. This was a very important technique to study the role of leucocyte antigen matching for skin graft survival as a model for kidney grafts.

New York was (and still is) a fascinating place: the seminars at Rockefeller, the Saturday morning seminars at Columbia University on nucleic acid research, but also the city itself, the museums, the music, the river. We had several family members in and around the city which made it easier to get a taste of American life, a fantastic experience. I met there also many of my lifelong friends: Peter Elsbach, a Dutchman working at NYU on bactericidal substances from leukocytes, and Pablo Rubinstein, a Chilean immunohematologist (who was in Fred Rosenberg's Blood Bank in Mount Sinai Hospital), highly intelligent, technically very gifted, and far too modest. I visited him in Chili and he came to Leiden for several short sabbaticals. Many others could be mentioned, such as the day I was talking to Kurt Hirschhorn, a creative genetic researcher at NYU, and who knew an incredible amount of great Jiddisch jokes. A small dark-haired student sitting behind a microscope interrupted our discussion several times by patting himself on the shoulder and exclaiming: “Look what a beautiful blast! Wow, I did a real fantastic job!” It was Fritz Bach! This was the start of the MLC (Mixed Lymphocyte Culture) test [32,33]. We remained close friends ever since.

During my stay in NY, I had regular contact with Leiden, where work went on, especially on the clinical importance of matched platelet transfusions. During my absence, Rugiero Ceppellini and Bernard Amos visited Leiden. Aad van Leeuwen and George Eernisse received them and they seemed very impressed with our work. This was the direct trigger for Bernard Amos to organize the first histocompatibility conference in 1964, in Durham, North Carolina.

After our stay in New York, we travelled through the US with two kids, and the third on the way, Anna Katinka (“Tinka”). First to Boston, where we visited Bernie Carpenter and John Merrill, who together with Joseph Muray had performed the first successful kidney transplant in the world [34]. The chef-de clinique was Jeejyboy. When I had spoken about 4A and 4B, he advised: “Jon, don't get involved in renal transplantation with your leukocyte antibodies. It is really awful.” He was certainly right. The mortality of transplanted patients was very high. A proper immunosuppressive regime had still to be developed. After visiting the Niagara Falls, we went further west to the Bad Lands and Denver, where we met Tom Starzl. Their results of kidney transplants were much better thanks to a better immunosuppressive protocol [35]. That evening I sent a telegram to Prof Mulder, head of Internal medicine in Leiden, with the message that Starzl had solved the problem and we should start kidney transplants in The Netherlands. On this trip we also visited Schilling who had developed the vitamin B12 uptake test, and Bob Good. We continued our journey through Glacier Park to Seattle, where I met Don Thomas for the first time, as well as Bob Epstein who in the 70ies would take a sabattical in Leiden. Thomas was already involved in bone marrow transplantations and had confidence in the potential of leukocyte typing. From there, we drove along the coast to San Francisco, where we revisited Rose Payne, who introduced me to Herb Perkins and Walter Bodmer, and then went all the way to La Jolla, and, through Mesa Verde, to Death Valley and back to Chicago. It had been a fantastic experience. Querido was right: my sabbatical not only introduced me to immunology, it also opened the (scientific) world to me.

Rose Payne and Walter Bodmer were the first to apply our computer approach. A few months later I got a letter from Rose saying:” Dear Jon, could you please send us another copy of your thesis? The copy you sent to me has travelled so often between Walter's lab and mine that it is completely falling to pieces and we would like to have a new one”. We sent it immediately. In 1964, they identified the LA/HLA-A (LA1, LA2, LA3) and later HLA-B locus antigens using the defibrinated leukoagglutination technique [27].

In that same period, two important technical improvements were realized. The complement-dependent cytotoxicity assay and especially the microcytotoxicity test developed by Terasaki appeared to be a truly revolutionary improvement [36]. If you were very good you could do 20–30 agglutination tests with one ml of serum, but with Terasaki's microcytotoxicity test you could do a thousand cytotoxicity tests!"(Fig. 9).

13. The first histocompatibility testing workshops, or how we lost our innocence

"Bernard Amos received money from the National Institutes of Health in order to organize a workshop on histocompatibility. I was invited to attend with about thirty people, and the meeting was held in the building of the National Academy of Sciences, especially known because of their Proceedings (PNAS). For us, the meeting was a great success and it was clear that we were miles ahead. I could present 6a, 6b, 6c (later 7c = B7), and everybody was very impressed, while the chairman of my session, Dausset, did not look too pleased. Since his presentation in Rome in 1954 where he presented the MAC antigen (HLA2), nothing significant had emerged from his lab. Rose Payne showed the data obtained together with Walter and Julia Bodmer, which had led to the recognition of LA1 and 2; now HLA-A1 and A2. Until now unexplained, the agglutination technique with defibrinated blood (in contrast with our EDTA agglutination technique) seemed clearly better to identify the A locus antigens than those of the B locus, while the EDTA agglutination technique did the opposite. Terasaki presented very preliminary data on the effect of matching using sera with leucocyte antibodies and of course the first version of the microcytotoxicity test. Kurt Hirschhorn demonstrated the "two way" MLC test that he and Bach had developed [37], just like Barbara Bain and Loewenstein, who independently (and maybe first) had discovered the same [38]. All names that hardly anybody knows any more. Sic transit gloria mundi!

Meanwhile, we had started with skin transplantations [39], initially using full thickness grafts, as used for severe burns. Someone had to show that this procedure could be done in volunteers (there were no ethical committees) and surprise, that was me. I received the first experimental transplant that failed miserably. From the second series we could get more information. Two donors were selected, one, whose HLA type resembled the first donor as much as possible, and the second whose HLA type was very similar to mine. We hoped that the first donor would be rejected quickly and the second one much later. A peculiarity of this skin transplant technique was, that the only way to determine a rejection was by histology. For this, we had a tiny apple drill (with a 2 mm diameter), to remove a tiny piece of the transplant. On a warm Sunday afternoon in August, I was waiting at the Blood Bank for the person who was going to take the biopsy. It lasted and lasted, and I decided to do it myself. I put the apple drill on top of the transplant, pushed the button and was hit by a screaming dash of pain through my whole arm. A few days later the pathology report came in: normal nerve tissue! It took a while till the innervation of my left lower arm had returned to normal. These data were presented at the 1964 Conference on Histocompatibility Testing in Washington DC, and although no conclusions could be drawn, Leslie Brent concluded enthusiastically that someone was finally performing scientific research on transplantation in humans!

After Washington, we went to Duke University in North Carolina, where in Amos's laboratory, the "wet" workshop was held. All participants brought their own sera with leukocyte antibodies, used their own technique, but, regrettably, also their own donor material, e.g. frozen lymphocytes. Ray Shulman had for instance brought his own platelet suspensions. The first wet Histocompatibility Workshop in 1964 focused on the results of the agglutination test (van Rood, Dausset, Amos, Payne, Ceppellini, Lalezari), mixed cell agglutination, complement fixation, and mixed leucocyte culture. A young researcher, Paul Terasaki, showed the complement-mediated cytotoxicity test, using lymphocytes, and very small quantities of serum [36,40]. Being so smart to register his techniques, he was able later to donate millions of dollars to his university, UCLA, to found the Terasaki Research Institute.

Later, Fleming Kissmeyer-Nielsen [41] and Terasaki [42] described that cytotoxic antibodies were present in failed kidney transplantations and suggested to perform crossmatches. This raised hope that matching for leukocyte antigens might pave the way for rejection-free organ transplantation, just as blood-group typing did for safe blood transfusions.

When I was asked to summarize this first workshop, I could only conclude that it was impossible to draw a conclusion, and that I therefore invited everybody to come to Leiden the next year, so that, with everybody using their own sera and techniques, we could all type the same set of donors.

My visit to Duke also taught me a not so pleasant lesson. As said, Rose Payne and Walter Bodmer had copied our computer analysis using my thesis. Rose Payne had even asked for a second copy. At Duke, she gave me a preprint of her paper. To my utter regret there was a short sentence in the introduction that they had used the same approach as Van Rood et al., but without mentioning a reference to our contributions. In my naivety I had expected that they would be as generous in their paper as they were in their correspondence.

Back in Leiden, we immediately started to prepare the workshop as a kind of military operation. The organizing team, made up of Aad van Leeuwen, George Eernisse, Hans Bruning, Ali Schippers, Thea Thoenes, Ria Castelli, met every week. Basically, it was quite simple. We had selected 45 donors who were willing to provide the required quite large amount of blood. We could use the new laboratories of molecular biochemistry for the workshop, and could use the Eysinga mansion nearby the hospital, for the meeting. Dausset came by at the end of the summer of 1964. He was very friendly. It was his first visit. He told me he was with Rapaport also working on skin transplantations and that intradermal injection with platelets did not lead to a shorter survival of the platelet donor's skin transplants. The preliminary conclusion was that these leukocyte-platelet groups might not be the transplantation antigens everybody was looking for.

Around this time, we were working with Hans Balner of the Primate Center in Rijswijk on a similar protocol, but we used buffy coat cells instead of platelets and we did not transplant one graft but two, one of which was closely identical to the immunizing donor, and the other one as much as possible identical to the recipient. Peter Medawar had shown in his classical experiment that a primary skin graft exchanged between two rabbits was rejected after 10 days, and a second skin from the same donor was rejected in 5 days, because the first skin had induced homograft sensitivity. In the next experiment, Medawar did not exchange a piece of skin but instead intradermally injected the buffy coat cells from a donor and thereafter transplanted a piece of skin from the buffy coat donor, that was rejected after 5 days. In other words, an intradermal injection of leukocytes could induce the same homograft sensitivity as a skin transplant, suggesting leukocytes must share transplantation antigens [43]. Next, we had to show that matching for these antigens improved graft survival. Thanks to Felix Rapaport, who had developed a very nice skin grafting technique, this could be sorted out.

Dausset came home with me and mentioned to Sacha "Votre mari a fait un grand travaille" (your husband did a great job). After Dausset left to a meeting in Stockholm for a blood transfusion congress, I contacted Hans Balner. He agreed that it was a good idea to show our results in Stockholm. I travelled to Stockholm and gave a short presentation in the same symposium as Dausset. Van Loghem was chairing the session and pointed out the potential large implications of our findings. Afterwards, Dausset came to me and wanted to hear it all again and I explained our protocol to him in my best French. I had no problems with this, because of an agreement with Rapaport that he was

coming to Europe to do a series of skin transplants in our department, and I knew he was also going to visit Dausset. I therefore regarded Dausset as a partner in this project. That was a mistake. The first proof was already at the airport in Stockholm, where Rapaport addressed me in a very aggressive way and ordered me to give all anti-HLA antisera to Dausset. I was pissed off by his behavior. This was not only the time prior to ethical committees but also prior to considering patenting anything. In spite of my anger I decided that developing this area was the most important and I sent Dausset a nice set of antisera that could identify nine different specificities.

Balner's experiments were finished [44,45] and I left for Paris to discuss future experiments and Rapaport's visit. It would be one of my worst ever visits to Paris. When I arrived at Dausset's laboratory I was received very friendly, I gave a talk (in French!) and was praised extensively for all the fantastic data we had collected. We then retired to Dausset's room where he told me bluntly that he had decided to continue his work with Rapaport but that he did not need the collaboration with the Leiden group. I was more sad than angry. Till that moment I had regarded him as one of the great, and I had always recognized his leadership in the field. This behavior made it clear that I had made a wrong assessment.

Rapaport had extensively published his technique [31,46], and albeit with some difficulty, I managed to convince Edith Frederiks, the plastic surgeon, to use Rapaport's technique and she ultimately performed close to a hundred transplants in about forty volunteers.

Most of the volunteers were colleagues (Eernisse, Jaap de Graeff, Don Smeets and Stuyveling) and myself (Fig. 10). The remnants of these transplants, some being bright white due to acute ischemic rejection, another hardly visible, as if it was an autologous graft, remained for decades.

The protocol was quite simple: we took someone who for instance was 7c (B7) negative, injected him with 7c (B7) positive cells, waited for 2 weeks and then transplanted a 7c-positive and a 7c-negative piece of skin. The compatible transplants had a mean survival time of 11 days, the incompatible ones 6 days (Fig. 11). George Eernisse was the champion: his incompatible transplant was rejected as a white graft (= no blood circulation) and was scored as 0 days, while on the other hand, his compatible transplant survived 12 days! With this we had proven that 7c was a transplantation antigen."

Fig. 10. Jon van Rood looking at the skin grafts on the arm of a colleague.

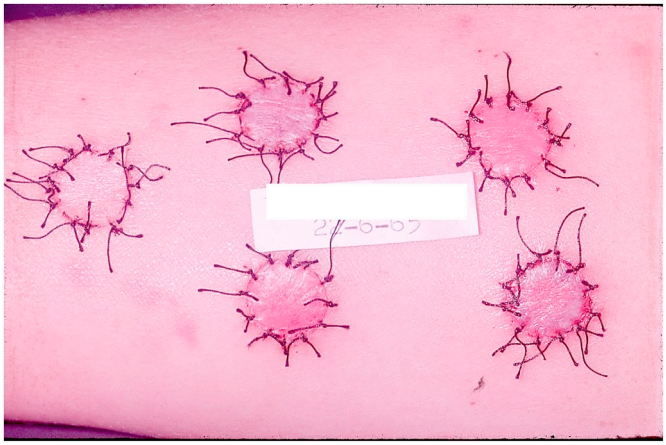


Fig. 11. Skin grafts on the arm of one of the recipients.

Fig. 12. From left to right: Jon van Rood, Bernard Amos and George Eernisse, the organizers of the Leiden Histocompatibility Workshop.

14. The Leiden workshop, 1965

"Everything was ready when August 1965 arrived. Bernard Amos was the president of this workshop while I served as secretary general and George Eernise as the secretary (Fig. 12). The donors were lined up. We would first have the workshop and then the symposium. The day we started, Dausset came running in very excitedly together with Rapaport and a big pile of pre-prints of the talk he was going to present: "Tissue allo-antigens in humans. Identification of a complex system (Hu-1)." Dausset had been able to reproduce the work we had done since 1961 in less than a year. This had only been possible because at that time, Pavel and Dagmar Ivanyi from Prague were doing a sabbatical with him. As they told me later, they already started to work on the afternoon that they had arrived and they did not stop until the train to the workshop in The Netherlands was leaving. It was indeed a fantastic piece of work. That they already had our sera of course enabled the testing and Dausset added a new finding, which was that asides from the existence of several alleles, there were significant associations between some alleles.

He called the "system" that he demonstrated Hu-1 [47]. We had shown the same associations prior to my PhD but we had not published this because the genetics did not make sense. A second preprint "Tissue allow-antigens and transplantation" described how they had reproduced our pre-immunization technique. That of course was nice, but it was not nice that they did not refer to our work.

I did not realize this at that time, as we had to get the workshop going and that worked perfectly. The workshop proved for the first time, that our approach of testing a fixed panel with different sera

Fig. 13. Characterization of HLA antibodies by among others Paul Terasaki, Walter Bodmer and Rose Payne during the Leiden workshop.

Fig. 14. Analysis of the computer print-outs by Walter Bodmer and Aad van Leeuwen.

indeed offered the possibility to compare different techniques (Fig. 13). We had 28 participants from twelve centers in the workshop. The panel had been typed for 4a to 9a. In total, 102 sera were tested, of which 43 with the EDTA test; 47 with the cytotoxicity test; 7 with the defibrinated agglutination technique, and 6 with the complement fixation technique. When we managed to show a computer analysis of the data on the last day of the symposium that followed the workshop (Fig. 14), everybody was convinced that this was the way forward. It was a glorious moment. And it became the central procedure of all subsequent workshops.

The conference itself was also a success. Ceppelini gave a marvelous talk in which he could calculate the number of loci that played a role in transplantation on the basis of a large number of cutaneous transplants that had been performed between relatives, parent-child or child-child. This study was the basis for the next workshop. Terasaki demonstrated his micro technique for cytotoxicity and showed the preliminary findings of a few donor-recipient pairs that he had been able to test for compatibility. We showed our data on the genetics of 4a (BW4) and 4b (BW6) and demonstrated that 7b, 6c (B7) and 7c (B8) were probably alleles. And then of course the skin transplant data.

The meeting was a great success, with 70 participants, including the technicians.

At this workshop, sera from different laboratories had been tested against a common panel of cells. A strong correlation was present for one specific antigen, that was recognized by several groups: Mac (Dausset), B1 (Shulman), LA2 (Payne and Bodmer), group 2 (Terasaki) and 8a (van Rood). The same specificity, later known as HLA-A2, had been detected by leucoagglutination, platelet complement fixation, and lymphocyte cytotoxicity.

For us, it was in many ways a milestone. In the first place, because it placed us firmly in the histocompatibility world. Secondly, because this formed the basis for the exchange of reagents and data, and thirdly, because we now knew that we were in serious competition with a lot of groups and not only with the French. Coming from “Leiden village” where biomedical research had hardly any scientific tradition at an international level, we clearly were “country yokels”. The way in which LA1, LA2, Hu-1 and the skin transplantations were presented opened our eyes. We had learned our lesson and lost our innocence.”

15. The 1967 workshop in Turin, Italy and what happened before it

During the Workshop in Leiden, Ruggero Ceppellini had offered to organize the next workshop in Turin, where he had established a new institute for human genetics, acting as director and professor, something quite common at the time, giving the person an almost complete power over his staff. Ceppellini had not been successful in implementing the leukocyte agglutination technique and leukocyte typing in his lab. He performed skin transplants without typing. As a good geneticist, he selected transplants within families, between a brother or sister and vise versa, and from parents to children or the other way around. In order to get volunteers, he often gave a lecture just prior to the exams. He explained the importance of the experiments and how much he would appreciate it when students and their family members would participate. He always had enough volunteers! He concluded on his data that there was one dominant system with a large number of alleles, but that other factors played a role as well. He also registered the influence of the ABO blood groups. He could not make a link with the HLA system, as he had no typing facilities. For this purpose, I had suggested that we would come to Turin prior to the meeting to type donors and recipients. “We” were Aad van Leeuwen, Ali Schippers and myself. It was a memorable period in our life's. The Italians, hearty by nature, overextended themselves in hospitality. Every evening we were taken to a trattoria or ristorante, where we enjoyed the nicest meals.

The results of these studies were exceptionally interesting and important. A distinct difference was noticed between transplants between brothers and sisters who seemed identical, versus those who differed for one or more antigens. It was furthermore shown, that group 5, identified by Aad, with the alleles 5A and 5B [48], was not relevant in transplantation.

A tradition was that the person who had organized the workshop would become the president of the next, in other words, I was president of the conference in Turin and Ceppellini the secretary-general. One evening, we were discussing how to organize the Workshop. Ceppellini wanted to give the information on the families, that we had typed, to the participants without mentioning who is who. The participants had then to come up with the segregation patterns. He did not tell me that there were monozygous twins among the brothers and sisters. Drinking a glas of good wine, we more or less organizing the workshop. I asked him whom of his staff was going to take care of it all. He thought for a moment and said: “No problem. Sergio Curtoni can do that”. Sergio was commanded to the house (it was 11 PM) to see “Il Professore”. He arrived, listened to Ceppellini's story, who ended in saying: “And you have the great honor to be my assistant and to be responsible for all organizational aspects”. Sergio was silent for a moment and answered: “Professore, it is indeed a great honour and also a catastrophy!”. Fortunately, with the help of many, everything went smoothly.

We did our laboratory work in labs that were put at our disposal by Fiat, and the conference was at Saint-Vincent. We had in the meantime

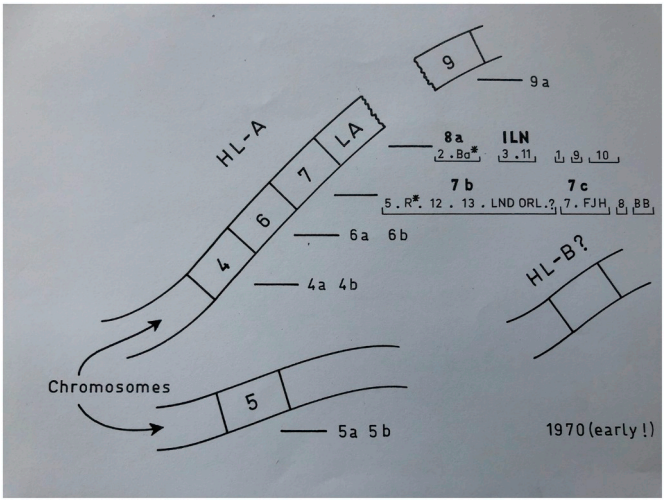


Fig. 15. Historical impression of the genetic organization of the antigens recognized by leuco-agglutination.

studiously continued with our work on the genetics. The help of Laurens Nijenhuis of the CLB was extremely important. He performed the complete genetic analysis and showed that aside from the 4A and 4B, and the 6A and 6B antigens (which we had already shown in Washington), there was a third antigen system, 7A, 7B, 7C (now HLA-B7) and 7D (now HLA-B8). It led to a schematic representation of the HLA system (Fig. 15). We knew that 4, 6 and 7 were closely linked on one chromosome and that 9 was probably not linked. Later, Martine Jager did her PhD on the 9 system, the leucocyte/granulocyte system, and proved beyond any doubt that the locus is not close to HLA [49]. We already knew that group 5 was not linked to HLA.

The computer analyses of this Workshop were performed by Dr. Serra, who flew with all the data to Leiden and happily returned with the analyzed dataset during the closing dinner. It is during this conference that Ceppellini introduced the term haplotype, which means that the genetic information for HLA-A,-B,-C etc., is transferred together from parent to child [50]. At that time, we did not know that those were different loci, between which cross-overs could develop. Another term that was introduced after the workshop, was Linkage Disequilibrium, which means that for instance the alleles HLA-A1 and HLA-B8 are more often inherited together (as a haplotype) than one would expect based on the occurrence of the two alleles in the population.

As I already mentioned, Ceppellini organized Turin and therefore was the Secretary General and I was" Il Presidente". That did not mean much. Ruggero Ceppellini was not the person to ask much advise.

The workshop in Turin was also very important for another reason. Everybody was convinced that the HLA system was important for skin transplantation, but the real question whether it was relevant for kidney transplants was still unanswered. During a flight, I was sitting next to a surgeon from Edinburgh, Michael Woodruff. He had performed a number of kidney transplants from parents to child and invited me to Edinburgh, "so that you can type my patients and their donors and we can see whether the HLA system is important for kidney transplantations". No sooner said than done. We, Aad van Leeuwen, Ali van Berkel, Hans Bruning and myself, travelled not only to Edinburgh but also to Brussels, Louvain, London, Boston, Minneapolis, New York, Durham, Virginia and Denver, where Tom Starzl had done the largest series of transplants with the best results. When we arrived in Denver, Fritz Bach was also present and perfectly performed the vena punctures. One morning I was in the elevator to go to the blood sampling room, and next to me was a man chained to a sheriff. I asked Tom Starzl what this was about. He told us that that man had committed a murder, and to compensate that a little bit, he had donated a kidney.

Also, the visit to Boston was memorable. We typed patients at the

	transplanted	alive
HLA-identical	10	10
HLA non-identical	29	8

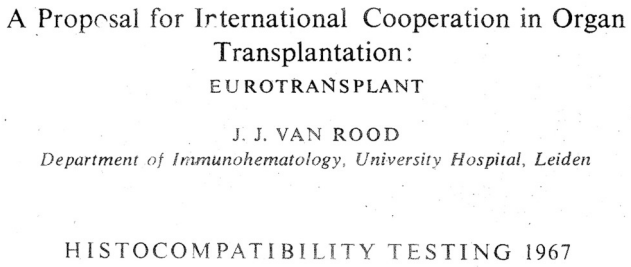
Fig. 16. Trip around the world leading to the conclusion that HLA matching leads to better graft and patient survival. Data derived from (51).

Peter Brent Brigham where John Murray worked (who later received the Nobel prize), and in the Massachusetts General Hospital, where Paul Russell resided. On the last day I was asked to go to his room. It was a beautiful semi-circled room with an enormous mahogany wooden table, behind which Russell was seated. The whole wall behind him was filled with impressive books and antique statues. Russell looked rather sour and pointed to a chair on which I was allowed to sit. He said" Jon, I heard you have been here typing our patients". I said" Yes, that is indeed true, and also your donors". "Did it go well?" "Yes, it went very well". "I also hear that you have been to the Peter Brent Brigham and did the same. Is that true?" I replied: "Yes, that is indeed true, we typed the donors and recipients in the Brigham". He became red in the face and said: "Jon, do you realize this this is the first time in 150 years that the Peter Brent Brigham and Massachusetts General have participated in the same project?" I answered that I did not know and that I did not consider it important.

Back home with the data, the results indeed showed that matching was relevant [51] (Fig. 16). At that time, chronic dialysis did not exist, and when a kidney was rejected, most often the patient died.

At the end of my talk on these data, that I was preparing for the conference in Saint-Vincent, I suggested to found Eurotransplant. Vincent Eijsvoogel talked to me after this talk and said:" I think this is such an important proposal that you should not hide it in the discussion of your presentation, but publish this proposal in a separate publication in the Conference Proceedings." No sooner said than done ([3] (Fig. 17). This advice is probably one of the reasons that the proposal to start Eurotransplant was so visible, and I think this had a great influence on the possibilities to get it realized."

After the Turin workshop, it was clear that the different antigens were inherited as one system and using different sera, four haplotypes


A Proposal for International Cooperation in Organ Transplantation:

EUROTRANSPLANT

J. J. VAN ROOD

Department of Immunohematology, University Hospital, Leiden

HISTOCOMPATIBILITY TESTING 1967

Fig. 17. The paper that led to the foundation of Eurotransplant.

could be identified within families, supporting the notion of a single MHC locus Ruggero Ceppellin introduced the terms linkage disequilibrium and haplotype, to describe all the HLA genes on one chromosome, inherited together, as in mice. A total of 13 specificities could now be recognized. Following the workshop, the first WHO conference on nomenclature was held in Williamsburg, Virginia. All laboratories had developed their own nomenclature, and in the end, nobody's nomenclature was adopted. A new nomenclature was agreed, and the complex was to be known as HL-A. The naming of new HLA genes and allele sequences and their quality control has been carried out by the WHO Nomenclature Committee [52].

At the 1967 Turin workshop, Bruning reported on a fluorescent micro test to identify cytotoxic antibodies [53]. Also, the first disease association (HLA-B5 with Hodgkin's disease) was reported by Amiel, which was followed by many other HLA and disease association studies. Terasaki showed that among 218 kidney transplant patients, as many as 23% of males and 46% of females had preformed antibodies. Such antibodies were found in seven patients who suffered immediate rejection, and in every instance, the antibodies were cytotoxic to the transplant donor. Six of the 7 patients were females, and all had been pregnant. Why evolution has developed a system where women can make antibodies against their child is a question left unanswered till this time.

16. HLA is finally taken seriously

“At the end of the workshop in Turin in 1967, I made a proposal to found Eurotransplant [3]. The fact that the HLA typing in family transplants indicated that HLA matched transplants had the best chance of survival, convinced us this should be applied also for unrelated transplants. After I had made it, Dausset came to me with the comment that he had already set up something similar in France, followed by a correction from Rapaport, that this was not the case.

I had not thought that my comment would have any consequences in the short run. When we came back from Turin, it was obvious that my proposal had not gone unnoticed. I received one invitation after the other and many to write reviews. Reviews about what? We had hardly any data. In spite of that, we published 22 reviews between 1967 and 1970 i.e. [54–56]. Looking back, a waste of energy, but one does not think of that at that time.

We had just started meetings about how to setup Eurotransplant, and had already made a list of patients in Leiden, when we received a phone call from Leuven. A helicopter was on its way with a kidney from a donor who had died shortly before in a traffic accident, and would Eurotransplant please arrange that it would go to the right recipient! Panic! Aad van Leeuwen, George Eernisse, Ali van Berkel, Hans Bruning, myself: everybody was drummed up to prepare everything. We were indeed able to indicate which patient would, based on the HLA data, have the best chance of success. Soon, more kidneys followed. In 1967, there were 5 transplants, in 1968, already 54. We had to build an organization before we had any organization, or as the English say: “to run before you can crawl”. With the dedication of everybody, we started to really enjoy it and the waiting list grew steadily.

Our work regarding HLA, the leucocyte antibodies during pregnancy, the platelet transfusions, and the skin and kidney transplantations attracted more and more people, including foreigners who wanted to work with us. One of the first and most valuable was Joe d'Amaro, who after a career at the Blood Bank in New York indicated that he wanted to work with us in order to fit himself more in the HLA happenings. The work of the regular Blood Bank combined with a leukocyte typing laboratory, and increasing numbers of clinical consultations, made it necessary to expand the staff. The first spacial expansion was to house a biochemistry lab (headed by Hans Bruning) and a real isotope laboratory, where George Eernisse performed survival studies and scans.

Meanwhile, in the Primate Center (part of the Radiobiological Institute), Dick van Bekkum and co-workers had spent years figuring out how one could perform bone marrow transplantations. One day they came with the statement that one needed the stem cells from the bone marrow to engraft and van Bekkum said: “why not do this in the clinic?” Because the experiments had only been performed in mice, we made it clear that the experiments had to be confirmed in monkeys first [57,58].

Thanks to the Primate Center, an institute funded by TNO, Dick van Bekkum and Hans Balner were able to reproduce the work, what had previously been done in mice in the monkey. This really opened up the way to clinical transplantation [59].

At the end of ‘68, a patient was admitted to the Pediatric department with Severe Combined Immune Deficiency Syndrome (SCID), a congenital disease of the immune system, which would cause the death of the child in a few weeks to months from infections. This is the perfect patient for a bone marrow transplant, as one does not have to condition the patient. After an intense discussion between the pediatric department, Rijswijk and our group, it was decided to type the family and luckily, the patient, Johan, had a sister with the same HLA groups. A bone marrow transplant was performed in the Isolation Pavilion. It was very successful, and at the moment Johan is the longest surviving bone marrow transplant patient in Europe and probably in the world (Fig. 18).

A few weeks before Johan was transplanted, a similar bone marrow transplantation was performed in two patients in the USA. In a few months' time, there were three successful bone marrow transplants in

Fig. 18. The first successful bone-marrow transplantation in Leiden. The picture to the right shows the patient and Jon van Rood more than 45 years after the transplantation.

children with congenital immune deficiency and all of this was possible thanks to HLA. I have to add that one of the donors that had been transplanted by Bob Good (in Minneapolis) had not been selected on the basis of serology, but on the basis of the MLC test, as then performed by Fritz Bach.

Fritz Bach is really a story by itself. As said above, I first met him during my sabbatical in New York in 1962. He was then involved in the MLC [32], a technique at that time still met with some doubts. The HLA world was made up of serologists. However, Ceppellini and our group were taking him seriously. In 1968, he facilitated, with the MLC test, the first bone marrow transplantation in the world, a few weeks before we transplanted Johan in Leiden.

Although we saw value in the MLC test, it was out of the question to introduce this test in the crowded space that we had available. We could only make the MLC work after we moved to a new laboratory, the so called Building 23 [60].

Johan's transplantation brought a whole new set of activities and duties. Performing the HLA typings for bone marrow transplant patients was clearly on our to do list, and the fact that we could provide HLA-matched platelet transfusions was an important asset.

One of Ruggero Ceppellini's favorite statements was: “You cannot push in all directions at the same time.” And that was exactly what we were doing. We of course still had the Blood Bank which required more and more attention, and where more and more technical possibilities were being introduced, a hematology service with an isotope laboratory, including patient scans, and an immunochemistry lab, where Hans Bruning had developed immunoelectroforeses. The “HLA-typing laboratory”, Eurotransplant, it was all getting too much. It was clear that we needed some heavy weights to keep control of it all. I visited the insurance company Delta Lloyd, and explained what Eurotransplant was doing. We soon came to an agreement that the insurance companies were going to pay the development costs of Eurotransplant, including the salary of the administrator and a secretary. We placed some advertisements for an economist, interviewed a couple of people and hired Henk Schippers, a guy with a good sense of humor and an enormous enthusiasm to work on Eurotransplant (Fig. 19). He started in March 1970 and made Eurotransplant grow.”

In 1972, Eurotransplant contained 30 typing laboratories located in The Netherlands, Belgium, Western Germany, Austria and Switzerland. There was a collaboration with 70 dialysis centers and 150 potential donor hospitals. On 1-1-1972, already 634 kidneys had been transplanted. Prior to a transplantation, a crossmatch test is performed with serum from the potential recipient and leukocytes from the kidney donor. Of each patient, a serum sample was available in every typing center, which was replaced every 3 months.

Fig. 19. Jon van Rood and Henk Schippers at the 10 year anniversary of Eurotransplant.

17. Clinical applications

“After a sabbatical in New York, in 1964, the first patient shown to me was Mrs. B.L., a woman with severe aplastic anemia due to chloramphenicol and with severe thrombocytopenia, who was bleeding from all orifices. The resident suggested abstaining from further treatment, lacking therapeutic options. Unknown to him, George Eernisse had investigated the impact of immunity against HLA on platelet transfusion survival. He had also developed technologies to make platelet transfusions, but did not bother to publish that achievement until much later, because we were far too busy to explore its potentials. We had shown that platelets carried HLA antigens and that antibodies against HLA could shorten platelet survival. Hence, I advised to give the patient platelet transfusions and the first random platelet transfusions were given in May 1964 and were successful. The patient stopped bleeding and was very grateful. We continued the random platelet transfusions [19] until a patient, who had been pregnant, appeared to have antibodies against the platelets and platelet recovery after the platelet transfusions dropped to zero and she started bleeding again.

Her case was discussed plenary and it was suggested that as the 9 “HLA” antigens we had recognized at that time in Leiden appeared to segregate together (the term haplotype had not yet been introduced by Ceppellini), it might be of interest to see whether some of her eight brothers and sisters might turn out to have a negative leucocyte agglutination cross match with her serum. This was indeed the case and every week one of these sibling donors came to Leiden to donate platelets, which all had an excellent recovery (Fig. 20). A splenectomy was done a few months later and the patient recovered, and continued to visit our outpatient clinic.

We realized we had found something new and when I got an invitation to come to New York in 1964 where the Blood Bank of New York University organized a meeting on new aspects in Blood Transfusion, I presented our findings. Afterwards, Ray Shulman, the NIH expert on platelet antigen group genetics, who I had befriended during my sabbatical, came up to me and said: “nice story Jon, but I do not believe this can be true!” Much later this was confirmed [61,62], without quoting us. This was not their fault, because our paper got published in a Belgian medical journal and was never quoted! [99] However, this patient stimulated research to answer the question whether solid organ transplants similarly depended on matching between these antigens. “.

Van Rood's career was in the meantime flourishing: he was first made a Lecturer in 1965 and then a Professor in Internal Medicine (1969) at the young age of 43. He had already founded the Eurotransplant Foundation and became its first President in 1967. Van Rood's vision on that time period (partly published in [63]):

“The platelet transfusions kept George Eernisse quite busy and showed a steady increase and turned out to be a good learning ground for immunogenetics in the sense that donors homozygous for a haplotype (e.g. HLA -A1, -B8; HLA-A3, -B7 etc.) turned out to be “universal donors” for patients carrying such a haplotype in a single dose.

Our close interaction with the clinic had several interesting consequences, such as the first example of MHC restriction in a patient (Mrs. R.) suffering from aplastic anemia and treated by ATG and a haploidentical stem cell transplant from her brother. The donor's stem cells caused a temporary chimerism but then disappeared and, post or propter, the patient recovered. Els Goulmy, who at that time was working as a technician with Ben Bradley and Annemarie Termijtelen in the cellular immunology section of our department, had learned the Cell Mediated Lympholysis test (CML) developed by Ceppellini, Miggiano and Laughterty and found that the blood of Mrs. R. lysed only about 50% of the HLA-A2 positive donor cells, while the patient and her donor were both HLA-A2 positive! Allan Munro from Cambridge, who was spending a sabbatical as a visiting Boerhaave Professor in our department, suggested to check the gender of the donors, because he had been informed that Elisabeth Simpson working in Mill Hill had made a

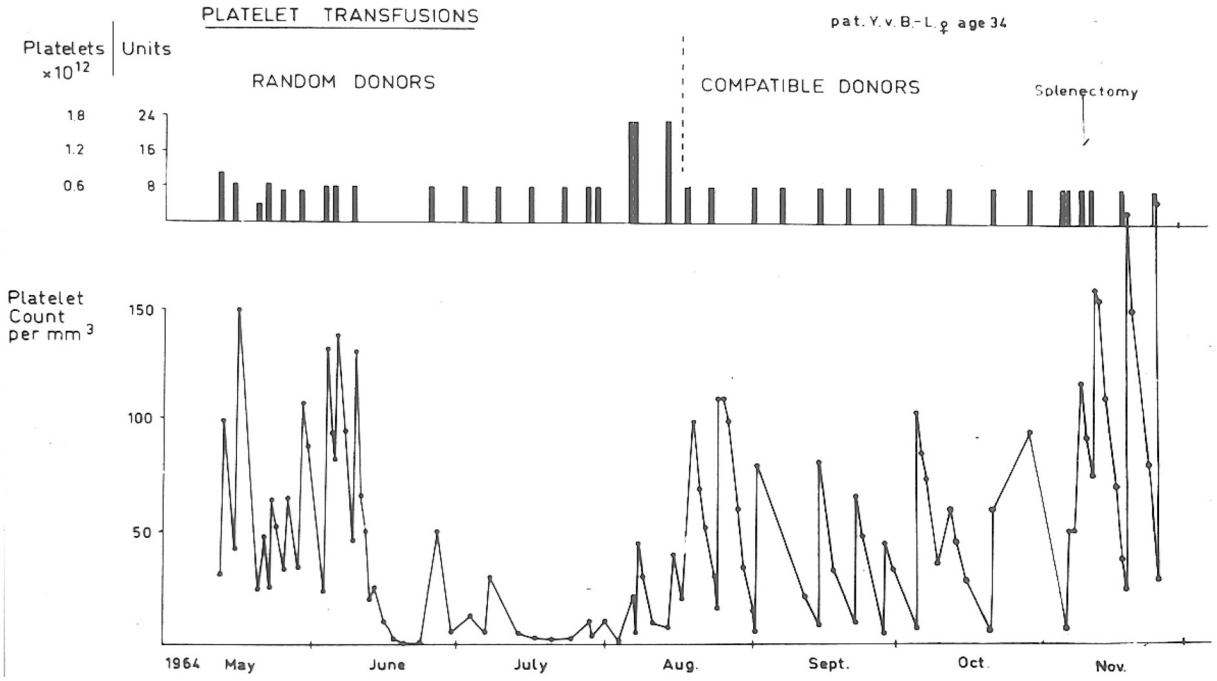


Fig. 20. Data showing that compatible platelets do survive in this patient, who was refractory to random platelet transfusions.

similar observation in mice: In mice, T cells could recognize HY peptides presented by Class I antigens [64], in fact confirming and expanding the findings of Zinkernagel and Doherty a year earlier [65]. This new observation [66,67] opened the field of Minor Histocompatibility Antigens in man, including their genetics and physiology, their role in epithelial malignancies and therapeutic potential, which is still going on."

18. HLA solutions

Due to the importance of the discovery of the HLA antigens for transplantation, a series of new journals was set up: Transplantation Proceedings, Tissue Antigens, Immunogenetics, Journal of Immunogenetics, Human Immunology.

"Chris Barnard had performed the first heart transplant in the Grote Schuur hospital in Kaapstad, and Roy Calne and Tom Starzl were searching for the possibilities to transplant livers. Germany was slowly getting out of the nightmarish time of the war, and biomedical research (preclinical and clinical) had hardly developed. Many Germans refused to recognize that and harked back to the glorious days before the war, at which time it had been self-evident that when you wished to specialize in Internal Medicine, Surgery etc., one went to Germany (Berlin) or Austria (Vienna). At the end of the sixties, the situation was quite different. Germany had to rebuild itself: Professor Walter Brendel, Head of Experimental Surgery in the largest University Hospital in München, was one of the few who realized this, and invited key figures in preclinical and transplantation immunology to Kitzbühl, a top ski area in Austria. The guests were invited to present their work and to discuss their findings between each other and especially with students from München. An impressive group of people had gathered, which included Sir Peter Medawar (Nobel prize winner), Avrion Mitchison (expert in low and high dose tolerance), Leslie Brent (Medawar's crown prince), Richard Bachelor (pupil of Peter Gorer, the real inventor of H-2), Liz Simpson (minor histocompatibility antigens), Dick van Bekkum (bone marrow transplantation), Tony Monaco (tolerance induced with bone marrow). In total, there were around 20 people. Brendel's students were similarly impressive: they had apparently been selected according to a couple of criteria, which included speaking perfect English (which was then quite unusual in Germany), being very good skiers, preferably teachers, and they had to be able to sing well. Obviously, they all had reached A levels in immunology. Although Germany had not yet developed its science yet, industry and trade had been actively developed thanks to the Marshall plan, leading to the Wirtschafts Wunder. Brendel had asked industrial giants for financial support and among the contributors were Biotest and Behring werke, and money was plentifull. The meeting was held in a small farmer's castle above Kitzbühl, beautifully located, and of 5 star level. When one was invited to this hotel, one received a pair of new skies (Pfitzenmayer), which one was allowed to take home! Nothing was lacking.

During this first meeting, Roy Calne presented that pigs that had received a non-related mismatched liver transplant would not reject after stopping immunosuppressants. This was the first description of the presence of tolerance in a large "outbred" animal. The hypothesis was that this was induced by the liver transplant itself. During the discussion, I wondered whether in agreement with prior experiments by Avrion Mitchison, low dose tolerance had been induced by soluble HLA antigens shed by the liver. Mitchison commented that indeed such a possibility might exist. Back in Leiden, we decided to investigate this. At first, the results were not very impressive. We had added a drop of serum from an HLA-A2 positive individual (control serum of an A2-negative individual) to a dilution series of anti-HLA-A2, but the inhibition effect was very small and badly reproducible. However, by adding an increasing amount of serum from A2-positive and negative individuals to the anti-A2 antiserum we could show that serum of A2-positive donors was able to inhibit anti-A2, while sera from A2-negative individuals could not. We observed the same phenomenon for other HLA antigens, especially for HLA-A9. We wrote a paper and submitted it to Nature, that sent it on to Roy Calne to review it. Roy Calne returned the paper the same day to Nature with the advice: "Rush to publish". Which indeed happened [68].

Independently, Chester Zmijewski at Duke University in North Carolina had found the same phenomenon and sent it to Science [69]. Both papers were published in 1970.

Although we demonstrated HLA-antigens in the serum, the question was whether they were able to induce tolerance, or, instead of tolerance, would rather lead to immunization. The reason Roy Calne found

it so exciting was that the release of HLA antigens from the liver into the blood might be one of the reasons why liver transplants could induce tolerance. At that time, experimental skin transplants were still being performed on volunteers at the department. I mentioned already that "volunteers" were the investigators themselves, colleagues and friends. We designed a nice protocol, inspired by Ceppellini, with a built-in genetic control.

We performed three experiments. 1) Plasma from a donor was infused and subsequently a skin transplant was performed. There was no prolongation of transplant survival and even one "white graft" occurred (a sign of acute rejection). However, in spite of proper centrifugation, the plasma still contained platelets. Previously, Dausset had already published that giving platelets prior to a skin transplant did not lead to rejection.

Nevertheless, to remove platelet remnants, we centrifuged the plasma in an ultracentrifuge, a quite unusual procedure in those days, and indeed, in three out of four experiments, we observed a slight extension of 1–2 days of the donor's transplant survival after infusion of the donor's plasma. It was at this moment that the medical faculty decided to set up a Medical Ethical Committee and the first act was forbidding experimental skin transplantation!

This work was stopped and was resumed 25 years later with the findings of Zavazava and Kronke [70], who suggested that sHLA had pro-apoptotic capacities. Whether that explains the tolerance that was induced by Roy Calne's liver transplant still remains to be seen!"

19. Fourth workshop in Los Angeles (1970) hosted by Paul Terasaki

"For the first time, sera were exchanged by mail, and the standardized micro-lymphocytotoxicity test was used everywhere. Data were entered into a computer, and the HLA specificities HLA 1 to 13 were established. I have to say that I have mixed feelings of this workshop. It was the first meeting with Ekkehard Albert, who was doing a sabbatical in Terasaki's lab. He had done the whole analysis of the panel and knew all computer results by heart. I remember him sitting with the large computer printout in front of him. Always correct and polite, a typical young German doctor with a clear scepsis towards the results from anywhere else than Los Angeles. We were rather sensitive regarding the analysis as the very existence of 4a and 4b as separate epitopes was being questioned. Prior to the discussion of the Workshop results, Paul Terasaki had called me aside for a private talk. He told me that because 4A and 4B "did not exist" and were only a mixture of different sera, he "regrettably" had not used them in his analysis.

I had expected this view and we had just written a paper with as the title "4A and 4B do they, or don't they?" What further happened with 4a (Bw4) and 4b (Bw6) is well known [71]. It took years of fighting to have the sera included in the workshops. The battle was only won when Peter Parham handed me a slide with the location of the Bw4 and Bw6 epitopes on the HLA-B molecules.

Different centers were now evaluating the relevance of histocompatibility testing with the outcomes in clinical organ transplantation, especially kidney transplants. Terasaki raised much attention to the question: Is typing worthwhile? In the meantime Gerhard Opelz showed that blood transfusions increased kidney transplant survival rates [72]! Confusion all over.

Other things were also happening: these were the years when Bian Tan and George Eernisse studied the possibility of leukocyte transfusions [73]. This was for the first time becoming a real option and it was especially applied in patients with a severe neutropenia and infections.

Around this time, IBM developed its first continuous flow centrifuge. I had heard about this during a visit to the Presbyterian Hospital in New York and contacted a representative. A meeting was set up, and I was expected to visit the factory to have a look at a prototype. I was amazed when the next morning a driver in livery and a huge Cadillac was waiting to take me to the factory just north of New York. I then realized that I (that little Blood Bank doctor) and our department were taken seriously by a giant such as IBM! We got our first continuous flow cell separator through this contact and George Eernisse managed to make products and treat patients with this machine."

20. Building 23 or the Hemo palace

"Our activities had expanded so much that new lodgings were an absolute necessity. The department consisted of 40 women and men, as well as the blood collection service with its waiting room. Even the Board of the hospital thought this was too much and plans were made to create a new building.

Also, the proposal to found Eurotransplant had caused quite a stir, also of the lay press. During those first years, 1968 and 1969, we often received journalists, and, at that time very unusual, even television crews. That was not always fun. Especially journalists from Germany were aggressive and tried to evoke comments that could be interpreted as if one wanted to let people die on purpose in order to get their kidneys for transplantation. However, in general, the press was very positive, and especially when Chris Barnard had performed the first heart transplant with an unrelated donor, they bombarded us with questions regarding the future options of organ transplantation.

The fact that in 1969, I became a full professor, made it for me much easier to knock on the door of the director of the hospital and also, what was very important, to visit the Ministries in The Hague. Drawings were made of a new building. Hans Bruning was the building chief and took care of all the connections and technical details in which he excelled. Drawing and building took more than a year, and meanwhile, we discovered that, although it was fantastic to get a new building, by the time we would move in, we would not have enough space. A large part of the new building was paid by the Institute for Radiopathology and Radiation Protection, the IRS. They had also paid for the building of the Isolation Pavilion, where the first bone marrow transplants had been performed. When we finally moved in, everybody was happy (Fig. 21).

In 1970, the Third Congress of the International Society for Organ Transplantation was held in the brand-new Congress Building in The Hague (Fig. 22). Hans Balner, Dick van Bekkum, Jochem van Loghem and I were the organizers, with Hans Balner clearly in the lead. It was all organized to perfection and was a great success. The foreigners, especially the Americans, remember the congress foremost because of the plentiful beer (Heineken), jenever, herring, smoked eal and especially the large number of lady friends of Hans Balner, who organized and accompanied the social activities. For the organizers, one of the highlights was being invited by Queen Juliana to have tea at Huis ten Bosch, together with the Board of the Transplantation Society (Fig. 23).

Fig. 21. The happy team of the department led by Jon van Rood (in the middle).

Fig. 22. sAnnouncement of the conference and workshop on histocompatibility testing in Leiden, August 15–21, 1965.

Fig. 23. The participants of the conference (among others Jean Dausset, Sir Roy Calne, Sir Michael Woodruff, Dick van Bekkum, Hans Balner, Jochem van Loghem and Jon van Rood) and the Board of the Transplant Society visiting Queen Juliana of The Netherlands.

It was of course our intention that we could present a paper about HLA matching and transplant outcome. With all the activities regarding the building, moving, the organization of the conference, the analysis of the data had been delayed. When they finally were completed, we really could not find a matching effect, and that was the whole idea behind Eurotransplant. We solved it by comparing the Eurotransplant data with historical data collected till that time. Those who had received a kidney under the auspices of Eurotransplant showed a much better survival than the results previously published by Murray and others [74]. We assumed this was due to the matching. We could proof this more than a decade later with the then analysis of Hans van Hooff, regarding the 10-year patient survival. Indeed kidneys that had been well matched for the 9 old Leiden antigens (4a, 4B, 6A, 6B, 7A, 7B, 7C, 7D, 8A) did much better than mismatched kidneys [73]. However, we have not been able to reanalyze the typing data with the current techniques and maybe we should not attach too much value to the findings as the numbers were low.

As if this was not enough for 1970, there was another landmark, the intention to start another foundation called Europdonor. There is a story behind this. I received a letter that stated that I had become an honorary member of the Deutsche Bluttransfusions Gesellschaft and was invited to come to Giessen to be invested with this honor and to give a talk. Of course, I accepted this honor full invitation, also because the previous honorary member was Bob Race, the man who together with his wife Ruth Sanger had written the book about Blood groups in man. With the success of Eurotransplant in mind, I finished my presentation, which was mainly about the importance of HLA typing and matching in platelet transfusions, with a proposal to establish what I then called Europdonor: the aim was to establish a shared file of circa 20,000 HLA typed donors, willing to donate platelets, derived from 20 centers all over Europe. I gave the manuscript to Aad van Leeuwen and George Eernisse for a last check. To my surprise, this was one of the rare occasions where George, who usually did not have any problems with my wild ideas, uttered a clear “no”. He argued: with the current workload, we have the greatest problems to take care of our patients, and we really cannot organize Europdonor and fulfill requests for matched platelets for immunized patients as well.

Although George opposed the activation of the concept of Europdonor (a large international file of HLA-typed platelet donors) at this time, he of course welcomed routine HLA typing of blood transfusion donors. Already in 1963, during my sabbatical in New York, George had shown, together with Leo Bosch, that thrombocytopenic patients with leukocyte antibodies could be kept alive with HLA-matched platelets. At first, those cases occurred sporadically, but as described above, in 1964 a woman with bone marrow aplasia became refractory to platelet transfusions due to the development of leukocyte antibodies. We found among her large family a number of donors who were for the most part identical with the patient, and this is as far as I know the first patient who received really HLA-matched platelet transfusions, that saved the life of the patient. She attended the 60th anniversary of the Blood Bank. Matched platelet transfusions gradually became a common request, but many people did not have a suitable family donor. Again, with the help of the Institute for Radio Pathology and Radioprotection, we got extra funds and soon had 20.000 HLA-typed blood donors and that turned out to be quite helpful to provide platelets to immunized thrombocytopenic patients. Later, we asked these donors whether they would also be willing to be registered as potential bone marrow for stem cell transplantation.

All attempts to help patients with an unrelated donor failed in that time, probably because routine typing for HLA-D was not yet possible [75]. The first successful unrelated donor transplant in a leukemic patient was performed in 1980 by John Hansen in Seattle, USA [76]. That changed, when we could perform the MLC test (see below). With this, the search for suitable unrelated donors became feasible for clinical routine. Nevertheless, the main achievement of Europdonor in the nineteen seventies and eighties was to provide HLA-typed cross-match negative donor platelets for thrombocytopenic patients, which formed a large part of George Eernisse's and Anneke Brand's work [77]. Building up a platelet donor base in Europdonor since 1970 has certainly saved as many life's as now is being achieved with bone marrow.”

21. Introduction of the MLC test

“In 1962, Fritz Bach, together with Kurt Hirschhorn, was working on the development of what later would be known as the Mixed Lymphocyte Culture or MLC [78]. The MLC test was published by them around the same time as by Barbara Bain and Loewenstein [38]. It has always remained a big mystery whether this was accidental or not. Fritz Bach tells the story that he was standing in an elevator at New York

university together with Gowans and Medawar attending a conference in New York. Gowans told Medawar that the lymphocytes, to which at that time no function had been attributed, and which were considered a kind of end-phase cells, were able to proliferate. When Fritz heard that, he wondered what would happen when you co-culture cells from two individuals? The two-way MLC test was born, later to be refined by Ceppellini to the one-way MLC test by irradiating one of the two, so that they cannot divide and only function as stimulator cells. The non-irradiated cells from the other person are the responder cells. If the HLA Class II antigens differ, one will see dividing lymphoblasts.

In the beginning, the test was performed by counting the number of cells that showed transformation into a blast [79]. Vincent Eijsvoogel, who worked at that time at the Central Laboratory of the Blood Transfusion Service in Amsterdam, had been given the task to set up the MLC test [80]. One of the most important contributions of Eijsvoogel has been the study of two large families in which a cross-over occurred. One of the families was named … Melief! Thanks to Cees Melief (my later successor) we had easy access to the families. There was a cross-over between HLA-A and -B, and another one between B and DR: in some cases, brother/sister combinations that were identical for HLA-A and -B did give a positive MLC. This was the beginning of the identification of the HLA-D locus.

Following our move to the Hemopalace (Building 23), we had sufficient space to set up the MLC test properly. Around that time, I was called by the pathologist in Utrecht, who mentioned that he had an outstanding PhD student and asked whether we could use a postdoc. It was Jan van den Tweel, who really made the cellular techniques working. [79]. With Eijsvoogel's work, we showed that unrelated individuals could sometimes be MLC negative. It was clear that HLA-D was polymorphic. But how to show that? Around that time, Jojan Keuning entered the department to work with Jan van den Tweel with the cells of children of nephew/niece marriages [60,81]. We could expect that some children would be homozygous, and could subsequently be used as stimulator cells in an MLC. If the MLC test would be negative, one could conclude that the responder shared the same HLA-DR type with the homozygous stimulator. The next question was, where to find the children of nephew/niece marriages. Johan van der Does made an important contribution to solve this. An uncle of him was Nuntius at the Vatican and he knew, that when a catholic man wanted to marry his niece, he needed permission from the Pope. The Vatican would of course keep a registry and record all of this very well. I wrote a beautiful letter to the Pope. We were giggling about it, but in truth, after 3 months, I received a letter from the bishop of Haarlem inviting me to a visit: the Vatican had given permission!

I went to see the bishop in Haarlem, we received a list of 16 nephew/niece marriages and found 8 homozygous typing cells (HTCs). We were of course delighted. Jan van den Tweel and Jojan Keuning immediately went ahead to identify the HLA-D groups. We soon realized that 8 cells were not enough to identify all HLA-D groups. We had a meeting and it was proposed that we would approach the “Libelle”, a ladies/housewife glossy. We wrote a nice “sob” story about babies with a lot of eczema that could be cured with a bone marrow transplant and in no time we had over 30 families whom we subsequently invited and collected 24 homozygous typing cells (Fig. 14). The thesis of Jojan Keuning, entitled “Typing for HLA-D", is one of the most important ever produced by the department. The homozygous typing technique and the first results were presented at a conference in 1972 organized by Fritz Bach. It was at a beautiful hotel close to St. Paul-de-Vence, on top of a steep mountain, overlooking the Mediterranean. It could not be prettier. During the conference, Ekkehardt Albert and Bo Dupont came with similar studies, using, if I remember correctly, one homozygous typing cell. As so often, the same idea had arisen at the same time. We had been involved in the preparations of the conference because Fritz and his wife Marilyn and Barbara Alter (his “Aad van Leeuwen”) spent a sabbatical with us. We were happy that during this period the Bach's and Barbara Alter facilitated the introduction of the Cell Mediated Cytotoxicity Test (CML), originally developed in Turin by Ceppellini, Migano and Lightbody, in our laboratory in Leiden [82].

When Dausset heard about the homozygous typing cells and the role that the Pope had played, he went to the Bishop of Paris but the Bishop answered negative to the request to get the data of the nephew/niece marriages. It was to his credit that he did not stop, and he sent Colombani with Degos to the Tuaregs in North Africa. The men of this tribe always marry their niece and they have quite good genealogical data. They collected a large number of blood samples, but when they typed them, only one child instead of the expected 24 was found to be homozygous. The reason behind this was never found. The data are certainly important. Bodmer thought that it was related to a “lethal gene”, but as far as I know there is absolutely no proof for this. Personally, I think that it is more likely that these people had a decreased resistance against infections, and that that becomes evident in the primitive circumstances in which the Tuaregs live.

The Leiden HTC's (homozygous typing cells) have for a long time been the gold standard for HLA-D typing. They were for instance used in Seattle till the early 1980s to select unrelated bone marrow donors. All of this changed when the DR serology was discovered.”

22. The world charted by HLA

“During the workshop in Los Angeles in 1970, extensive discussions took place regarding the subject of the next workshop. Whereas Terasaki was especially interested in getting the right definitions for the different HLA-A, -B and -C specificities (while throwing the baby, i.e. 4A and 4B, out with the bathwater, under the assumption that they had to be antibody mixtures), the group of councilors in contrast decided that one would now chart many different people and animals. It was a proposal by Ceppellini, supported by Bodmer, who as good geneticists saw the importance of this effort. Dausset had already indicated that he wanted to organize this workshop, which would take place in 1972 in Evian.

The world was really divided between different laboratories traveling to the most remote areas, to take blood samples and perform HLA typing's. This was quite a task. Despite the micro cytotoxicity technique having been introduced as the standard technique, transporting cells over great distances had not yet been properly arranged. A lot of different approaches emerged, including one from Roy Walford, that cells would be ok after a week of travel. We regrettably believed that, which led us to loose much of our material, as it was not true.

During our lab meeting we discussed which populations we could claim. A guest at that time was Marika Palfy, a Hungarian technician. The Central Laboratory in Amsterdam would go to Indonesia, so that was out of the question. Marika proposed to take one of the valleys of Switzerland. The people there had been isolated for a long time, with a lot of inbreeding, and that could be an interesting subject. We decided to do that, as a preparation for our contribution to the workshop, which would be the analysis of HLA groups in Bushmen. This was inspired by our excellent relation with the South African group of M.C. Botha and Ernette du Toit whom we met after the first heart transplants by Christian Barnard.

We did as planned. We contacted the local doctor in Fiesch, a small village at the bottom of the Jungfrau gletscher. Their area had only recently been connected with an asphalted road and previously, only a small number of families had lived in this uninhabitable very isolated spot. Aad van Leeuwen, Johan van der Does and I went to Fiesch with a car loaded with equipment to take blood samples. The blood was sent to Leiden, but we had of course to show the results to the village community and were faced with a problem. When we analyzed the genealogy, we noticed that there were quite a number of illegitimate children, much more than we had seen in The Netherlands. When I started to discuss this very carefully, the audience broke out in a hearty laugh. Afterwards, one of the elder villagers told me that it was customary that: “die jungen Kerlen im Fruhling durch das Fenster zum

Fig. 24. Blood collection of Bushmen for population studies on HLA.

Hasensprung kamen". This excellent work was never properly published, except in the thesis of Johan van der Does. This operation improved our logistics for our trip to South Africa.

We flew to Johannesburg and from there to Windhoek, where four-wheel army trucks took us into the bush. After traveling for a day, we arrived at Botswana land where we were lodged at the Red Cross, where we could take blood samples (Fig. 24). It was a unique experience. The Bushmen were one of the kindest and gentlest groups of people I have ever met (See Millne's book:" the Harmless people"). We gave them something sweet or tobacco. A few days later, we moved into the bush ourselves and collected blood in their very primitive camps. One time we saw a herd of elephants, about two miles away. In spite of the distance, the bushmen immediately wanted to leave as they were clearly scared.

Botha organized a small plane that took us to the "Etosha Pan reserve", where we stayed a few days. They provided us with a car and we went into the reserve. It was astounding how many animals one saw. We saw twelve different types of deer, wildebeest, elephants, giraffes, lions and of course enormous numbers of monkeys. It was a fantastic trip.

It had of course been our intention to collect all the data and prepare a kind of flow sheet of the distribution of the HLA genes and their frequencies around the world. We hoped this would give us an idea about the migration of the different races in the world. Dausset was the organizer and had managed to rent a beautiful hotel on the lake of Geneva. It was early spring, prior to the main season, and he had been able to get the very expensive hotel for a cheap price. When we arrived in Evian we were told that, regrettably, there were no people in Paris to enter our data into the computers and would we please fill out the forms again. We therefore spent the first 2 days in our hotel room on our belly, registering our data again. I was very angry about this and it led to a fight with Dausset, which was made up later.

The conference was excellent with outstanding talks about anthropogenetics and phylogeny. The results were interesting, especially for Ceppellini, his collaborator Alberto Piazza, and Walter Bodmer who mainly performed the genetic analyses. For most of the participants, the direct use was not clear.

The reason for these studies was the assumption, that the differences in gene frequencies in various parts of the world would show a relationship with infection pressure from viruses and bacteria. Ceppellini had been the great pioneer in this field, with a beautiful study of Sardinia, where he had HLA-typed four villages, two in the mountains where there was no malaria, and two villages on the coast, where malaria was prevalent. He demonstrated significant differences between a number of polymorphic genetic markers, especially HLA and Gm [83]. This has been a hallmark study, posing as the basis of many HLA and disease association studies.

1972 was certainly not only the year of St-Paul-de Vence and the Histocompatibility Workshop in Evian. In 1972, Bob Epstein, who previously worked with Don Thomas in Seattle, joined us as Boerhaave professor. He lived in Oegstgeest for a year, with his wife and three children. We had the ambition to build a dog colony, similar to what Seattle had done to study bone marrow transplantation. We could afford this by using the private income of the clinical practice. During 3 years we performed studies on immunogenetics and the use of ATG (Anti-Thymocyte Globulin), work also reported in Johan van der Does' thesis. However, the facilities were too primitive to perform bone marrow transplants in dogs.

23. HLA rally won by serology (thanks to Aad)

Thanks to the help of nephew-niece marriages, the Pope and the Libelle, a panel of homozygous typing cells (HTC) was established and used worldwide to select unrelated bone marrow donors. For organ transplantation, this was too time consuming, but it logically led to the question whether one could identify the HLA-D determinants serologically. And now, serendipity once more came to the rescue (P. Medawar, personal communication: serendipity = looking for a needle in a haystack and coming up with the farmer's daughter).

In early 1972, Carl Koch came to my room and said: "I gave Mrs. P., who volunteered for our research, a skin transplant from an HLA-AB identical, MLC positive male donor. I had expected a transplant survival of 14 days, but we are already at day 16 and the transplant is not being rejected". I was of course curious to meet her, and we inspected the graft, which showed no trace of rejection.

At her next visit, a few days later, I sat down with her and we went through her complete prior history. It was clear that Mrs. P. had always been in good health, never had a transfusion and was the mother of three children. We of course wondered whether she might have made antibodies, that might have anything to do with the prolonged skin graft survival. A crossmatch between her serum and skin donor lymphocytes was negative. Thus, we were faced with a recipient of a skin transplant which showed an unexpected long survival, for which we did not have an explanation. We decided to develop a new protocol. The MLC test with Mrs. P. as responder and the skin transplant donor as stimulator was set up again, but now not only with AB serum but also with the serum of Mrs. P. And see, the positive MLC test was completely inhibited [84]. That something similar could happen also with HLA-A and -B anti-sera had been shown before by Ceppellini, but this was a completely different situation. According to the cross match, Mrs. P. had no antibodies to the skin donor who was HLA-AB identical.

This is one of the many times that Aad with her technical knowledge and enormous devotion made the impossible possible: a panel of individuals was composed, who were HLA- and-B identical with Mrs. P. and with the skin transplant donor. In addition, donors had to give a positive MLC test with Mrs. P. as the responder. We found that some of the combinations were inhibited by Mrs. P's serum and others not, suggesting polymorphism. The next question was whether this corresponded with the HLA-D determinants.

To identify the antibody that was responsible for the MLC inhibition, we performed all possible variations of the cross match, such as longer incubation times, medium adaptations, etc. but we did not get more than some indiscriminate reactions without any pattern. Prof Willy Hijmans and his technician Riek Schuit had moved to the Radio-Biological TNO Institute working on the aging process. They had developed an immunofluorescence technique, detecting a broader antibody spectrum than the micro cytotoxicity test. They thought their test could detect the MLC-inhibiting antibodies. The cells of the stimulators were incubated with the serum of Mrs. P. and an indirect fluorescence test was applied. They used as second antibody an anti-human globulin not reacting with the Fc receptor, which later was shown to be essential. It all sounds quite easy, but doing it was quite complicated. There was

one fluorescence microscope in Rijswijk (those things were extremely expensive in those days), and that one was being used all day. Aad was very welcome, after 6 PM! She left our department each evening with the prepared cells, and started a real Sisyphus job. Of each sample, at least 200 and sometimes 400 cells had to be counted, to calculate the percentage fluorescing cells This meant endless switching between phase-contrast and fluorescence microscopic fields. Aad told me that when she was driving home at 10 PM, she saw fluorescing cells everywhere and could not discriminate between a red or green traffic sign or a fluorescing cell. After many evenings, the work was done. For a generation that has grown up with FACS devices, it may be hard to imagine what it meant, especially because we were dealing with dozens of experiments and the necessary controls. It was in particular difficult because the number of fluorescing cells was quite low, while in spite of the anti-human gamma globulin not reacting with Fc, the negative controls still showed 5 to 10% positive cells, leading to a complex scoring.

The proportion of fluorescing cells after incubation with serum from Mrs. P. never reached more than 20–40%. Some cases were clearly much lower (4–10%). Were these all variants of a negative test, or was this a pattern? Aad thought the latter, and was close to desperation when I proposed to repeat all the tests. But she did it, and showed that most samples that were below 10% the first time, still gave responses under 10%, while the stronger reactions were also reproducible. The big moment came, when the results of the homozygous typing cell (HTC) panel and of the panel that Aad had used came in. Yes, there was a correlation! (Fig. 25) The serum of Mrs. P. reacted with the HLA-D3 determinant. We called the test "The MLC Inhibition test using SD (serologically defined) Identical Stimulator cells (MISIS)". It was a pity that the panels contained only 8–10 different cell populations (it had been difficult enough to get these), and the results were therefore not statistically significant, but showed a strong trend. This is one of the most important breakthroughs the department ever made, and this had not been possible if Aad had not been counting the green and red cells

Serum Pl.
Population data

Donor		Immunofluor.	LD XI	HL-A 8
	1	19	+++++	yes
	2	21	-	no
	3	15	+++	yes
	4	17	++	no
	5	17	++	yes
	6	25	-	no
	7	15	++++	yes
	8	16	++++	yes
	9	17	++++	yes
	10	19	++	yes
	11	7	-	no
	12	8	++++	no
	13	6	-	no
	14	8		yes
	15	6	-	no
	16	3	-	no
	17	2	-	no
	18	8	-	no
	19	6	-	yes
	20	6	-	no

Fig. 25. Early study showing that the HLA-D antigens detected in the MLC (LD = Lymphocyte Determined) can also be serologically determined by immunofluorescence.

Fig. 26. Aad van Leeuwen, the right hand of Jon van Rood, who played among others a crucial role in the serological detection of the HLA-DR antigens in Leiden.

all those evenings in Rijswijk.

We happily went to St Paul-de-Vence where Fritz Bach had organized a cellulology conference in a beautiful brand-new hotel, dug out in a mountain, and each chamber had its own little swimming pool. In addition, the meeting was excellent. We showed our data, the audience started off critically, at the end there was as Vincent Eijsvoogel later said: "a deep silence, and one realized that HLA-D could be identified with antibodies." [85,86] During the break, Dausset came to me and said: "My compliments! I am jealous." This test was the basis for the DR serology, but regrettably, the fluorescence techniques were too laborious for routine use in organ transplantation. That became only possible after we realized that the low percentage of positive cells was caused because HLA-DR was only expressed on monocytes and B cells. Aad was also the first to demonstrate that HLA-DR was present on activated T cells (Fig. 26). Regrettably, we never published that.

A variant of the test was developed thanks to Robert Winchester from the group of Kunkel from the Rockefeller Institute. He had made B cell lines (at those times not common everywhere), and the serum from Mrs. P. showed 100% fluorescence with these B cell lines; obviously because the DR antigens are constitually expressed on B cells. Winchester had come to Leiden because Willy Hijmans had organized a conference on immuno-fluorescence together with the New York Academy of Sciences. He was one of the keynote speakers and we also gave a presentation, with Winchester as co-author. We worked together on a draft and Winchester took it home to clean it up. When the paper came back, to our astonishment, his boss at the Rockefeller Institute, who had not been involved in the study at all, was senior author and not me. We had an "open" discussion in which he tried to convince me that this was as expected. Finally, the name of his boss was removed from the paper, and only Winchester was a co-author [85].

Hans Balner at the Primate Center had followed our activities closely and was the first to develop a cytotoxicity test with enriched B cells, which we copied quickly. We were now able to type a large panel and indeed, significant correlations were found for all the different specificities.

In 1975, we travelled to Aarhus, where Flemming Kissmeyer-

Nielsen organized the Sixth Histocompatibility Workshop. He had decided to decorate the meeting with posters, on which each department could show their presentations in a frivolous way. Ben Bradley, head of the cellulology lab, decided to publish a newspaper with the help of many others. The lead article had as heading: "HLA-D rally won by serology". It was very nice of him, as he was a real cellulologist! To our surprise, all the English groups had also discovered antibodies against HLA-DR antigens, using exactly the same techniques that we had used! It was never clear whether one of the reviewers leaked, or whether it was really coincidental that so many laboratories made the same discovery. A little later we developed a modified cytotoxicity assay in which the B cells were labeled by anti-human immunoglobulin in order to discriminate these cells from the T cells without the need to isolate the B cells [87]. This so called two-color fluorescence (TCF) assay has been used for quite some time for HLA-DR typing enabling the establishment of a clear HLA-DR match effect in renal transplantation. [88].

The long survival of the skin transplant of the HLA-AB-identical MLC positive donor due to the presence of MLC-inhibiting antibodies remained intriguing and raised the question again whether so-called enhancing antibodies existed. These antibodies would protect the transplant. There were some ideas that this could be the mechanism that might play a role in the spreading of cancer. In kidney transplantation there were several cases that survived very well in the presence of a class II antibody. As far as I know, a systematic study to analyze this has not been performed. Nevertheless, the HLA work really bloomed after the DR serology became a reality. The complexity of the HLA system could be studied now in a more efficient way. HLA-D and the serological equivalent seemed very closely related, if not identical, and at the 7th workshop, it was decided by Ceppellini, Walter Bodmer and me to introduce the name HLA-DR, standing for D related. The impact of HLA-DR serology on HLA and disease associations cannot be underestimated."

24. Building a department

"In 1972, the Department in Leiden was becoming well-structured. The Blood Bank had already expanded to 150 employees, with 25 academicians. The Department of Hematology had now four sections: clinical hematology led by Bruno Speck, hematomorphology led by Lopes Cardozo, coagulation led by Fredi Loeliger and Immunohematology led by van Rood. Our focus was histocompatibility related research that in order to define the optimal match between donor and recipient, which would permit a reduction in the use of immunosuppressive drugs, and thereby improve the resistance to infections.

25. Missed opportunity

Under this topic comes one of my major mistakes! In 1964, I was preparing the printing and defense of my thesis, and my main interest was the genetics of the newly discovered HLA (Group Four) antigens. I had arranged with the head nurse of the Diabetes department that of each patient, from whom blood was taken, an extra tube was collected and sent to Aad van Leeuwen, who was working on the genetics of these antigens. Aad showed me the data and pointed out that she was surprised to see that almost all patients had a positive reaction with serum no 16. I was busy and missed the clue! Much later, I realized that serum 16 recognized HLA-B8 in close linkage equilibrium with HLA-DR3, not so surprising in a population of diabetes patients! Subsequent studies on HLA associations were initiated in our department included both infectious and autoimmune diseases such as leprosy, rheumatoid arthritis, Type I diabetes and Celiac disease under the inspired guidance of Rene de Vries but these studies are outside the scope of this review. In the 1980s, the role of the HLA molecules greatly expanded beyond transplantation. Ceppellini once said, "There can be little doubt that the motivation of nature in selecting for a genetic polymorphism of this complexity, was not an a priori hostility against transplantation surgeons". In 1983, seven HLA loci and around 90 antigens were known (HLA-A: 20, HLA-B 42, HLA-C 8, HLA-D 12, HLA-DR 10 polymorphisms). Luckily, many antigens are often inherited together, a phenomenon known as linkage disequilibrium. Leiden had from the beginning been involved in the study of the relationship between HLA and autoimmune diseases and with infections.

26. Epilogue

26.1. 1981 The Nobel Prize does not go to van Rood

In 1980, the fever that Leiden might get a Nobel prize went to a top level. When van Rood attended a gala evening, CEO's of industry even congratulated him on the coming award. However, when the winners of the Nobel Prize in Physiology or Medicine 1980 were announced, the laureates were Baruj Benacerraf, Jean Dausset, and George D. Snell, for "their discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions".

Twenty years later, van Rood discussed this in a movie: "As a doctor, I had always shared my findings openly with other researchers, and not kept them secret till the papers had been published." He had also always been gallant to his peers, not blowing his own horn.

Following his retirement in 1991 [89], van Rood started a self-appointed volunteer job at Europdonor, among other things to raise the number of bone marrow donors, and continued his research on the significance of non-inherited maternal and inherited paternal HLA antigens, as a co-worker in the laboratory of Frans Claas.

26.2. Bone marrow transplantation

HLA matching provided the opportunity to find the appropriate bone marrow donor, initially from family members. The first three patients with immunodeficiencies were transplanted in 1968, two in Minneapolis and one Leiden. In Europe, the European Bone Marrow Transplant Group was founded in 1979. In 1985, van Rood was the founding president of the European Foundation of Immunogenetics and in 1988 he founded Europdonor (nowadays known as Matchis, the Dutch stem cell donor bank). He started to promote international exchange through Bone Marrow Donors Worldwide (1988), which started as a thin telephone book containing the HLA types of the available bone-marrow donors in the world (Fig. 27) and is currently a file of over

Fig. 27. Van Rood and van Leeuwen look at the first version of Bone Marrow Donors Worldwide. At the moment more than 32 million donors have been registered.

Fig. 28. Impression of one of the courses organized by Jon van Rood on a sailing ship.

Fig. 29. Jon van Rood carrying the signs of his many honorary doctorates.

32 million potential hematopietic stem cell donors worldwide. Furthermore, he established the Leiden Institute for Immunology (1987) and the World Marrow Donor Association (an initiative of John Goldman), which was incorporated in 1994. After his retirement, in 1991, Van Rood continued to do research, during the last years from a nice office at Europdonor, located at the corner of the LUMC campus, with a view on the trains at the Leiden railway station. During the last 25 years, he worked on a new area of research, the Non-Inherited Maternal Antigens (NIMAs).

26.3. NIMA: Non-inherited maternal antigens

Van Rood et al. describes the history of the discovery of the role of NIMA [63] as follows: “In the 1980s, the access to Homozygous Typing Cells obtained from cousin marriages offspring made it possible to identify so called acceptable mismatches in sera from highly immunized end-stage renal patients waiting for an unrelated renal allograft [90]. This finding has been instrumental for the acceptable mismatch program of Eurotransplant, which aims at the: identification of HLA antigens towards which the patients have not made antibodies in order to use this knowledge for kidney allocation in highly-sensitized patients. This program has enabled successful transplantation of many highly sensitized patients [91,92].

It turned out that the acceptable mismatches were often HLA-A and -B antigens known to be in high Linkage Disequilibrium. This could be explained by exposure to the Non-Inherited Maternal haplotype during fetal life, which induced tolerance for immunization in adult life (grandmother theory) as first described for the Rhesus-D blood group antagonism by Ray Owen in 1954 [93]. Although these Rhesus data have not been confirmed, the NIMA effect, as it was baptized, could be demonstrated in renal transplant recipients, where NIMA-mismatched haplo-identical sibling renal allografts did as well as HLA-identical sibling grafts [94].

The Inherited Paternal and Maternal antigens (IPA and IMA) are genetic markers that a fetus receives from its parents. Due to feto-maternal cell traffic the Non-Inherited Maternal antigens (NIMA) are seen by the child and, vise versa, the mother sees fetal cells that express the IPAs. Van Rood described this as follows: “Both mother and child recognize the “non-self” of each other and are able to regulate this recognition in such a way that rejection does not occur. The memory of this mutually-maintained balanced recognition for instance explains why a stem cell transplantation from mother to child is much more effective against leukemia than a stem cell transplantation from father to child".

Indeed, sharing a NIMA in a haploidentical sibling stem cell transplantation significantly reduced Graft-versus-Host disease [95]. Studies on bone marrow transplantations from the National Cord Blood Program (NCBP) in New York (the largest umbilical cord bank in the world) in collaboration with Europdonor revealed that patients who share a NIMA with the umbilical cord stem cell graft more often accept the graft, thereby expanding the pool of potential donors [96]. A role has also been attributed to the maternal immune responses against the IPA in the prevention of the relapse of leukemia. Microchimeric elements in the cord blood unit, most likely maternal T cells with anti-IPA immunity, are thought to exert a graft-versus-leukemia effect by attacking cells expressing IPA [97]. This anti-IPA immunity may also play a more general role in controlling malignancy in an offspring.

In 2016, when he turned 90, van Rood was still working daily at Europdonor, mainly on projects involving NIMA's and IPA's in stem cell transplantation: a project to reduce the recurrence of leukemia after cord blood stem cell transplantation with anti-IPA immune responses [97,98]responses. The last paper was published in 2017 and actually in the month when he passed away [98].

26.4. Inspirator

As a professor at Leiden University, van Rood stimulated many youngsters to go into research and become basic or clinician scientists. One of the ways how van Rood introduced young researchers to the

Fig. 30. Jon van Rood receiving the Robert Koch medal.

international research world was through meetings on a sailing ship, first the Eendracht, later the Dageraad. A group of around 30 people would spend several days on board of a ship, having lectures in the morning, sailing in the afternoon (Fig. 28), and eating and drinking, offering many opportunities to discuss science. One third of the researchers would be PhD students, one third settled Dutch researchers and one third international experts. Youngsters had access to the experienced researchers while working on the sails or while peeling potatoes or preparing scrambled eggs.

In the early 1970's, van Rood had an own sailing ship built, called “de Zeehond" (the Seal), that offered extra sleeping facility during the Dageraad courses and offered the opportunity to the PhDs of the department to discuss science while sailing on warm summer afternoons.

For many years, the Department of Immunohematology and Blood Transfusion was considered one of the most prestigious places for doing a PhD, but students had to work really hard and many never finished their PhD. Nevertheless, Van Rood was promotor of a total of 72 PhD students, including the authors of this review. Many of van Rood's PhD students became principal investigators and professors all over the world, making van Rood scientific grandfather of many more PhD students.

26.5. Honors

Although Jon van Rood did not received the Nobel Prize, he received many other signs of recognition including eight honorary doctorates from different universities in all over the world i.e. in The Netherlands, Belgium, Italy, France, Chili and Germany (Fig. 29). He became a Fellow of the Royal College of Physicians and the Royal College of Pathologists in London and a Foreign Associate of the National Academy of Sciences, USA. Van Rood has given many special lectures, including the prestigious van Loghem lecture of the Dutch Society for Immunology, the Ceppellini lecture of the ˜European Federation for Immunogenetics (EFI) and the Rose Payne award lecture of the American Society for Histocompatibility and Immunogenetics (ASHI). He was an honorary member of the Dutch Society of Immunology, received the Distinguished Service Decoration of the Dutch Red Cross, a Knighthood in the order of the Dutch Lion, and in 1991, on his retirement, he was made a Commander of the Order of Oranje Nassau. International awards include the Karl Landsteiner Memorial Award, the Robert Koch Medal (Fig. 30), the Wolf Price (Israel), The Amsterdam Prize for Medicine of the Royal Dutch Academy of Sciences (The Netherlands), the Medawar Prize (TTS) and the Artois-Baillet-Latour Prize (Belgium).

During the summer of 2017, van Rood was on holiday at his house in Lemmer, working on a grant application. After enjoying outdoor swimming, he did not feel well in the evening and died that same night. At the end of his inaugural lecture in 1965, van Rood addressed the members of the audience: “After my family it is you who through your friendship and team spirit have provided content to my work and my live. That is why I so often used the word “we”.” Indeed, his whole life has been focused on stimulating collaboration, with a focus on improving the lives of patients through scientific discoveries.

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