

On a Long and Winding Road: Alloantibodies in Organ Transplantation

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Abstract. Today we know that both the humoral and the cellular arm of the immune system are engaged in severe immunological challenges. A close interaction between B and T cells can be observed in most “natural” challenges, including infections, malignancies, and autoimmune diseases. The importance and power of humoral immunity are impressively demonstrated by the current coronavirus disease 2019 pandemic. Organ transplant rejection is a normal immune response to a completely “artificial” challenge. It took a long time before the multifaceted action of different immunological forces was recognized and a unified, generally accepted opinion could be formed. Here, we address prominent paradigms and paradigm shifts in the field of transplantation immunology. We identify several instances in which the transplant community missed a timely paradigm shift because essential, available knowledge was ignored. Moreover, we discuss key findings that critically contributed to our understanding of transplant immunology but sometimes developed with delay and in a roundabout way, as was the case with antibody-mediated rejection—a main focus of this article. These include the discovery of the molecular principles of histocompatibility, the recognition of the microcirculation as a key interface of immune damage, the refinement of alloantibody detection, the description of C4d as a footmark of endothelium-bound antibody, and last but not least, the developments in biopsy-based diagnostics beyond conventional morphology, which only now give us a glimpse of the enormous complexity and pathogenetic diversity of rejection.

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EARLY TRANSPLANT EXPERIMENTS AND THE BIRTH OF TRANSPLANT IMMUNOLOGY

The turn of the past century was a time of remarkable medical advances, including the establishment of immunology as an academic discipline and the beginning of experimental transplant research (Figure 1, Table 1). In the 1880s, Elie Metchnikoff championed the concept of cellular immunology by recognizing phagocytosis as an

elementary component of immunity.² At the same time, Emil von Behring and Shibasaburo Kitasato made their discovery of antibodies as key immunologic components.³ Von Behring envisioned the existence of antibody-producing cells, although plasma cells were not characterized until half a century later.²¹ Paul Ehrlich proposed a model for an antibody molecule with multiple branches for antigen binding and activation of complement,^{22,23} which had previously been discovered to help in killing bacteria.^{4,5} These early observations formed a critical basis for subsequent research in transplantation.

The first systematic skin transplant experiments started in the 19th century with George David Pollock being an early pioneer.¹ Skin transplantations figured prominently in research and were performed by many researchers, including Leo Loeb²⁴ and Georg Schöne.¹⁶ It was soon discovered that foreign skin could not be used to form permanent grafts^{25–28} and that second transplants were prone to accelerated loss,²⁹ which was in contrast to the success of transplantations between monozygotic twins.²⁷ In 1899, Alfred von Decastello conducted the first experimental kidney transplant,⁷ followed a few years later by Emerich Ullmann (both of whom worked in Vienna).^{8,9} In 1902, Ullmann attempted the first human organ transplant, anastomosing a pig's kidney into a young uremic woman. When the transplant failed, he noted that he could not overcome the technical difficulties.^{9,30} Ullmann did not relate his observations to rejection, although he was familiar with the principles of immunization, having been trained in rabies vaccination by Louis Pasteur (Ullmann himself

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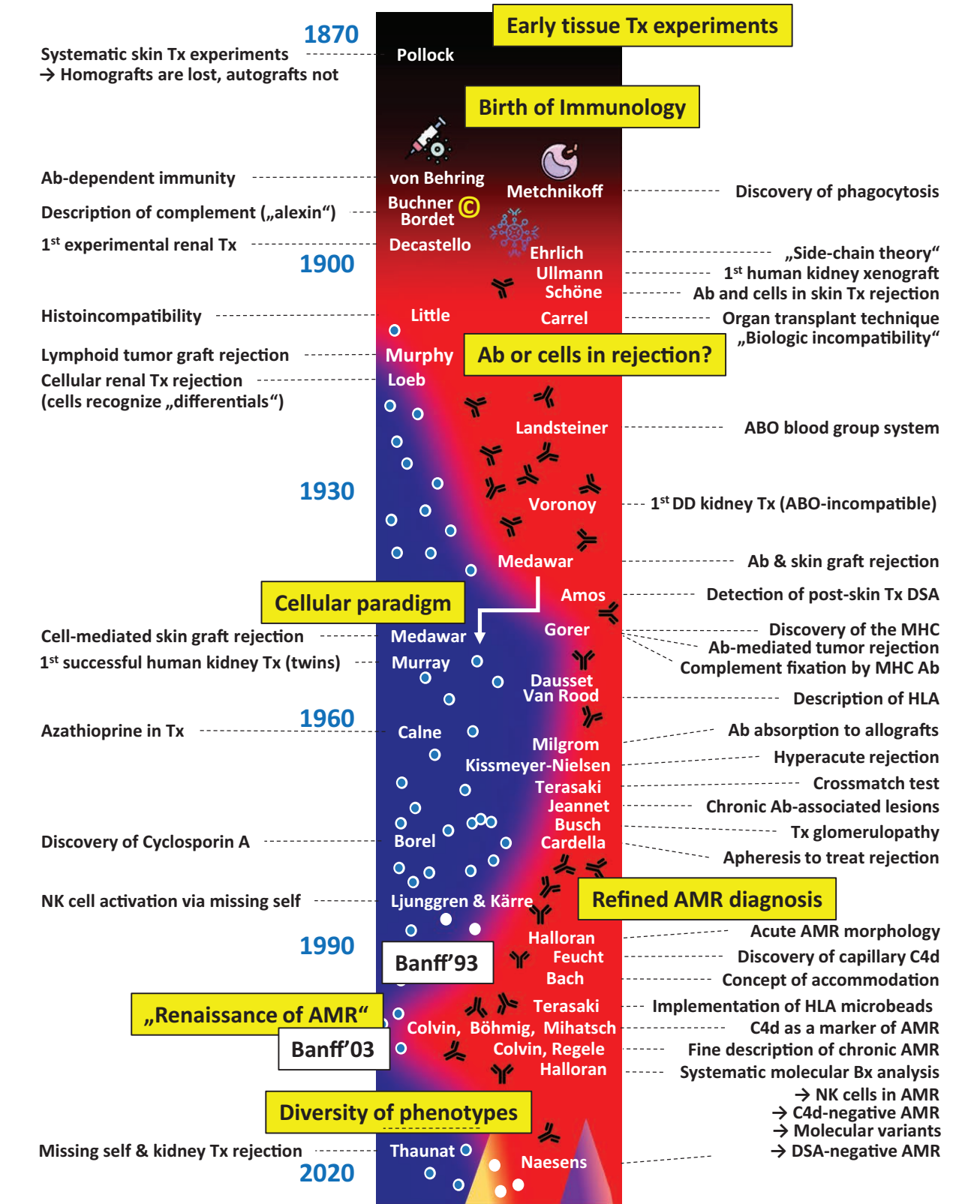


FIGURE 1. Discoveries contributing to our understanding of cellular-mediated rejection vs AMR. The scheme provides a selection of discoveries and researchers that have contributed to our understanding of rejection. Ab, antibody; AMR, antibody-mediated rejection; DD, deceased donor; DSA, donor-specific antibody; MHC, major histocompatibility complex; NK cell, natural killer cell; Tx, transplantation.

started a vaccination program in 1886).³¹ In the following years, Mathieu Jaboulay and Ernst Unger performed similar xenotransplantations, which also failed.^{14,15} It can be speculated that these early xenografts were lost because of hyperacute rejection, perhaps the first-ever clinical cases of antibody-mediated rejection (AMR).

TABLE 1.
Milestones in transplantation immunology from pre-1900 until 1930

Time	Achievements	Impact
1871	G.D. Pollock noted that skin allografts, unlike autografts, regularly failed. ¹ Numerous scientists subsequently performed systematic skin transplant experiments.	Foreign skin is not suitable as a permanent graft; second skin grafts from the same donor are prone to accelerated loss; skin grafts between monozygotic twins, however, are successful.
1893	E. Metchnikoff described phagocytosis and intracellular killing in host defense. ²	Can be regarded as the first report on innate cellular immunity (Nobel Prize awarded in 1908).
1890	E. Behring and S. Kitasato detected the antibacterial activity of sera from infected animals. ³	First report on acquired immunity; the existence of antibody-producing cells was envisioned (First Nobel Prize in Medicine for E. Behring in 1901).
1900	P. Ehrlich formulated the “side-chain theory,” a model of a branched antibody molecule with multiple binding sites, and coined the term “complement,” the former “alexin” described by H. Buchner ⁴ and J. Bordet. ⁵	Description of the interaction between innate and acquired immunity via complement (Nobel Prizes for P. Ehrlich in 1908 and for J. Bordet in 1919).
1900	Discovery of the ABO blood group system by K. Landsteiner. ⁶	First “transplantation antigens” (Nobel Prize in 1930); in his Nobel lecture, he already suggested that “ <i>serum reactions may be employed in the future for the important problem of transplantation.</i> ”
1902	E. Ullmann and, independently, A. von Decastello (both in Vienna) published their kidney transplant experiments in dogs. ^{7,8} The first transplant was performed by von Decastello in 1899.	All kidney transplants were a failure within the first days.
1902	E. Ullmann attempted the first xenotransplantation, a pig kidney, into a uremic woman. ⁹	Ullmann noted technical difficulties that he could not overcome.
1905	A. Carrel developed a new operative technique for vascular sutures and performed a variety of auto-, allo-, and xenotransplantations. ¹⁰⁻¹²	Technical basis for the transplantation of vascularized organs (first Nobel Prize for work related to transplantation in 1912). Noticing that allografts, in contrast to autografts, fail immediately, he suspected a role of “biologic incompatibility” in his Nobel lecture. ¹³
1906	M. Jabulay performed 2 xenotransplants in 2 women (pig and goat as donors). ¹⁴	Immediate graft failure.
1909	E. Unger transplanted an ape kidney into a young girl. ¹⁵	Graft never functioned. Unger suggested a “biochemical barrier.”
1911	G. Schöne described different outcomes of skin allo- and xenotransplantations in different experimental settings. ¹⁶	Recognizing the importance of relatedness between donor and recipient, the phenomenon of sensitization, the possible role of antibodies but also of cellular elements, the concept of “transplant immunity” emerged.
1914	J. Murphy performed heteroplastic tissue grafting (Ehrlich mouse-sarcoma into rats). Lymphoid reactions in tumor transplants could be abolished by X-ray treatment. ^{17,18}	Early hint to a “lymphoid barrier” in tumor transplantation.
1917	L. Loeb, after performing skin transplantations since 1897, recognized differences between auto- and allotransplantation of renal tissue. ¹⁹	Microscopic examination of kidney tissue revealed a characteristic morphology, including lymphocyte infiltrates into the lumen of tubules.
1933	Y. Voronoy transplanted a kidney from a deceased donor into a patient with mercuric chloride poisoning. ²⁰	First cadaveric kidney transplantation in man; multifactorial graft loss.

THE CONCEPT OF TRANSPLANTATION IMMUNITY—EMERGENCE OF AN “ANTIBODY PARADIGM”

At the beginning of the 20th century, Alexis Carrel revolutionized vascular surgery by developing a triangular anastomosis technique. This allowed him to overcome the technical difficulties encountered by Ullmann and to perform a variety of auto-, allo-, and xenotransplantation experiments.¹⁰⁻¹² Carrel—the first scientist to receive a Nobel Prize for work related to transplantation, particularly for developing vascular anastomosis—recognized early on that allografts inevitably failed. In his 1912 Nobel Prize lecture, he noted that “while the problem of the transplantation of organs has been solved from a surgical

point of view, we see that this by no means suffices to render such operations of definite surgical practicability, and it will only be through a more fundamental study of the biological relationships existing between living tissues that the problems involved will come to be solved ...”¹³ Carrel may be considered the “father of transplant surgery,” but he was also the first to note “inflammation” in rejected transplants: “The histological examination of the transplanted kidneys showed that the organs presented some lesions, very slight in some cases and more marked in others. The lesions were of diffuse nephritis. It is very probable that in the transplantation of the kidneys, as in the transplantation of the limbs, a reaction of the organism against the new organ takes place after a few days ...”¹³

Carrel’s “biologic incompatibility” was further elaborated by Leo Loeb, who considered a role of both antibodies and lymphocytes, the latter sensing the “finest degrees of difference.”³² In 1916, he had already described the distinct morphology of cellular kidney allograft rejection, including the penetration of lymphocytes into the lumen of tubules.¹⁹ In a book published in 1911, Georg Schöne detailed his skin and tumor transplant experiments, their association with the relatedness between donor and recipient, and the phenomenon of sensitization and pronounced rejection.¹⁶ Schöne, who coined the concept of “transplantation immunity,” speculated on antibody-mediated injury and a “fight of cellular elements” at a time when the principles of histocompatibility were not yet established.¹⁶ A role of antibody responses as an obstacle to transplantation was also proposed by Karl Landsteiner, the person who discovered the ABO blood group system. In his 1930 Nobel lecture, he noted that “serum reactions may be employed in the future for the important problem of transplantation, although the knowledge available at present justifies no more than a hope in this direction.”⁶ In 1933, Yuri Voronoy performed the first allogeneic transplant of a deceased donor’s kidney. The immediate loss of this transplant may have been multifactorial, although ABO incompatibility may have contributed considerably.²⁰

TRANSITION TO A “CELLULAR PARADIGM”

Early tumor transplant experiments contributed significantly to our understanding of immunology.³³ Clarence C. Little showed in inbred mice (including the “Japanese Waltzing Mouse” developed by 19th-century “mouse fanciers”) that isografts succeed and allografts fail, a phenomenon he described to be controlled by Mendelian inheritance rules.^{34,35} He postulated the codominantly expressed immunogenic products encoded by a large number of loci, essentially laid the groundwork for transplant genetics, and provided the basis for what George Snell later formulated

as the “5 laws of transplantation.”³⁶ Important achievements made between 1930 and 1960 are listed in Table 2. Among them are Peter Gorer’s groundbreaking discoveries. Gorer identified the H-2 histocompatibility antigen in mice and showed the existence of anti-major histocompatibility complex (MHC) antibodies (mice have H-2 class I on red cells; he used hemagglutination to discover H-2 and anti-H-2).^{39,45-47} He may have been the first to demonstrate alloantibody-triggered rejection (of tumor grafts),⁴⁸ and he discovered that anti-MHC can fix complement in vitro,⁴⁹ an important foundation for Paul Terasaki’s cytotoxic crossmatch test. Gorer, whose work was beautifully described in a tribute by Peter Medawar,⁵⁰ can be considered the “father of MHC” and perhaps the “father of AMR.” In 1961, he wrote, “Five years ago, all of us argued about ‘the homograft problem’ as if there were only 1 possible method of graft destruction. Today, no well-informed person takes this view. We all agree that specifically sensitized cells play a major role and that humoral antibodies may be found at high titer. In those cases where both types of immune response are known to occur, we are very interested in finding out the relative importance of each and the ways in which the sensitized cells and humoral antibody may interact ...”⁵¹ He died that same year and was therefore not eligible for Nobel Prize consideration.

George Snell showed that the H-2 “gene” was a complex of at least 2 genes.⁵² Later, Jean Dausset and Jon van Rood independently described sera containing antibodies against antigens that turned out to be HLA.^{43,44} In 1980, Snell and Dausset, together with Baruj Benacerraf, received the Nobel Prize for the discovery of the MHC. The groundbreaking achievements of these pioneers formed a crucial basis for subsequent research.

By the 1950s, several observations heralded the transition to a broadly accepted “cellular paradigm.” This is perhaps best illustrated by the work of Peter Medawar, one of the most influential transplant researchers. In a series of skin transplant experiments, he characterized

TABLE 2.
Milestones in transplantation immunology from 1930 to 1960

Time	Achievements	Impact
From 1940	Using skin transplants in animals, P. Medawar characterized the “homograft problem”—first by the action of antibodies, then by lymphocytes. ^{28,37,38}	Transition to a “cellular paradigm” in transplantation (Nobel Prize for P. Medawar in 1960, together with Macfarlane Burnet, for the discovery of acquired immunological tolerance).
1948	Identification of the H-2 histocompatibility antigen in mice by tumor transplantations (independently) performed by P. Gorer and G. Snell. ³⁹	The beginning of “histocompatibility research” (Nobel Prize for G. Snell in 1980—P. Gorer died in 1961).
Around 1950	A series of “unmodified” human kidney transplantations (also involving ABO-incompatible transplants).	Graft failures—role of antibody-mediated injury was suspected.
1954	D. Amos found the formation of antibodies in response to skin allografts. ⁴⁰	The start of serological tests, in this case, agglutination tests, for the detection of alloantibodies.
1954	J. Murray performed renal transplantation between genetically identical twins. ⁴¹	Long-term graft survival (without immunosuppression); Nobel Prize in 1990 (together with E.D. Thomas, founder of bone marrow transplantation).
1955	D. Hume summarized contemporary knowledge and analyzed the results obtained with 9 human kidney transplants in the absence of immunosuppression. ⁴²	Based on these early clinical results, Hume concluded that transplantation for the treatment of human disease was not yet justified.
1958	J. Dausset and J. van Rood detected alloantibodies against leukocyte antigens in human sera. ^{43,44}	The emergence of the Human Leukocyte Antigen system (Nobel Prize for J. Dausset in 1980). J. van Rood later became the founder of Eurotransplant.

the “homograft problem” in great detail.^{28,37,38} Medawar, who, together with Macfarlane Burnet, was awarded the 1960 Nobel Prize for the discovery of acquired immunological tolerance, described the immunological nature and donor specificity of skin graft rejection, which he found to be dominated by cellular infiltrates. Notably, in his early publications, he favored “serum-borne antibodies” as the cause of rejection, pointing out that “the antibody generated by skin homografts is such that it specifically prevents the completion of nuclear division in the cells of homologous grafted skin ...”^{28,37,38} Later, however, Medawar became a strong advocate of a “cellular theory,”⁵³ supported by Avron Mitchison’s observation that immunity to tumor grafts could be transferred with lymphocytes but not with antibodies.⁵⁴ It should be remembered that another early mastermind, James Murphy, already advocated a cellular theory in the 1910s; he described a lymphoid reaction against tumor grafts and used X-ray treatment to overcome this “lymphoid barrier.”^{17,18} Indeed, Murphy may have been the first person to conclusively establish a role of lymphocytes in rejection, but contemporary researchers paid little attention to his investigations.⁵⁵

In the early 1950s, a number of kidney transplants were performed in France and in the United States. The frequent finding of cellular infiltrates, as has previously been described in experimental models,^{56,57} indicated a role of cellular immunity. However, it was difficult to put these observations into a clear immunological concept. It must be borne in mind that at that time, the basics of T- and B-cell immunity and the principle of T cell help for B-cell activation, first described by Henry Claman in 1966,⁵⁸ were not yet established, and the close interaction between these 2 branches of adaptive immunity—common knowledge today—was only gradually being recognized. In an impressive, unadorned report published in 1955, David Hume provided a detailed description of the results obtained from 9 human kidney transplants.⁴² Five transplants failed to develop measurable function, and the clinical course in all patients was dominated by infections, bleedings, and infarction. When discussing rejection mechanisms, he was in favor of acquired immunity based on the causative role of antibodies. Hume thereby challenged Loeb’s theory of lymphocytic infiltration brought about by “differentials”: “We agree with Medawar, Dempster, and Simonsen that Loeb’s theory of differentials is very confusing, and at times ‘impenetrably metaphysical’” However, at the same time, he pointed out that “the chief objection to the theory of acquired immunity at the present time is that it has never been possible to detect circulating antibodies either following the initial transplant or on repeated ‘immunization’ of the animal ...”⁴² At this time, Hume may not have been aware of the first descriptions of serologic donor-reactive antibody detection.^{40,59} Apparently unimpressed by the disappointing results of early attempts of kidney transplantation, but building on the previous genetic and immunologic discoveries and René Küss’s innovative surgical method of placing kidneys into the iliac fossa,⁶⁰ Joseph Murray went ahead and performed a successful renal transplant between identical twins in December 1954.⁴¹

Along with the growing prestige of T cells, there has been a long-running competition between proponents of a cellular versus a humoral theory in transplant immunity (Figure 1). The cellular paradigm finally prevailed, and although it may have evolved in a roundabout way, it came to dominate

transplantation medicine for decades. It should be remembered that many key observations were made in skin transplant experiments. However, skin may not be an ideal target for AMR.^{61,62} Several days must elapse until recipient and donor capillaries come into contact (inosculation). Thus, preformed alloantibodies do not reach their target, hyperacute rejection cannot occur, and ABO incompatibility is irrelevant. In the later phase of vascularization, donor capillaries are replaced by recipient capillaries and antibody-dependent reactivity appears to be blunted, whereas T cells dominate rejection.^{61,62} It is questionable whether it was justified to generalize the results of skin grafting and apply them to the transplantation of vascularized organs.

EMANATIONS OF THE “CELLULAR PARADIGM”

The cellular paradigm has greatly influenced research and clinical practice in 3 important areas of transplantation medicine. Conversely, results in these areas have strengthened this paradigm for many years (important discoveries made in the period between 1960 and 1990 are listed in Table 3).

Development and Application of T Cell-directed Immunosuppressive Therapies

With the experiences in Boston, Paris, and elsewhere, it was clear that the endogenous immunosuppression associated with uremia would not be sufficient in preventing rejection. After a short period when total body irradiation was used,^{82,83} the era of therapeutic immunosuppression began in the 1960s with the availability of azathioprine, a derivative of 6-mercaptopurine, which had been shown by Robert Schwartz and William Dameshek to exert profound immunosuppressive activity.⁸⁴ Azathioprine was first used by Roy Calne in transplant experiments with dogs.⁶³ Shortly thereafter, it was used in patients, usually along with radiation, actinomycin C, or steroids.^{64–66} Last but not least, these early developments in immunosuppression made the first human heart transplant, performed by Christiaan Barnard, possible.⁷⁰ As a next step, lymphocyte-depleting antisera were generated in animals, and after a series of transplant experiments, including Byron Waksman’s studies in guinea pigs,⁸⁵ they were applied in patients to counteract rejection. Thomas Starzl, famous for having performed the first human liver transplant,⁶⁷ was one of the pioneers who introduced antilymphocyte globulin into clinical transplantation.⁸⁶ Later, George Köhler and Cesar Milstein’s 1975 invention of the hybridoma technique⁷⁴ allowed for the generation of monoclonal anti-T-cell antibodies, with OKT3 being the first used in clinical routine.⁸⁷ A real breakthrough came in 1976 when Jean Borel discovered the immunosuppressive activity of cyclosporine A.⁷⁵ It was Calne who then reported on the use of cyclosporine A in clinical transplantation,⁷⁷ and in subsequent years, large trials have confirmed the superior immunosuppressive potency of this compound, which was licensed for use in renal transplantation by the early 1980s. Another calcineurin inhibitor, tacrolimus, even surpassed these favorable results, and after the publication of the Elite Symphony multicenter trial,⁸⁸ this agent remains the preferred immunosuppressant today. T-cell suppression is presumed to be the “gold standard” of immunosuppression to this day and fostered the “cellular paradigm” in transplantation.

TABLE 3.
Milestones in transplantation immunology from 1960 to 1990

Time	Achievements	Impact
1960	Use of 6-mercaptopurine as immunosuppressant in dogs by R. Calne. ⁶³	Nobel Prize (1988) for G.B. Elion and G.H. Hitchings for development of purine analogs; Alternative to whole body irradiation
1961	Azathioprine became available as immunosuppressant in humans. ⁶⁴⁻⁶⁶	Standard immunosuppression for many years together with steroids.
1963	T.E. Starzl performed the first liver transplantation. ⁶⁷	The concept of the acute rejection process as a reversible phenomenon was developed.
1964	Development of the microcytotoxicity test by P.I. Terasaki and J.D. McClelland. ⁶⁸	Beginning of clinical liver transplantation.
1966	F. Kissmeyer-Nielsen described hyperacute rejection of kidney allografts caused by preexisting antibodies against donor cells. ⁶⁹	Enabled and standardized large-scale tissue typing and antibody screening.
1967	C. Barnard performed the first heart transplantation in humans. ⁷⁰	Proof that grafts can be destroyed immediately by humoral factors in the absence of cellular infiltrates.
1969	Introduction of the crossmatch test by R. Patel and P.I. Terasaki. ⁷¹	Beginning of clinical heart transplantation.
1970	M. Jeannet observed the de novo production of alloantibodies in recipients of renal grafts. ⁷²	Eliminated hyperacute transplant rejections.
1971	G.J. Busch described a distinct glomerular lesion in renal transplants (transplant glomerulopathy). ⁷³	Suggested humoral alloreactivity as an important trigger of chronic rejection.
1975	Development of the hybridoma technique and production of monoclonal antibodies by G. Köhler and C. Milstein. ⁷⁴	Still a leading criterion of chronic antibody-mediated rejection.
1976	J. Borel detected antilymphocytic activity in an antifungal agent. ⁷⁵	Enabled fundamental improvements in diagnosis and therapy.
1977	C.J. Cardella used plasmapheresis to counteract renal graft rejection. ⁷⁶	Nobel Prize for G. Köhler and C. Milstein in 1984 (together with N.K. Jerne).
1979	Introduction of cyclosporine A as a clinically used drug by R. Calne. ⁷⁷	Basis for the development of cyclosporine A.
1982	G. Opelz initiated the international Collaborative Transplant Study. ⁷⁸	First promising therapeutic approach in otherwise untreatable cases with suspected humoral rejection.
1983	M.R. Garovoy introduced flow cytometry in crossmatch testing. ⁷⁹	Beginning of the era of calcineurin inhibition in immunosuppression.
1983	K. Mullis developed the polymerase chain reaction. ⁸⁰	Provided a platform for the analysis of factors relevant for transplant survival.
1985	First description of the principle of missing-self-triggered natural killer cell activation by H.G. Ljunggren and K. Kärre. ⁸¹	Greatly improved sensitivity of crossmatches.
		Revolutionized genetic analyses; Nobel Prize for K. Mullis in 1993 (together with M. Smith).
		A groundbreaking discovery that shed light on the principles of activation.

The armamentarium of anti-T-cell reagents has continued to expand and a better understanding of the molecular mechanisms behind T-cell activation has ultimately led to the development of the costimulation blocker belatacept.⁸⁹ For a long time, it was not known that effective T-cell suppression would also inhibit B cell- and antibody-dependent alloreactivity. As such, the frequency and severity of AMR may have been reduced or postponed to the late posttransplant period. Thanks to the favorable results obtained with immunosuppressive compounds targeting T cells, no need was felt to develop therapies specifically directed against B cell- and antibody-driven alloimmunity. This need came up much later when it was recognized that “conventional” immunosuppression failed to fully prevent immunologic graft damage and did not allow for indefinite long-term graft survival. Even today, rejection still constitutes the leading cause of graft failure.^{90,91}

No Routine Immunological Monitoring in the Posttransplantation Period

In the early period of the “cellular paradigm,” the most dangerous attack on a transplant still was hyperacute rejection, a rejection phenotype first reported by Paul Terasaki in

1964,⁹² and then described in detail by Flemming Kissmeyer-Nielsen.⁶⁹ Starzl’s interpretation was that of an immune-mediated coagulopathy resembling a Shwartzman reaction, a thrombohemorrhagic phenomenon triggered by injection of endotoxin.^{93,94} Terasaki solved the problem by establishing the lymphocytotoxicity crossmatch to exclude recipients with preformed complement-fixing DSA.^{68,95} The test was quickly adopted into clinical routine and (combined with ABO compatibility) has virtually eliminated hyperacute rejection. Once this “humoral danger” could be avoided, T cell-mediated rejection (TCMR) became the focus. Nevertheless, several researchers have continued to collect evidence on the relevance of AMR beyond hyperacute rejection. It was known from animal studies, including those by D. Bernard Amos in the 1950s,⁴⁰ that skin grafts can induce the formation of de novo alloantibodies. Stetson and Demopoulos were able to demonstrate rejection of skin grafts in rabbits triggered via passive serum transfer.⁹⁶ Rejection was also induced by sensitized lymph node cells held away from skin grafts by placing them within diffusion chambers, a strong hint toward the role of antibody-mediated injury.⁹⁷ In 1963, Altman et al⁹⁸ showed cytotoxic antibodies appearing after skin or kidney transplantation in dogs. In their experiments, they could also

passively transfer homograft immunity. Notably, in his early experimental work, Terasaki reported on antibody-triggered renal damage in mice via injection of homologous antisera.⁹⁹ Other experimental models, however, pointed to a phenomenon of “enhancement”—the ability of alloantibodies to enhance graft survival—which dominated the literature in the 1970s and 1980s and probably contributed to the confusion around whether antibodies could mediate rejection in humans.¹⁰⁰

In the 1960s and 1970s, some authors also described posttransplant alloantibodies in clinical transplantation. These turned out to be associated with rejection.^{71,101–104} Among them were Milgrom et al¹⁰¹ who also observed the phenomenon of antibody adsorption to allografts. In 1970, Jeannot et al⁷² suggested a role of chronic AMR. In their landmark study, they described distinct morphologic changes, such as thickening of the glomerular basement membrane and obliterative vascular lesions.⁷² However, posttransplant antibody monitoring in those early days was primarily performed in the context of research protocols and had not found its way into the broad clinical routine.

Bias in the Histopathological Assessment of Transplant Biopsies

In the early days of clinical transplantation, the immunological status of an organ transplant was primarily assessed via biopsies. However, some pitfalls have emerged in their interpretation. In a solid organ transplant, the endothelial surface is the first biological border encountered by the recipient's immune system (does not apply to skin, bone marrow, or experimental tumor grafts). Because endothelial cells carry important transplantation antigens (MHC; blood group determinants), they not only stimulate immunological attacks but are also their main targets. Antibodies and complement factors cannot easily cross this barrier, whereas the components of cell-mediated immunity can exit the vascular space to invade the surrounding tissues, resulting in cellular infiltrates.

Kendrick Arthur Porter published a comprehensive overview of kidney transplants in 1965.¹⁰⁵ In this work, he detailed lesions that sometimes appeared without typical cellular infiltrates. These included necrotic vascular lesions associated with immunoglobulin (Ig)G deposits in the walls of arteries and arterioles and obliterative lesions in grafts with prolonged survival.¹⁰⁵ Lindquist et al¹⁰⁶ noted the role of interstitial capillaries as a site of alloimmune challenge. A few years later, Busch et al⁷³ created the term “transplant glomerulopathy” to describe a distinct glomerular lesion associated with antibody binding to the endothelium. Some experimental models revealed IgG binding and complement fixation (C3 deposits), albeit with great variations.^{94,107–109} In a short-term perfusion model of renal allograft rejection, Sellin et al¹¹⁰ observed vascular C3 fixation along with IgG deposits as an indicator of local complement activation. Their results suggested a direct role of antibodies in the graft destruction process, supported by distinct morphologic features of endothelial injury.¹¹⁰ Interestingly, the localization of immune deposits in Sellin's experiments differed from the primarily peritubular localization found in rat experiments reported by Lindquist et al.¹⁰⁹ Several authors have reported comparable immune complex deposition in the vasculature of

human renal allografts, even outside of hyperacute rejection.^{111–114} It was also repeatedly recognized that in some cases of rejection, there was a conspicuous margination and accumulation of cells within the capillaries.

Despite these observations, the detection of Ig and complement deposition was rather inconsistent. Interest in the humoral part of rejection, therefore, waned within a few years. AMR, as we now recognize it, is not associated with IgG staining, perhaps because of the high turnover of endothelial surfaces, and this probably contributed to the failure to recognize AMR for decades. Instead, pathologists turned to cell-mediated lesions in arteries/arterioles, glomeruli, and the tubular system, particularly interstitial infiltrates and described them extensively. The prominent glomerular capillaries could not be overlooked, whereas peritubular/interstitial capillaries were no longer in focus and may even have escaped detection. For example, in the first Banff classification of renal allografts, published in 1993,¹¹⁵ the term “peritubular capillaries” (PTC) appears only once in a table, in the context of “hyperacute rejection.” In the updated version in 1997,¹¹⁶ interstitial capillaries gained 2 citations. AMR was “suspected” at best in some cases with endarteritis, glomerulitis, and appearance of interstitial polymorphonuclear leukocytes. At the height of the cellular paradigm in the 1980s, fine needle aspiration was advocated to evaluate parenchymal and graft infiltrating cells.¹¹⁷ Without anatomical coordination, a diagnosis of AMR was practically impossible using this (short-lived) method (which was complicated by contamination of the aspirate by blood cells). In hearts, interstitial capillaries were overlooked as well. In the first working formulation for the standardization of heart transplant rejection,¹¹⁸ the term “capillary” does not appear. As a result of this, precisely this biological border received little attention in 2 major diagnostic disciplines, namely histopathology and immunopathology. This was the case for many years.

RENAISSANCE OF HUMORAL IMMUNITY—THE EMERGING CONCEPT OF AMR

The renaissance of AMR in transplantation began in the early 1990s with the reappraisal of rejection-associated capillary lesions. These observations led to a fundamental revision in pathology/pathophysiology and, finally, to the establishment of a comprehensive concept of AMR (important achievements of the past 3 decades are provided in Table 4).

Return of the Capillaries

Within the mammalian nephron, 2 capillary beds are arranged in sequence. One is the glomerular capillary convolute, embedded between 2 arterioles in a unique anatomical structure. The other is the peritubular capillary plexus, which originates from the glomerular efferent arteriole, is then distributed in the interstitium along the tubular system, and finally drains into the venous system. Because of marked differences in hemodynamic forces, extravasation of alloreactive lymphocytes probably occurs much more easily in PTC than in turbulent glomerular capillaries. In cell-mediated rejections, this may explain the focal distribution of interstitial lymphocytic infiltrates, leading to tubulitis. In a highly inflammatory situation involving humoral immune reactants such as alloantibodies and complement proteins, which are not constrained

TABLE 4.
Milestones in transplantation immunology from 1990 to today

Time	Achievements	Impact
1990	P. Halloran defined a special morphology of rejection associated with alloantibodies in serum. ¹¹⁹	Beginning of comprehension of AMR as an entity.
1991	Detection of capillary deposition of complement C4d in renal transplants by H.E. Feucht. ¹²⁰	Proof that alloantibodies can act directly on graft endothelium; strong evidence for AMR as an entity.
1991	Based on his observations in xenotransplant experiments, F.H. Bach first suggested a role of transplant accommodation. ¹²¹	Description of a new principle of graft protection from antibody-mediated tissue injury.
1993	Results of the first Banff Conference on kidney transplant pathology published by K. Solez. ¹¹⁵	Generally accepted scheme for the pathological classification of (cell-mediated) transplant rejections.
1998	Development of microbead assays for the detection of alloantibodies by R. Pei. ¹²²	A new test system to amend or even replace cell-based detection systems.
2001	G. Böhmig reported on successful use of immunoadsorption to treat acute C4d-positive humoral rejection. ¹²³	Proof that humoral rejection is principally reversible.
2002	Fine description of the phenotypic presentation of chronic a by S. Mauiyyedi ¹²⁴ and H. Regele. ¹²⁵	Major basis for the standardization of the criteria of chronic AMR phenotypes.
2002	J. Platt describes positive C4d staining in stable ABO-incompatible kidney grafts and proposes a role of “accommodation.” ¹²⁶	First description of C4d staining without morphologic rejection evidence, an immunological peculiarity of ABO-incompatible transplantation.
2003	Detection of complement on microbeads by M. Wahrmann. ¹²⁷	First description of a bead-based technology to characterize the complement-activating capability of anti-HLA antibodies.
2003	Incorporation of AMR into Banff scheme. ¹²⁸	Forced pathologists to consider AMR.
2005	P. Halloran initiated the Edmonton project to systematically establish and validate a molecular platform for assessing rejection phenotypes. ¹²⁹	Critical contribution to a better understanding of the pathophysiology, dynamics, and phenotypic variants of transplant rejection and their clinical relevance. The objective evaluation of gene expression patterns enables a major improvement in biopsy-based diagnostics.
2005	Revision of the ISHLT criteria for the rejection of heart transplants. ¹³⁰	Formal acknowledgment of AMR in heart transplantation.
2005	D. Dragun first described a potential role of antibodies against the angiotensin II type 1 receptor in transplant rejection. ¹³¹	Early description of an alternative pathomechanism of AMR involving non-HLA autoantibodies.
2009	Description of C4d-negative AMR by B. Sis. ¹³²	Expansion of the spectrum of AMR phenotypes.
2010	L. Hidalgo described gene expression data suggesting a pivotal role of NK cells in AMR. ¹³³	This work brought NK cells into focus as important players in rejection processes.
2015	First use of imlifidase to cleave antibodies. ¹³⁴	A new therapeutic principle of removing antibodies has found its way into transplantation medicine.
2019	A. Koenig et al ¹³⁵ systematically deciphered the role of missing self-recognition by NK cells in the context of organ transplantation.	Description of a new antibody-independent pathomechanism potentially contributing to DSA-negative AMR.
2020	Systematic description of DSA-negative morphologic and molecular AMR by J. Callemeyn ¹³⁶ and P. Halloran. ¹³⁷	Further extension of the multifaceted phenotypic pattern of AMR.
2022	First transplantation of a genetically engineered pig heart into a human by B. Griffith. ¹³⁸	The graft was lost after 49 d, but without typical features of rejection. A milestone on the way to a possible solution to the organ shortage worldwide.

AMR, antibody-mediated rejection; DSA, donor-specific antibody; ISHLT, International Society of Heart and Lung Transplantation; NK cell, natural killer cell.

by hemodynamic forces, leukocytes may also invade the glomerular space, leading to glomerulitis.

Not all researchers lost interest in interstitial capillaries during the “cellular paradigm,” some continued to gather valuable knowledge and suggested an important role of PTC as a sensitive alloimmune target.^{119,139-143} In the 1990s, Philip Halloran^{119,141,142} made a key contribution to the field by characterizing the distinct morphological picture of AMR, which turned out to be very different from that of TCMR. He suggested that PTC are a primary target of anti-HLA antibodies based on a phenomenon of margination of inflammatory cells (granulocytes and mononuclear cells) in congested PTC.^{119,141,142} Halloran’s descriptions also included other features, such as capillary microthrombi and fibrinoid necrosis of transplant vessels,¹⁴² findings that were reminiscent of those previously

described for hyperacute rejection, albeit less pronounced (and not consistent features of AMR). Subsequent studies by others characterized marginating mononuclear cells in AMR as being predominantly monocytic cell populations.^{144,145} It was also recognized that rejection can lead to typical ultrastructural changes in the long-term, such as the multilayering of PTC basement membranes, which was first described by Monga et al,¹⁴⁶ or transplant glomerulopathy, a lesion characterized in detail by Zollinger et al.¹⁴⁷ Later it became clear that these lesions were characteristic manifestations of chronic AMR.^{125,148} Ginette Lajoie suggested that AMR can even cause the fragmentation and disappearance of PTC endothelial cell lining in some intertubular areas (interestingly without features of tissue necrosis and infarction),¹⁴⁸ but such observations need to be confirmed.

Pathologist's Dilemma: Hidden Targets Attacked by Invisible Missiles

Ever since the work of Albert Hewett Coons made immunofluorescence studies in tissues possible,¹⁴⁹ the components of humoral immunity have been sought in transplant biopsies, but with inconsistent results. This was probably the key reason why alloantibodies were not considered important in rejection. In the 1980s, however, Giuseppe Andres and Jan Brentjens¹⁵⁰ noted that because of the rapid redistribution of antigens or because of capping, shedding, or internalization of Ig and complement, along with clearance mechanisms in the blood stream, antibodies, or immune complexes do not adhere to endothelial surfaces for long, which impedes their detection.¹⁵⁰

The solution to the problem would come with the implementation of C4d staining. This complement split product is covalently bound to the endothelium, where it can serve as a “footprint” of complement activation through otherwise undetectable alloantibody binding.¹⁵¹ In the 1980s, a small group of young researchers in Munich studied the renal metabolism of C4, established a panel of monoclonal antibodies, and came across C4d. Coincidentally, they also applied C4d staining to transplant biopsies. Their surprising findings were, however, met with great skepticism, first by the local authorities (pathologists, nephrologists, and transplant surgeons) and then by the reviewers/editors of the leading medical journals. After “rejections” there, the description of capillary C4d in renal grafts finally appeared in 1991¹²⁰ in a journal loved by clinical immunologists but less so by the transplantation authorities. Neither a second report demonstrating the clinical relevance of capillary C4d¹⁵² nor the subsequent publications^{151,153} were able to attract the community's attention. For certain reasons (still obscure to author H.E.F.), almost 10 y passed until the “C4d technique” was generally accepted, when centers in Boston, Vienna, Basel, and other universities used C4d staining and published their results.¹⁵⁴⁻¹⁵⁸ In this regard, Harvard pathologist Robert B. Colvin played an important role in bringing C4d to the attention of the transplant community. The Vienna group generated a polyvalent anti-C4d antibody that could be applied to paraffin sections and thus contributed to the dissemination of the technique.¹⁵⁶ In this context, it is also noteworthy that Halloran actually presented the concept of AMR as a microvascular disease at the first Banff meeting in 1991, but there was resistance among pathologists to the inclusion of AMR in the Banff system, and it was only the widespread confirmation of C4d staining that led to its acceptance.

The discovery of capillary C4d can be regarded as a powerful catalyst for our current understanding of transplant rejection, which was entirely in the spirit of a lecture held by the famous physiologist Ivan Petrovich Pavlov who emphasized that “Science moves in spurts, depending on progress made in its methods. With each step forward in methods we rise, so to speak, to a higher step, from which a wider horizon opens to us, with subjects previously unseen ...” C4d staining has led to widespread acceptance of the concept of AMR beyond hyperacute rejection, which also included the phenotypic presentation of chronic AMR.^{124,125} Pathologists may initially have been reluctant because of the large discrepancies between conventional histology and immunopathology—in numerous cases,

capillary C4d would indicate severe, refractory rejections even in the absence of relevant cellular infiltrates.

A critical change occurred in 2003 when C4d was included as a diagnostic criterion in the Banff classification, along with glomerulitis and capillary margination as important morphologic features.¹²⁸ The Banff '05 meeting report included glomerular double contours (transplant glomerulopathy) and PTC basement multilayering as diagnostic criteria for chronic active AMR¹⁵⁹ and the Banff '07 scheme introduced an algorithm for scoring of PTCitis.¹⁶⁰ Rejection criteria were also reformulated in heart transplantation, and in 2005, AMR was finally addressed in a revised scheme created by the International Society for Heart and Lung Transplantation.¹³⁰ In 1999, 2 studies had already shown C4d deposits in transplanted hearts. One connected C4d with immunological rejection,¹⁶¹ the other primarily with ischemic injury¹⁶² (the International Society for Heart and Lung Transplantation initially preferred the ischemic variant). The upcoming use of xenotransplants, now with the first use of organs from genetically engineered pigs in human recipients,^{138,163,164} will, nevertheless, require a permanent focus on immunological injury of capillaries.

Evolution of Anti-HLA Antibody Testing

Two tests developed by Paul Terasaki played historical roles: the microcytotoxicity test in the 1960s and the Luminex bead assays in the 1990s. Viable cells, unlike severely damaged cells, do not take up trypan blue *in vitro*. This phenomenon was first discovered by Alwin M. Pappenheimer in 1917¹⁶⁵ and later formed the basis of various biological assays. In 1956, Gorer and O’Gorman first described the detection of anti-MHC antibodies via complement-mediated cell lysis.⁴⁹ This principle was significantly refined by Terasaki, resulting in the establishment of the microcytotoxicity test for crossmatch and panel reactivity testing.^{68,71,95} In the 1980s, however, it became clear that standard lymphocytotoxicity testing could not detect clinically relevant sensitization in all patients, and Garovoy et al,⁷⁹ along with other groups, introduced flow crossmatching to uncover complement-nonactivating alloantibodies. Although independent from cell lysis, flow cytometry also allowed for the discrimination between complement-activating and nonactivating antibodies by simultaneously detecting C4c deposition on lymphoblasts.¹⁵³ In 2001, Terasaki et al published an excellent “serologic” review article that summarized the emerging evidence for a role of antibody in rejection, and this may have significantly contributed to the “renaissance” of AMR.¹⁶⁶

The invention of a totally cell-free, highly sensitive test system, once again by Terasaki's group, was the next breakthrough. This assay used fluorescent microbeads for the detection of anti-HLA antibodies. Originally, it was set up as a tool to measure panel reactivity using pools of alloantigen-coated beads,¹²² but later, the method was significantly improved by using single-antigen beads coated with recombinant HLA molecules and Luminex technology.¹⁶⁷ In 2003, Wahrmann et al¹²⁷ demonstrated fluorescent C4d staining (and detection of other complement products, such as C1q and C3d) on flow beads exposed to alloantibodies, a solid phase strategy to dissect the complement-fixing

ability of anti-HLA antibodies. Later, other groups focused on solid-phase detection of C1q and C3d using single-antigen beads.^{168,169} The actual clinical relevance of such assays is, however, still controversial.^{170,171}

PROOF OF CONCEPT—ANTIBODY DEPLETION TO COUNTERACT AMR

If the concept of AMR as an entity was valid, then changes at the level of antibodies should have profound consequences. Indeed, the transfer of alloantibodies or of cells producing them was shown to trigger rejection in experimental models.^{96,97} Conversely, successful attempts to counteract AMR via depletion of antibodies or inhibition of their production would be a clinical breakthrough. In 1970, in a xenotransplant model, Bier et al¹⁷² were the first to demonstrate that plasmapheresis could impact the intensity of AMR. Afterward, several groups reported the use of plasmapheresis in clinical transplantation. Slapak et al¹⁷³ described its successful use in a case of unintentional ABO-incompatible transplantation, which was the starting point for the implementation of systematic desensitization to cross ABO barriers.^{174,175} Such protocols were later refined by Gunnar Tyden, who implemented the use of rituximab and ABO-specific immunoadsorption.¹⁷⁶ Cardella et al¹⁷⁶ were the first to successfully use plasmapheresis to treat anti-HLA antibody-triggered rejection. With 1 exception,¹⁷⁷ subsequent controlled trials published in the 1980s, however, failed to demonstrate a benefit in this context.¹⁷⁸⁻¹⁸⁰ At this time, AMR was not well defined, and many patients were included on the diagnostic basis of “refractory” or “vascular rejection,” which, as we now know, should not be mistaken for AMR. Later, immunoadsorption, a more selective technique, was successfully applied to prevent¹⁸¹⁻¹⁸³ or treat rejection.^{123,184} In 2007, Böhmig et al¹⁸⁵ conducted a randomized controlled trial of immunoadsorption in C4d-positive AMR, which suggested effective rejection reversal (and consequently led to its premature termination). This trial and more recent uncontrolled studies of plasmapheresis (usually in conjunction with intravenous immunoglobulin) in well-defined AMR have finally led to the broad consensus of apheresis as the standard of care in AMR.¹⁸⁶ A recent fascinating development is the therapeutic use of the bacterial enzyme imlifidase, which cleaves and inactivates IgG nearly completely, albeit only temporarily.¹³⁴ A landmark study by Stan Jordan¹⁸⁷ demonstrated effective use as a desensitization strategy.

Nevertheless, it has become clear that transient antibody removal may not suffice for the prevention of antibody-mediated injury in the long term. A major challenge remains the development of effective, safe strategies for interference with B-cell immunity, or important effector mechanisms such as complement or natural killer (NK) cell activity. Owing to the immense progress in transplant immunology, several promising concepts are now in the pipeline, but none have been approved for clinical use to date.¹⁸⁸ In this context, a series of nonhuman primate experiments launched a decade ago by Stuart Knechtle to systematically evaluate innovative therapeutic approaches with potential translational impact must be mentioned.¹⁸⁹⁻¹⁹¹

It has already been noted that inhibition of T-cell activation may well interfere with B-cell immunity. However, the opposite also appears to be true. The chimeric monoclonal antibody rituximab, reactive against CD20-bearing cells, is used in many desensitization protocols to inhibit the production of alloantibodies. CD20, a supposed prototypical B-cell marker, is, however, also variably expressed on a subset of T cells (first described in 1993¹⁹²). Depletion of these cells by rituximab was shown during the treatment of autoimmune diseases.¹⁹³ These intriguing findings have been widely ignored by the transplantation community.

MOLECULAR PHENOTYPING—TOWARD A NEW PARADIGM?

In 1993, Kary B. Mullis was awarded the Nobel Prize for his research on the polymerase chain reaction, a discovery that revolutionized genetic analyses.⁸⁰ At the same time, new molecular biological techniques to study tissue gene expression became available, including the microarray analysis established by Mark Schena.¹⁹⁴ Such techniques allowed for a systematic reevaluation of immunological processes in situ, including transplant rejection.

The Expanding Phenotypic Spectrum

The new techniques were first used to study the activation of T lymphocytes and effector molecules, such as granzyme B and perforin.¹⁹⁵ In 2003, Minnie Sarwal drew attention to the contribution of B-cell infiltrates to steroid-resistant rejections using DNA microarray profiling.¹⁹⁶ Such rejections still have to be classified as cell-mediated because nothing is known about locally produced alloantibodies and their potential targets.¹⁹⁷ Moreover, molecular studies by Halloran's group have indicated that B cells and plasma cells accumulate in areas of atrophy/fibrosis, which is unrelated to AMR.¹⁹⁸

In the past 2 decades, the initial view of a dominant diagnostic significance of C4d has been tempered, and C4d lost its position as an indispensable rejection marker.¹⁹⁹ Its value was perceived to be oscillating between “highly significant” and “useless.” This, of course, does not undermine its major contribution to our understanding of AMR. The antibody, its target, and the ensuing biological consequences of antibody binding are relevant, not C4d itself, which may even have immunomodulatory properties.²⁰⁰ For example, a low-titer antibody against blood groups (as is the case in the setting of ABO incompatibility) would not impair graft function and could even indicate a putative state of “accommodation” while still producing bright C4d staining.^{126,201} The concept of accommodation, in which the graft protects itself from immune injury, was first proposed for xenografts by Fritz Bach.¹²¹ However, whether, or to what extent, this state of immunological resistance plays a role in clinical transplantation is still unclear. It also became apparent that many patients will remain C4d-negative, despite detectable DSA and adverse graft dysfunction.²⁰² The existence of C4d-negative AMR was established via microarray studies¹³² and by observational studies analyzing serial protocol biopsies.²⁰³ This phenotype may, for example, be because of anti-HLA antibodies activating cells directly via signaling, independent of complement^{204,205} (other mechanisms, for example, those involving NK cells, are discussed below).

Getting Out of “Flatland”

Over the past 15 y, the Edmonton group led by Philip Halloran has established a microarray-based diagnostic platform to characterize rejection.¹²⁹ Setting up large reference sets of indication biopsies, they defined gene expression signatures that accurately dissect different types of rejection, in particular, AMR, TCMR, and their subphenotypes. These signatures outperform conventional histology in terms of predictive power.^{206–208} Analysis of the genes involved in rejection also contributed to a better understanding of molecular rejection mechanisms²⁰⁹ and has played a significant role in the discovery of C4d-negative AMR.¹³² Transcriptomic analysis has meanwhile extended to heart and lung transplantation.^{210,211}

A recent, intriguing observation was that a considerable proportion of AMR cases, defined according to AMR-associated transcript sets, may indeed be anti-HLA DSA (and mostly C4d) negative.^{136,137} This may involve different mechanisms, including DSA absorption to transplanted tissue, a phenomenon that was described many decades ago.¹⁰¹ There could also be a role of antibodies toward non-MHC antigens, which may escape standard tests. The issue has been studied since the 1970s, with Peter Stastny and Leendert Paul being among the early pioneers.^{212,213} Non-HLA reactivities may include autoantibodies directed against the angiotensin II type 1 receptor, which were first described by Duska Dragun in 2005.¹³¹

Last but not least, molecular analysis of biopsy material brought into play an important role of NK cells.^{133,214} This known, but still enigmatic type of cells is of interest because these cells could represent a link between cell-mediated and antibody-mediated graft rejection because of their unique biological properties. They can directly lyse targets that do not express certain self-determinants and by the engagement of special Fc receptors, they are also able to execute antibody-dependent cellular cytotoxicity. The principle of missing-self-triggered NK cell activation, elucidated in its mechanisms by Ljunggren and Kärre in 1985,⁸¹ but known in stem cell transplantation as a phenomenon of hybrid resistance since 1964,²¹⁵ has been studied for decades. Based on new results, it is now in focus again.¹³⁵ When discussing the role of NK cells in rejection, it is important to note that there are enormous differences in NK cell activity between mice and humans. In 1974, Halloran et al,²¹⁶ using an inhibition assay with xenogeneic chicken erythrocytes as target cells, demonstrated the ability of cell-bound anti-MHC class I and class II antibodies to engage Fc receptors in mice. Notably, because mouse NK cells, unlike human NK cells, are unable to lyse alloantibody-coated mouse lymphocytes, mice cannot mediate AMR like humans do.²¹⁶ Fundamental species differences, which also include inherent differences regarding patterns of MHC antigen expression (eg, murine renal microvascular endothelial cells, unlike those of humans do not express detectable MHC class II antigens²¹⁷), may have been a source of confusion. It appears that some of the conclusions drawn from mouse transplant models may have limited applicability to the pathogenesis of rejection in clinical transplantation. Hence, to gain insight into the cellular and molecular pathogenic mechanisms of AMR, we should rely primarily on human observations, maybe with the exception of transplant experiments in primates,

which have proven to be a suitable model of human AMR.²¹⁸

In a comment to one of the studies of the Edmonton group, Colvin cited Edward Abbot's satirical fantasy novella, *Flatland*, in which the 2-dimensional protagonist, a square, entered a world of 3 dimensions (*Spaceland*).²¹⁹ There, the square realized that it was “omnivident” and could see inside everything. One may speculate that using molecular diagnostic tools, perhaps in analogy to *Spaceland*'s “omnivalence,” the diagnosis of AMR could be reached in the near future without histological examination of tissue specimens, without C4d-staining, and even without alloantibody detection.

CONCLUDING REMARKS

According to Thomas Kuhn, one of the most influential philosophers of science, progress in a scientific field does not necessarily follow a linear path but rather develops in paradigms evolving in succession.²²⁰ These are temporary intellectual buildings that contain specific scientific problems and their solutions. Paradigms can compete for a while until 1 paradigm gains general acceptance. If discrepancies arise that no longer fit into an established framework of thought, then a paradigm shift may be necessary.²²⁰ It took quite a long time until the various mechanisms behind rejection were understood and could be reconciled. After a phase of early fact-gathering, the consensus oscillated between a “cellular” and a “humoral” theory of rejection. Then, the dominance of cellular rejection became a paradigm, exemplified by Medawar's overwhelming “cellular dogma.” Perhaps triggered by the game-changing discovery of C4d, we then witnessed a remarkable paradigm shift toward a primary role of AMR. Now, some researchers have begun to challenge the dichotomous view of rejection while new pathways have come into play, among them the different forms of NK cell-driven injuries. We do not know how organ transplantations will be performed during the next few decades, and if indeed, they will be necessary at all. However, there will be other complex challenges. It is to be hoped that they will be addressed with an open mind and in a more ambitious way than was the case with AMR. How could this be accomplished? We would like to suggest (1) follow your own hypothesis; (2) do not rely on any dogma and keep an open mind; (3) collect and incorporate essential knowledge in time; and (4) study the patients, and they will give you the truth.

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