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Matching for Human Leukocyte Antigens (HLA) in corneal transplantation – To do or not to do

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ABSTRACT

As many patients with severe corneal disease are not even considered as candidates for a human graft due to their high risk of rejection, it is essential to find ways to reduce the chance of rejection. One of the options is proper matching of the cornea donor and recipient for the Human Leukocyte Antigens (HLA), a subject of much debate. Currently, patients receiving their first corneal allograft are hardly ever matched for HLA and even patients undergoing a regraft usually do not receive an HLA-matched graft. While anterior and posterior lamellar grafts are not immune to rejection, they are usually performed in low risk, non-vascularized cases. These are the cases in which the immune privilege due to the avascular status and active immune inhibition is still intact. Once broken due to infection, sensitization or trauma,, rejection will occur. There is enough data to show that when proper DNA-based typing techniques are being used, even low risk perforating corneal transplantations benefit from matching for HLA Class I, and high risk cases from HLA Class I and probably Class II matching. Combining HLA class I and class II matching, or using the HLAMatchmaker could further improve the effect of HLA matching. However, new techniques could be applied to reduce the chance of rejection. Options are the local or systemic use of biologics, or gene therapy, aiming at preventing or suppressing immune responses. The goal of all these approaches should be to prevent a first rejection, as secondary grafts are usually at higher risk of complications including rejections than first grafts.

Contents

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1. Introduction

Cornea transplantations are performed frequently, and results are often excellent. However, many cases are not eligible for a transplantation as the chance of rejection is considered too high. Rejection may even occur in low risk cases and in lamellar grafts, and one wonders whether matching for the HLA antigens may contribute to better graft survival. We review the evidence for this, especially focusing on studies in humans, and we looked which potential new approaches may be available to clinicians in the near future.

Since the first successful corneal transplantation in 1905 (Zirm, 1989), many improvements have been made that significantly increased graft survival. The development of microsurgery and the operating microscope allowed more precise corneal transplantations, and the recognition of the function of corticosteroids in the 1950s helped to reduce inflammation and fight potential rejection episodes. In 1982, a major textbook on immunology mentioned that "The cornea, the transparent membrane of the eye, is normally not perfused with blood and does not contain lymphatics, so it is not easily accessible to immune cells; when transplanted, it enjoys the status of a privileged graft. However, even in this favorable situation, graft rejection sometimes occurs, presumably because of damage to the grafting site inflicted by surgery" (Klein, 1982). It is clear that immunological theory and animal experiments led to the idea that corneas were hardly susceptible to rejection. Indeed, when looking nowadays at all cases, corneal allografting is one of the most successful forms of solid organ transplantation. Still, in spite of the great results in experimental corneal transplantation in rats, and the excellent results in certain groups of patients and types of corneal transplants, immune-mediated graft rejection remains the single most important cause of short- and long-term corneal graft failure. This is also the case when a cornea is implanted in normal- or low-risk patients (Coster and Williams, 2005; Kuchle et al., 2002; Qu and Xie, 2010; Wilson and Kaufman, 1990). While overall survival rates for full thickness grafts are close to 90% one year after transplantation, they subsequently steadily decline (Ing et al., 1998; Williams et al., 2006). At ten years, in random transplants, the overall graft survival rate is 60%–80% (Coster and Williams, 2005; Ing et al., 1998; Inoue et al., 2000a; Thompson et al., 2003; Williams et al., 1997), with an immunological rejection rate after fifteen-years ranging from 21% to 29% (Patel et al., 2004). Due to changes in surgical techniques, we now see fewer full thickness grafts, and anterior and posterior corneal replacements are gaining popularity, but rejection has been reported as still having a major impact, also in lamellar keratoplasties (Allan et al., 2007; Mashor et al., 2010; Reinhart et al., 2011).

One assumed that matching of donor and recipient for HLA (human leukocyte) antigens was not necessary in corneal grafting due to the already protective ocular immune privilege; it is therefore not surprising that HLA matching studies were performed for kidney transplantation soon after the identification of the HLA antigens as a potential transplantation antigen system, but that HLA matching was only sporadically analysed for corneal transplants. When these studies were performed, the outcomes led to controversial results regarding the benefit of HLA matching to reduce corneal allograft rejection (Baggesen et al., 1991; Beekhuis et al., 1991; Ehlers and Kissmeyer-Nielsen, 1979; Foulks et al., 1983; Hargrave et al., 2004; Hoffmann and Pahlitzsch, 1989; Reinhard et al., 2003; Stark et al., 1978; The Collaborative Corneal Transplantation Studies Research Group, 1992; Vail et al., 1997; Vannas, 1975). A difference is especially observed between most European studies and the main study performed in the USA, the CCTS study. Subsequently, a long-term discussion on the benefit of HLA typing and matching and the strong opinions of some leaders in the field have discouraged research in this field, and one wonders whether that is correct. As every immune rejection starts with direct or indirect recognition of donor major or minor HLA-antigens as being foreign, there are still good reasons to unravel and not directly to disregard the role of HLA matching in corneal transplantation, and to properly evaluate the existing data.

It is essential to find ways to improve cornea graft survival in high risk cases, especially as a large group of patients with severe corneal disease is not even being considered for a human graft due the high risk of rejection, such as cases with severe burns or Stevens Johnson's disease. Limbal transplantation is not discussed in this review, but its application is severely limited due to the very high frequency of graft rejection (Fernandes et al., 2004).

We will therefore address 1) the role of transplantation immunology in corneal transplantation, and specifically the role of HLA antigens in antigen presentation; 2) the influence of HLA matching in corneal graft survival, for both the major and minor histocompatibility antigens, and 3) discuss immunological approaches that may increase graft survival. We will focus on studies that may be clinically relevant in the near future, and will therefore limit the description of new basic research data in mice.

2. Corneal transplantation immunology

2.1. High and low risk cases

The cornea is the most-commonly transplanted tissue in humans, with over 65,000 transplants being performed worldwide each year (Williams et al., 2009). Part of the success of corneal transplantations is based on the immune privilege of the cornea, and hence, corneal grafts have a survival benefit over organ allografts (Niederkorn, 2001; Niederkorn and Stein-Streilein, 2010; Niederkorn, 2006; Skelsey et al., 2001; Streilein, 2003). Notwithstanding the irrefutable role that the immune privilege plays in the success of corneal grafting, it will not completely protect the unmatched graft from immune rejection, but only curtail the effect of immune responses. Graft survival is related to the status of the recipient bed, such as the amount of vascularization, and the

underlying disease, and not so much to the recipient's age in the Australian data (Figs. 2–6). The latter finding is in contrast with other reports, such as Vail et al. (1997), who in a cohort of 2777 grafts studied in the UK Corneal Transplant Follow up Study observed less rejection with advancing age. While other causes of failure increased with age, graft survival did not differ. Vail's study showed that the risk of rejection was associated with the presence of glaucoma, inflammation, regrafting and large graft size. These associations point to the importance of the recipient bed: analyses of human corneas and animal studies have shown that the number of antigen-presenting cells in the receiving cornea play an important role: low- or normal-risk patients have an avascular cornea, no inflammation and thus only a few antigen-presenting cells, and have long-term graft survival rates ranging from 62 to 96% after 10 years (Williams et al., 1997). On the other hand, high risk patients with corneal vascularization in several quadrants of the cornea, active herpetic infection, and high densities of Langerhans' cells, have 10-year graft survival rates of 35%–41% (Thompson et al., 2003; Williams et al., 1997) Thompson et al. (2003) studied 3992 consecutive eyes that underwent a penetrating keratoplasty at a large tertiary care center, and reported that the most common causes of graft failure were endothelial failure and immunologic endothelial rejection. Keratoconus had the best 10-year survival, at 92%, while first regrafts had only 41% graft survival at 10 years. The presence of deep vessels at more than 3 clock hours led to 65% 10-year overall graft survival versus 82% in non-vascularized cases. As already mentioned, patients with highly-vascularized corneas are often not considered candidates for grafting, as their chances of developing rejection are too high. It is noteworthy that the transplants in the most successful cases, i.e. in keratoconus and primary endothelial dystrophy of Fuchs, are the conditions with the least amount of vascularization (Jager et al., 1988). Retransplants are often placed in vascularized recipient beds, and every subsequent regraft has a lower chance of surviving (Thompson et al., 2003; Williams et al., 1997).

If one also takes into account that upon failure of the primary graft, often a re-graft is performed (Bersudsky et al., 2001; George and Larkin, 2004; Qu and Xie, 2010; Williams et al., 1997; Yahalom et al., 2005), it is obvious that reducing the risk of immunologic primary allograft rejection and understanding its mechanism is of vital importance to improve vision, to reduce graft loss and to prevent retransplantations.

2.2. Immune privilege

The immune privilege of corneal allografts is based on at least six physiological mechanisms, which either block the induction of the immune response, deviate the immune response towards a tolerogenic pathway or help to escape the immune attack (Niederkorn and Larkin, 2010):

1) Absence of corneal lymph and blood vessels and blockade of lymph vessel formation, together with a relative lack of lymphatic drainage from the eye, ensures that antigens can only leave the eye via the aqueous draining system into the bloodstream (Albuquerque et al., 2009). The aqueous outflow will carry antigens to the spleen, rather then the draining lymph nodes, which will then act as the primary lymphoid tissue (Streilein, 1995).
2) Inducement of regulatory T cells by the allograft, which inhibits the induction and function of the alloimmune T cells (Chauhan et al., 2009; Yamada et al., 2005).
3) Protection from complement-mediated cytolysis (Bora et al., 1993; Hargrave et al., 2003; Hegde et al., 2002; Lass et al., 1990).
4) Induced apoptosis of neutrophils and T cells at the graft–host interface (Stuart et al., 1997; Yamagami et al., 1997).
5) Diminished corneal T-cell proliferation (Hori et al., 2006; Jager et al., 1995; Shen et al., 2007) and,
6) Diminished NK cell activation (Apte et al., 1998; Apte and Niederkorn, 1996).

Corneal immunosuppression is not a passive system based on the absence of antigen recognition, as was originally thought, but consists of active immunological interactions with many different players. The combined effect of these active mechanisms that inhibit immune response after introduction of antigen in the anterior chamber is known as anterior chamber-associated immune deviation (Niederkorn, 2006; Stein-Streilein and Streilein, 2002; Streilein, 2003). Antigens placed into the anterior chamber of murine eyes are taken up by specific macrophages in the iris, which subsequently migrate to the spleen. The presence of TGF beta in the anterior chamber modifies the antigen-loaded macrophages in such a way that they develop immunosuppressive characteristics (Wilbanks and Streilein, 1992). Leakage of donor corneal proteins into the anterior chamber of the eye may thus induce anterior chamber-associated immune deviation. Additionally, the compact architecture of the corneal stroma is believed to inhibit the infiltration of immune cells and the blood-aqueous barrier prevents immunologically-active cells and factors from entering the ocular tissue (Streilein, 1995). Although functional antigen-presenting cells (APCs) are present in the peripheral and paracentral cornea, they are scarce and mostly immature in the healthy central cornea, resulting in weak local antigen presentation (Mayer et al., 2007). As grafts are usually placed centrally, they encounter only a few APCs. This may of course be different when inflammation is present, bringing in massive numbers of APCs, as is the case e.g. in herpetic corneal infections (Williams et al., 1989). Corneal tissue by itself is able to produce cytokines to inhibit T cell responses (Jager et al., 1995); the nature of the factors that are involved has not yet been elucidated. In the clinical setting, a local immune response, elicited by the corneal transplantation, is further tempered by topical corticosteroids applied to the cornea post-operatively, supported (when needed) by oral immunosuppressive agents (The Collaborative Corneal Transplantation Studies Research Group, 1992). Mucosal tolerance can also be induced by conjunctiva-associated lymphoid tissue (Dua et al., 1995).

2.3. Histocompatibility antigens

The first descriptions, of what is now known as the Human Leukocyte Antigen (HLA) system, the major histocompatibility complex (MHC) in humans, date from 1958 (Dausset, 1958; Payne and Rolfs, 1958; van Rood et al., 1959). As many ophthalmologists who perform corneal transplantations are not immunologists, we will include a description of the development of the HLA system, which explains its weird nomenclature.

In 1936, Peter A. Gorer, studying allogeneic tumor transplantation in mice, discovered the presence of an antigen (antigen II) responsible for rejection (Gorer, 1936); additionally, he found that the sera of mice, that had received an allogeneic tumor, contained antibodies against this tumor (Gorer, 1937). In 1944–45, Peter Medawar confirmed that an immune response against the graft was responsible for rejection of allogeneic transplants (Medawar, 1944, 1945). After the Second World War, Gorer continued his work, together with George D. Snell, and found that his antigen II was encoded by a gene on the H locus in mice (Gorer et al., 1948). In 1958, Dausset (1958), van Rood et al. (1959), and Payne and Rolfs (1958) laid the foundation of what is now known as the HLA complex. They studied human sera obtained from multi-

transfused patients or multiparous women and noted that antibodies in these sera reacted with leukocytes from many other individuals, but not all, thus demonstrating the existence of a polymorphic system. The first named antigen was H2, for which Dausset gets the credits (Dausset, 1958). Van Rood noticed that serum from a multiparous woman who developed a blood transfusion reaction contained antibodies against white blood cells instead of erythrocytes, and using sera from many women and a leukocyte agglutination assay, he identified a series of antigens. Van Rood reported the existence of a bi-allelic system of leukocyte antigens, which he called 4a and 4b (now known as HLA-Bw4 and -Bw6) (van Rood, 1962). Other researchers identified other leukocyte antigens, and when the International Histocompatibility Workshops were established, different researchers were able to compare their work. This led to the discovery that most of these leukocyte antigens were genetically closely linked, and inherited at one chromosomal region, which was originally called HL-A (Human Leukocyte, locus A) (Thorsby and Lie, 2005), with the letters forming also a combination of the H of Dausset's H2 locus, and Rose Payne's LA antigens (Terasaki, 1990; van Rood, 1969). The chromosomal region that codes for the HLA antigens is located on the short arm of chromosome 6 and responsible for the polymorphic HLA complex. At the time it was discovered that there were different Class I loci (Kissmeyer-Nielsen et al., 1968), many antigens had already been identified and given a number, and this explains the strange numbering of A's and B's (A1, A2, A3, Bw4, B5, Bw6, B7, B8, A9, etc). The terms Class I for HLA-A, -B and -C and Class II for HLA-DR, -DQ and -DP were introduced by Klein (1977) and Thorsby and Lie (2005).

The primary biological role of these HLA antigens is not to impede tissue matching (although the name histocompatibility antigens seems to suggest that this is the most important function of these antigens), but to present all kind of antigens to the immune system. Antigen presentation is important in the recognition of environmental pathogens such as viruses, bacteria and fungi, to which the direct innate immune system and subsequently the adaptive immune system must develop an adequate response. HLA antigens contribute to the induction of immune responses, as well as to the effector phase, by presenting target peptides to helper and effector T cells.

As already mentioned, HLA antigens are divided into two classes, HLA Class I and Class II. HLA Class I antigens are derived from the three classical loci HLA-A, -B and -C and the non-classical loci HLA- E, -F, -G, -H, -I and -J, and are expressed on platelets and almost all nucleated cells, except most cells of the central nervous system. The HLA Class I molecules present peptides, derived from proteins that are broken down inside the cell, to cytotoxic CD8-positive T cells. HLA Class II consists of three main genetic loci, HLA-DR, -DQ and -DP, and these molecules are expressed on some immunological cells such as B cells and activated T cells, and especially on antigen-presenting cells such as dendritic cells, macrophages, and monocytes, and on endothelial cells and thymic epithelial cells. The Class II molecules present peptides from exogenous antigens to the CD4-positive helper T cells. Cytokines can modify the level of expression of Class I and II molecules. The HLA genes are the most polymorphic genes in the human genome, providing a great diversity of HLA alleles, with each specific allele having the ability to present certain antigens better or worse than other alleles.

Transplanting tissue from one individual into another introduces a new set of donor HLA antigens into the recipient. The peptides that are derived from the foreign HLA molecules can either be presented by the patient's own HLA molecules, or by the donor's that are present in the graft (Fig. 1). Differences in the set of HLA alleles between donor and host may result in recognition of

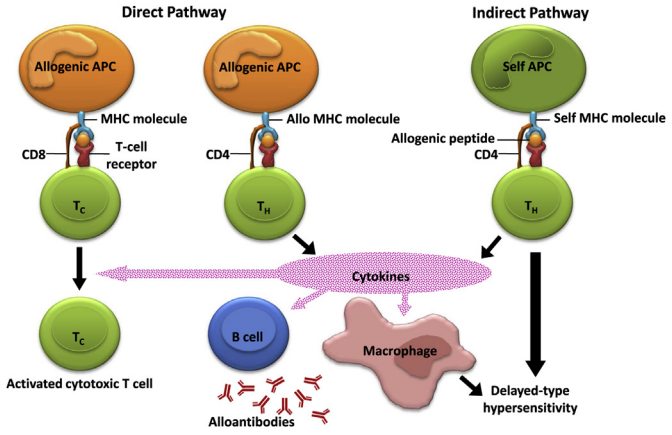

Fig. 1. Pathways of recognition of HLA antigens (MHC) and mechanisms of graft rejection.

the donor tissue as foreign, and this may induce an immune response that ultimately ends in irreversible graft rejection. Reducing the differences in HLA antigens between donor and host by HLA matching should reduce the risk of rejection. HLA matching as a method to reduce graft rejection in living-related kidney transplantation came in use in the 1970s, when it was widely acknowledged that transplantation between HLA-identical living-related individuals was superior to other methods to improve graft survival (Singal and Skinnider, 1970; Thorsby and Lie, 2005). Matching was first started using the HLA Class I antigens, but it took until 1978, after the identification of HLA-DR, before HLA-matching for cadaveric kidney transplantation showed beneficial results (Albrechtsen et al., 1978a, 1978b; Opelz, 1985; Persijn et al., 1978; Ting and Morris, 1978).

In spite of all the mechanisms that are involved in the immune privilege of the eye, corneal graft rejection can occur and is caused by the immune response generated by recognition of host antigens. In spite of the early assumption that transplanted corneas would enjoy immunological privilege and could not be rejected, clinical data and early animal experiments demonstrated that especially grafts in vascularized recipients can be rejected (Maumenee, 1951). Rejection can take place in all three distinctive corneal cell layers or in one layer only: the epithelium, stroma and endothelium (Khodadoust and Silverstein, 1969). Knowledge about the distribution of the HLA antigens is therefore important to comprehend the rejection process. As graft rejection occurs most commonly in the endothelium (Chong and Dana, 2008), one would expect this cell layer to have the highest expression of HLA antigens. This is not the case. HLA-A, -B and -C (Class I) are present at high levels on epithelial cells, especially near the limbus, at lower levels on the stromal keratocytes and even less on the endothelium, although conflicting reports exist regarding the latter (Baudouin et al., 1988; Pepose and Benevento, 1991; Treseler et al., 1984; Whitsett and Stulting, 1984; Williams et al., 1985).

HLA-C is expressed at 10–35% of the levels of HLA-A or -B (Bunce and Welsh, 1994; McCutcheon et al., 1995), and is considered a weak transplantation antigen with no known role in matching for allografts (Ferrara et al., 1978). The Class II antigens HLA-DR, -DQ and -DP (Class II) are not expressed by the three corneal layers in the healthy eye; however, Class II positive epithelial dendritic cells are present, mainly in the peripheral corneal epithelium (Baudouin et al., 1988; Pels and van der Gaag, 1984; Pepose and Benevento, 1991; Treseler et al., 1984; Whitsett and Stulting, 1984; Williams et al., 1985). Studies on rejected human corneas show that inflammation leads to expression of HLA Class II in all cell layers (Donnelly et al., 1985; Dreizen et al., 1988).

Of the 'non-classical' HLA antigens (HLA-E, -F, -G, -H, -I and -J), only HLA-E (four alleles) and -G (three alleles) are known to be polymorphic and little is known about their expression and physiological role in the cornea. They have no known relevance to clinical corneal transplantation (Taylor and Dyer, 1995), although HLA-G is expressed in all three corneal layers (Le et al., 2003). Histocompatibility antigen expression is higher in corneas of younger persons compared with older persons (Whitsett and Stulting, 1984). Indeed corneal rejection risk is higher for grafts from young (0–5 years) than from older persons (40–70 years) (Palay et al., 1997).

Aside from the major histocompatibility antigens, minor histocompatibility antigens (minor H antigens) exist. Minor H antigens are peptides presented by the HLA complex, and are derived from polymorphic proteins. A donor can have a different variant of the same protein as the host, which upon presentation of its peptides by HLA Class II molecules on donor or host APCs, will be recognized as foreign and can induce a strong immune reaction against the graft; this has been well documented in HLA-identical stem cell transplantations (Dickinson et al., 2002; Goulmy et al., 1996; Goulmy, 2006).

Known minor H antigens expressed on corneal tissue are HY, the male-specific minor H antigen, and HA-3 (de Bueger et al., 1992; Goulmy et al., 1995; Peeler et al., 1988; Ross et al., 1991; Sonoda and Streilein, 1992). Animal studies have demonstrated that minor antigens can be an important target for immune responses in corneal transplantations (Haskova et al., 2003; Sano et al., 1996, 1999).

2.4. Immune rejection

Once a recipient's immune system recognizes the allograft as foreign, rejection will occur. In general, rejection can be defined as hyperacute, acute or chronic (Bush, 1999). Hyperacute rejection is the most rapid and aggressive form and takes place immediately after implantation. This type of rejection has been described in solid organ transplantation and is mediated by circulating antibodies, directed against ABO-blood group or HLA antigens or both; such antibodies may have developed as the result of a previous antigen exposure, such as a blood transfusion or pregnancy (Wood and Goto, 2012). With regard to corneal allografts, early studies found an influence of ABO incompatibility in high risk patients (Inoue and Tsuru, 1999; Maguire et al., 1994), but more recent studies did not confirm this (Dunn et al., 2009; Soma et al., 2004; Stulting et al., 2012). In contrast to the clear role which anti-HLA alloantibodies have in solid organ transplantation, their role is not entirely clear in corneal transplantation. Although they can exacerbate a rejection, alloantibodies are not believed to be by themselves capable of acutely rejecting corneal grafts, most likely due to the lack of blood vessels in low risk grafts (George and Larkin, 2004; Goslings et al., 1998; Stein-Streilein and Streilein, 2002). This minor influence of alloantibodies, together with the avascularity of the cornea, explains that hyperacute corneal rejection, if at all, seldom occurs. However, it should not be ignored that allosensitization as the result of previous antigen exposure still has an important influence on corneal transplant survival, even if it does not lead to acute rejection. The negative effects of

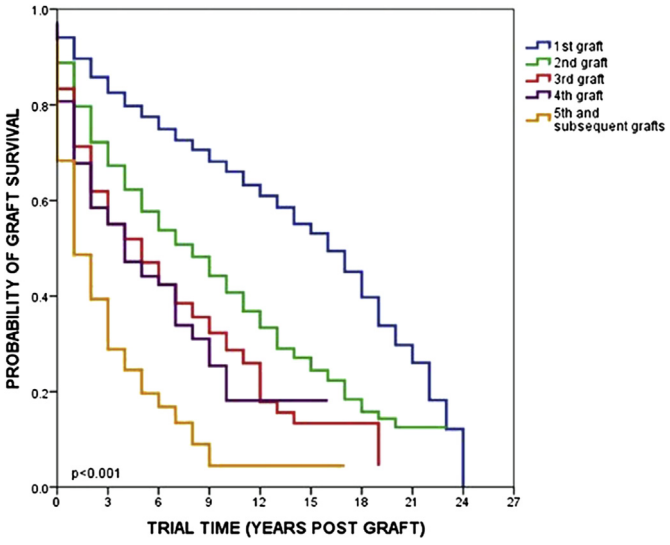

Fig. 2. Kaplan–Meier survival plot for corneal graft survival according to the number of previously-rejected ipsilateral transplants. Regrafts have significant worse graft survival (log-rank test). Used with permission from Department of Ophthalmology, Adelaide: The Australian Corneal Graft Registry 2012 Report; KA Williams, MT Lowe, MC Keane, VJ Jones, RS Loh, DJ Coster (Eds); 2012, pp. 1–246.

Number at Risk

Identity	Initially	3 years	6 years	9 years	12 years	15 years	18 years	21 years	24 years
1st graft	12988	5441	2727	1359	754	360	119	32	1
2nd graft	2412	887	411	181	86	31	14	4	n/a
3rd graft	607	191	82	32	16	6	3	n/a	n/a
4th graft	161	51	25	11	3	2	n/a	n/a	n/a
5th & subsequent graft	123	30	7	2	1	n/a	n/a	n/a	n/a

immunization were found to be independent of the degree of vascularization (Roy et al., 1992). Regrafting is undesirable as it leads to a higher chance of rejection and moreover, the fast occurrence of graft failure after a re-transplantation indicates the importance of absent prior allosensitization before grafting (Fig. 2) (Coster and Williams, 2005; Niederkorn and Larkin, 2010; Williams et al., 2012). The role of allo-antibodies in any type of corneal graft rejection is discussed below.

Acute rejection manifests within days to months and is initiated by the recognition of new foreign antigens by the immune system: this type of rejection is antibody as well as cell-mediated (Game and Lechler, 2002). With adequate and timely treatment, acute rejection can be resolved, although it can still predispose the host to chronic rejection.

Chronic rejection develops slowly and usually occurs after months or years and is believed to be mediated by antibody and cell-mediated immune responses with macrophage involvement (Game and Lechler, 2002). Despite improvements in immunosuppressive therapies which can prevent and control acute rejection, chronic rejection is still a major problem, and has become the major cause of solid organ (Bush, 1999; Racusen, 2003), and corneal allograft failure (Coster and Williams, 2005; Ing et al., 1998; Inoue et al., 2000a; Patel et al., 2004; Thompson et al., 2003; Williams et al., 1997, 2006).

With regard to the different corneal cell layers, epithelial rejection is, although probably common, generally quiet, asymptomatic, transient and usually does not affect graft survival (George and Larkin, 2004; Khodadoust and Silverstein, 1969; Prendergast and Easty, 1991)Stromal rejection is relatively common, can progress to endothelial rejection if not treated, and can be acute or chronic (George and Larkin, 2004; Khodadoust and Silverstein, 1969; Panda et al., 2007; Prendergast and Easty, 1991) as infiltrating stromal cells may persist for several years in the allograft (Macdonald et al., 2010)Endothelial rejection can be acute or chronic, and is the most symptomatic and serious one of the three types of rejection (Khodadoust and Silverstein, 1969). Endothelial cells persist for life as they are hardly proliferative. Therefore cell loss due to recurrent reversible rejection episodes may lead to graft failure due to endothelial decompensation (Bourne, 2001; George and Larkin, 2004; Joyce et al., 1996; Prendergast and Easty, 1991).

Once the immune privilege of the anterior chamber and cornea has been compromised, a local immune response may lead to graft rejection. The loss of immune privilege may either be due to inflammation caused by the allograft itself, or to other causes of inflammation, corneal vascularization, or ocular surface diseases. A well-known trigger of rejection is a loose suture, which will attract mucous and bacteria and become a focus for leukocyte accumulation (macrophages, Langerhans' cells, T cells) inducing local upregulation of HLA antigens (Jonas et al., 2002). Immune rejection involves both the innate and adaptive immune response. Major and minor H antigens can be recognized indirectly via presentation through the innate immune system and directly via the adaptive immune system as illustrated in Fig. 1 (Game and Lechler, 2002; Sayegh and Turka, 1998). The innate immune response

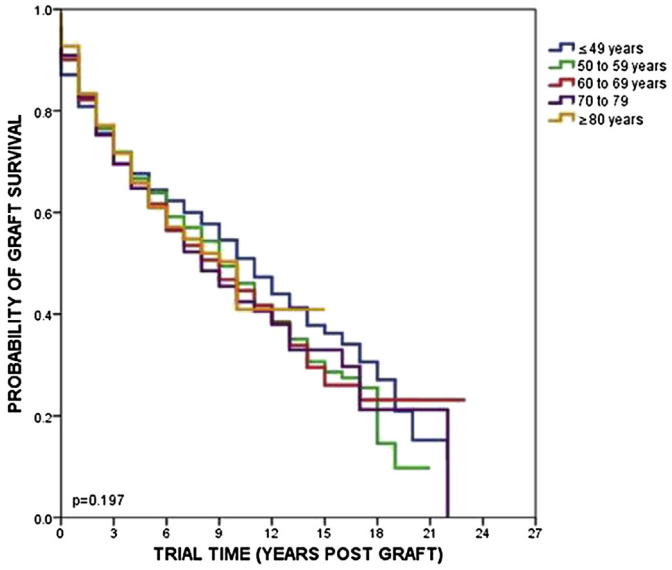

Fig. 3. Kaplan–Meier survival plot for corneal graft survival according to the recipient age (keratoconus excluded). Age at time of grafting had no significant effect on graft survival (log-rank test). Reprinted with permission from: Department of Ophthalmology, Adelaide: The Australian Corneal Graft Registry 2012 Report; KA Williams, MT Lowe, MC Keane, VJ Jones, RS Loh, DJ Coster (Eds); 2012, pp. 1–246. URL: http://hdl.handle.net/2328/25859.

Number at Risk

Identity	Initially	3 years	6 years	9 years	12 years	15 years	18 years	21 years
≤49 years	2060	868	454	235	142	73	26	5
50 - 59 years	1235	529	273	144	77	30	7	n/a
60 - 69 years	2102	907	471	224	109	34	5	1
70 - 79 years	3548	1316	541	176	62	14	2	1
≥80 years	2467	558	156	32	4	1	n/a	n/a

(neutrophils, macrophages) on its own is not capable of rejecting the graft and the involvement of the adaptive immune response (T cells) is required (Krensky et al., 1990; Niederkorn, 2007). The major bridge between the two is the interaction between APCs of the innate immune system and T cells of the adaptive system. APCs, of both the donor and host, are able to pick up material shed from the graft and activate the adaptive immune system by presenting these antigens to the CD4+ T helper cells. These CD4+ T helper cells play a central role in recruiting effector cells into the graft, as they are able to activate CD8+ cytotoxic T cells which subsequently attack the donor cells, and B cells which start producing antibodies against donor antigens. Furthermore, CD4+ T cells recruit macrophages, granulocytes, and NK cells, and activate the complement system. All of these players are able to kill corneal cells, leading to rejection.

In the adaptive immune system, CD4+ T cells of the host respond directly to donor HLA Class II antigens, which are expressed on APCs, while CD8+ T cells of the host recognize donor HLA Class I antigens, present on the cell surface of donor corneal cells (Game and Lechler, 2002; Sayegh and Turka, 1998; Wood and Goto, 2012).

Direct recognition plays a major role in acute rejection, while indirect recognition is additionally involved in acute and chronic rejection (Auchincloss et al., 1993; Lee et al., 2001; Sayegh and Turka, 1998). Involvement of the adaptive immune response and especially of the T cells is required for corneal graft rejection (Krensky et al., 1990; Niederkorn, 2007), but not necessarily simultaneously (Hegde and Niederkorn, 2000). While studies in mice showed that minor H antigens may even have a more important role in rejection than major MHC antigens, very few studies have been performed to determine the presence of T cells directed against minor antigens in human corneal transplant rejections.

2.5. Cellular and humoral anti-corneal immune responses in humans

Once antigen presentation has occurred, immune responses against the graft may be induced (Maumenee, 1951). It is possible that previous sensitization through an earlier graft or pregnancy has occurred, which has induced either an antibody or cellular immune response against the cornea, against minor antigens or against the HLA antigens. Many studies about the role of such immune responses have been performed in rabbits or mice, but only some studies regarding T cell or antibody responses are available regarding humans. In Leiden, crossmatches between recipient serum and cornea donor leukocytes have been performed since 1968, as antibodies were considered a risk factor for corneal graft rejection. Additionally, screening was performed in potentially-sensitized individuals to determine the presence of antibodies against a panel of leukocyte donors (van Rood et al., 1976). This was initiated after early studies had shown that anti-leukocyte antibodies are present in cornea recipients. While one study showed the presence of directly lympho-cytotoxic T cells in the blood of corneal transplant recipients but hardly of any antibody (Grunnet et al., 1976), a second study from the same group on one case correlated graft rejection with the presence of an antibody-dependent cell-mediated cytotoxicity response against peripheral blood lymphocytes (Ehlers et al., 1981). In the U.S.A., Stark collaborated with Terasaki, one of the first experts in identifying anti-HLA antibodies, to investigate the development of lymphocytotoxic

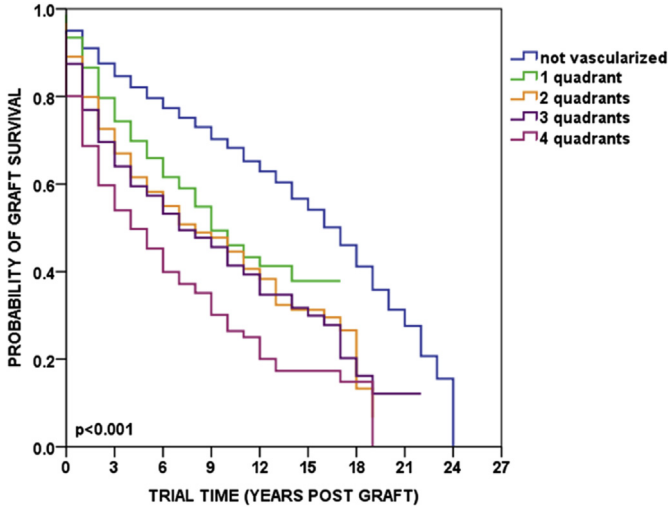

Fig. 4. Kaplan–Meier survival plot for penetrating corneal graft survival according to the pre-graft vascularization status. Graft survival was shorter when more quadrants were vascularized (log rank test). Reprinted with permission from: Department of Ophthalmology, Adelaide: The Australian Corneal Graft Registry 2012 Report; KA Williams, MT Lowe, MC Keane, VJ Jones, RS Loh, DJ Coster (Eds); 2012, pp. 1–246. URL: http://hdl.handle.net/2328/25859.

Number at Risk

Identity	Initially	3 years	6 years	9 years	12 years	15 years	18 years	21 years
Not vascularized	11168	4871	2488	1266	703	336	123	34
1 quadrant	1158	417	182	60	22	7	n/a	n/a
2 quadrants	1721	614	267	136	71	25	4	n/a
3 quadrants	844	312	153	67	34	18	5	2
4 quadrants	1400	386	162	63	30	14	4	n/a

antibodies in corneal transplant patients (Stark et al., 1973). The presence of allo-antibodies was determined on a panel of leukocytes from 90 to 100 individuals in the microlymphocyte cytotoxicity test. Three cases had antibodies prior to transplantation, and all three developed a rejection. Five out of six patients who were negative prior to transplantation and developed a corneal graft rejection developed such antibodies, versus only one of eight patients who did not reject and had a successful transplant (Stark et al., 1973). As this study clearly showed a correlation between a rejection and the development of antibodies and rejection, it led to a clear advice regarding testing of corneal transplant patients for the presence of cytotoxic antibodies: patients should be screened when there is a history of pregnancy, blood transfusion, or previous corneal transplant failure, upon which a cross-match test between the serum of the sensitized patient and cells from the potential donor should be performed, and mismatches of the same specificity as the cytotoxic antibodies of the recipient should be avoided. These criteria were not all based on scientific data from this study, but were similar to the screening advise given on the basis of data on kidney transplants (Opelz et al., 1973). Another study described that even in patients with 100% panel reactivity, acceptable mismatches could still be found and could be defined in 80% of these patients (Vannas et al., 1976).

The Collaborative Corneal Transplantation Studies Research Group (1992) included a prospective study to compare donor-recipient pairs with (37 cases) and without (419 cases) a positive serological crossmatch (lymphocytotoxic anti-HLA antibodies): a higher frequency of graft failure due to rejection was seen in the cross-match positive group than in the crossmatch negative group (Hahn et al., 1995). In another cohort, Des Marchais observed a negative influence of a positive crossmatch only in patients who had previously undergone a transplant or already rejected a cornea graft (Des et al., 1998). In a study on 1681 consecutive keratoplasties, Völker-Dieben et al. reported that a panel reactivity of more than 10% led to a significantly-increased chance of rejection in moderately to severely vascularized recipients (Volker-Dieben et al., 2000). When looking at an IgM crossmatch between patient serum and corneal donor rims, antibodies were found in 28%

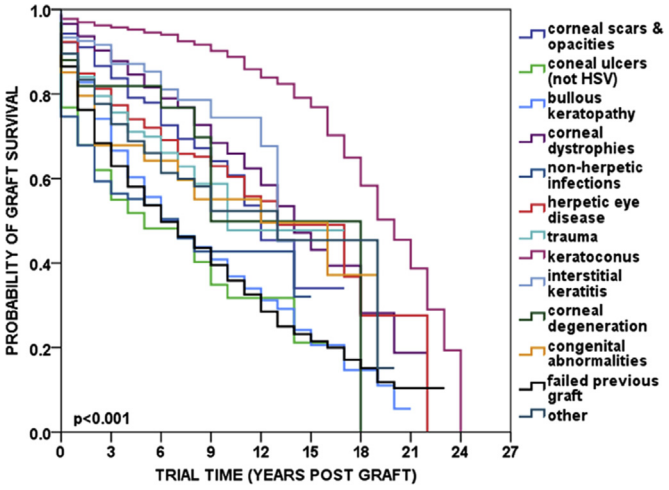

Fig. 5. Kaplan–Meier survival plot for corneal graft survival according to main indications for penetrating grafts. The variation in survival across the main indications was significant (log rank test); keratoconus had the longest survival, and corneal ulcers the shortest. Reprinted with permission from: Department of Ophthalmology, Adelaide: The Australian Corneal Graft Registry 2012 Report; KA Williams, MT Lowe, MC Keane, VJ Jones, RS Loh, DJ Coster (Eds); 2012, pp. 1–246. URL: http://hdl.handle.net/2328/25859.

Number at Risk

Identity	Initially	3 years	6 years	9 years	12 years	15 years	18 years	21 years
Corneal scars & opacities	314	118	68	22	13	2	n/a	n/a
Corneal ulcers (not HSV)	349	71	32	15	6	1	n/a	n/a
Bullous keratopathy	3814	1236	475	168	62	20	4	1
Corneal dystrophies	1806	872	429	188	96	35	7	2
Non-herpetic infections	221	60	32	12	7	3	n/a	n/a
Herpetic eye disease	692	287	151	87	50	26	4	2
Trauma	266	103	54	21	9	3	n/a	n/a
Keratoconus	4930	2444	1367	781	471	251	98	27
Interstitial keratitis	151	81	41	19	11	n/a	n/a	n/a
Corneal degeneration	117	33	16	7	3	1	n/a	n/a
Congenital abnormalities	67	25	15	10	10	5	3	n/a
Failed previous graft	3277	1152	522	225	105	40	17	4
Other	287	112	56	30	17	11	3	n/a

of recipients, but no association with the occurrence of rejection was observed (Borderie et al., 2004)However, an ELISA-based crossmatch procedure to detect donor-specific anti-HLA antibodies using outer scleral rim instead of blood lymphocytes to isolate the donors' HLA molecules showed that having no anti-HLA antibodies led to less immunological complications during follow-up: 77% of individuals without antibodies had no rejection during the mean follow-up period of 18 months, versus 25% of the recipients with preformed anti-HLA antibodies (Sel et al., 2012). Although these studies provide some evidence that the presence of anti-HLA antibodies has an adverse effect on penetrating corneal transplants, too few studies have reported on their importance in different types of corneal diseases, or their importance for re-transplants after rejection. Recently, Sel et al. studied the impact of pre-existing donor-specific allo-antibodies against donor HLA Class I or II prospectively using a novel ELISA-based crossmatch procedure: in a short-term follow-up study, they found a 50% lower rejection rate in patients without allo-antibodies (Sel et al., 2012). This is in accordance with the finding in solid organ transplantation that allo-antibody formation is related to acute and especially chronic allograft rejection (Doxiadis, 2012; Terasaki, 2003).

Antibodies can be directed against major and minor HLA antigens or against corneal antigens. Nelken analyzed whether a corneal transplantation induced the development of antibodies that bound to corneal antigens, and indeed, in 15/33 cases such antibodies were observed (Nelken and Nelken, 1965). Some studies analyzed responses against a specific soluble corneal protein, known as BCP 54 (Bovine cornea protein 54) (Jager et al., 1991), which has been identified to correspond to corneal aldehyde dehydrogenase (ADH) (Verjans et al., 1990). Antibodies against this antigen occur in healthy controls, but also in patients with Fuchs' heterochromic cyclitis or uveitis (Kruit et al., 1985; van der Gaag et al., 1989), and such antibodies were found to be present in over 40% of cornea transplantation patients. Similarly, patients often displayed a T-cell mediated reactivity against this antigen (Jager et al., 1994). No prognostic value could be attributed to the presence of a humoral or cellular anti-BCP 54 immune response, but it was interesting that five of the six patients who had a rejection episode during the study period had anti-BCP antibodies prior to transplantation and changed from a negative to a positive cellular anti-BCP 54 response (Jager et al., 1991). These studies suggest an increase in T cell reactivity against corneal antigens as the result of local inflammation, not necessarily as the cause of rejection. Furthermore, there may be a subgroup of patients who tend to develop any type of immune response.

Earlier, Stark et al. had used the leukocyte migration inhibition test to determine the presence of a cellular immune response in corneal graft recipients (Stark, 1980). Only patients who had rejected a graft at the time of testing showed a positive response.

Roelen et al. determined the presence of cytotoxic T lymphocytes (CTLs) against mismatched donor Class I antigens in patients

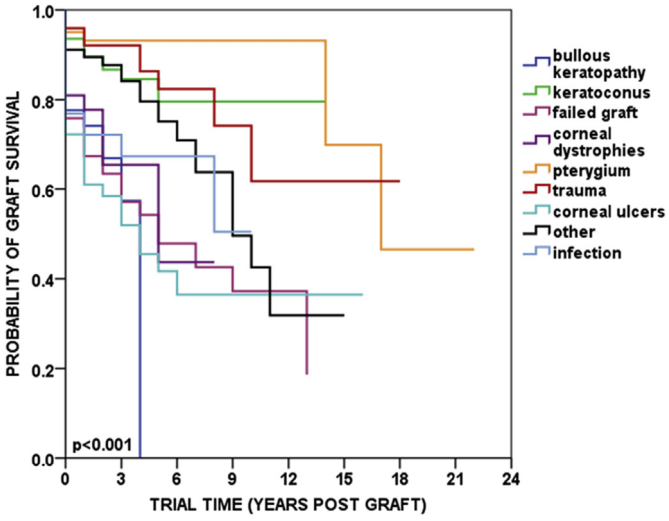

Fig. 6. Kaplan–Meier survival plot for corneal graft survival according to the main indications for lamellar grafts. The variation in survival across the main indications was significant (log rank test); pterygium had the longest survival, and bullous keratopathy the shortest. Reprinted with permission from: Department of Ophthalmology, Adelaide: The Australian Corneal Graft Registry 2012 Report; KA Williams, MT Lowe, MC Keane, VJ Jones, RS Loh, DJ Coster (Eds); 2012, pp. 1–246. URL: http://hdl.handle.net/2328/25859.

Number at Risk

Identity	Initially	3 years	6 years	9 years	12 years	15 years	18 years	21 years
Bullous keratopathy	341	7	n/a	n/a	n/a	n/a	n/a	n/a
Keratoconus	266	41	12	4	2	n/a	n/a	n/a
Previous failed graft	252	30	12	8	3	n/a	n/a	n/a
Corneal dystrophies	126	5	1	n/a	n/a	n/a	n/a	n/a
Pterygium	163	48	17	11	7	3	2	1
Trauma	124	37	18	6	2	1	1	n/a
Corneal Ulcers	126	18	8	2	1	1	n/a	n/a
Infections	82	15	4	2	n/a	n/a	n/a	n/a
Other	270	74	18	9	2	1	n/a	n/a

with and without a corneal graft rejection. CTLs were divided into naïve and primed CTLs based on their sensitivity or resistance to anti-CD8 or cyclosporine A in vitro. Rejections were strongly associated with the presence of primed donor-specific CTLs (Roelen et al., 1995).

All the different types of corneal transplantation and not only penetrating corneal transplants can lead to rejection as shown by data of the Australian Corneal Graft Registry for anterior (Figs. 7 and 8) and posterior keratoplasties (Fig. 9). Tan describes that in penetrating keratoplasty (PK), acute endothelial rejection occurs in 20% of the cases during 5-year follow up. While in anterior lamellar keratoplasty (ALK) there is no risk of endothelial rejection as the endothelium is not transplanted, stromal rejection occurs only in 1–2%. This lack of rejections is probably not directly related to the tissue characteristics, but to the disease, as ALK are mostly performed for keratoconus, a type of corneal disease that is not associated with corneal angiogenesis, and leads to infrequent rejection even in PKs. In endothelial keratoplasty (EK), endothelial rejection rates are probably similar as in PK (Tan et al., 2012).

3. Matching major histocompatibility antigens

Data from the European Eye Bank Association in 1995 indicate that over time, HLA matching has been performed less and less, although some confounding factors are present. In 1991, matching for HLA Class I was performed in 24% and for Class II in 19% of the transplanted corneas, compared to 9.5% for both Class I and II in 1995 (Maas-Reijs et al., 1997). This trend continued and in 2008, HLA-Class II matching in corneal allografting was performed in only 3.3% of the transplanted corneas reported to the European Eye Bank

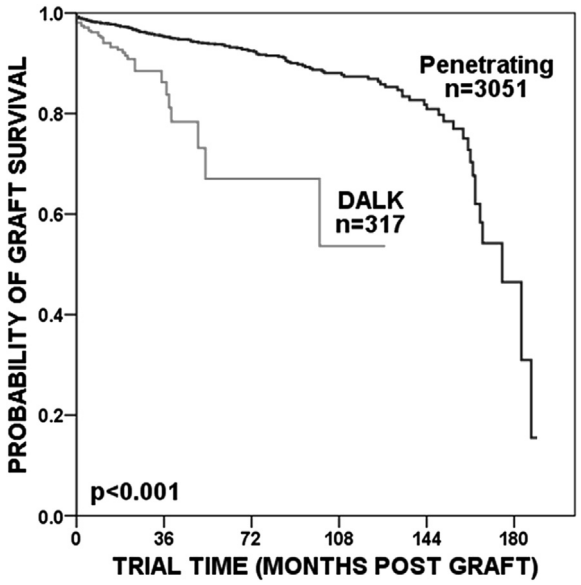

Fig. 8. Kaplan–Meier survival plots of observed penetrating corneal grafts (penetrating) and deep anterior lamellar keratoplasty (DALK) procedures performed from 1996 through 2013 for keratoconus. The numbers on the plot represent the number of grafts at risk in each stratum. The differences between the curves are significant at $P < 0.001$ (log-rank test). Penetrating grafts for keratoconus fared significantly better than DALK procedures for the same indication over the same era. Reprinted from Ophthalmology, Vol. 121/5, DJ Coster, MT Lowe, MC Keane, KA Williams, A comparison of lamellar and penetrating keratoplasty outcomes: a registry study, pp. 979–987, Copyright 2014, with permission from Elsevier.

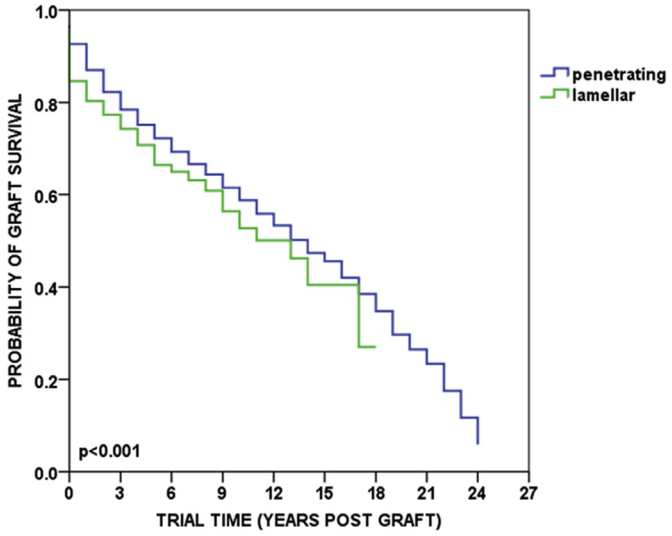

Fig. 7. Kaplan–Meier survival plot for corneal graft survival according to type of keratoplasty (irregardless of indication). Penetrating grafts survived longer than the lamellar grafts (log rank test). Reprinted with permission from: Department of Ophthalmology, Adelaide: The Australian Corneal Graft Registry 2012 Report; KA Williams, MT Lowe, MC Keane, VJ Jones, RS Loh, DJ Coster (Eds); 2012, pp. 1–246. URL: http://hdl.handle.net/2328/25859.

Number at Risk

Identity	Initially	3 years	6 years	9 years	12 years	15 years	18 years	21 years
Penetrating	16736	6960	3428	1761	973	465	187	43
Lamellar	1751	274	90	41	16	5	2	n/a

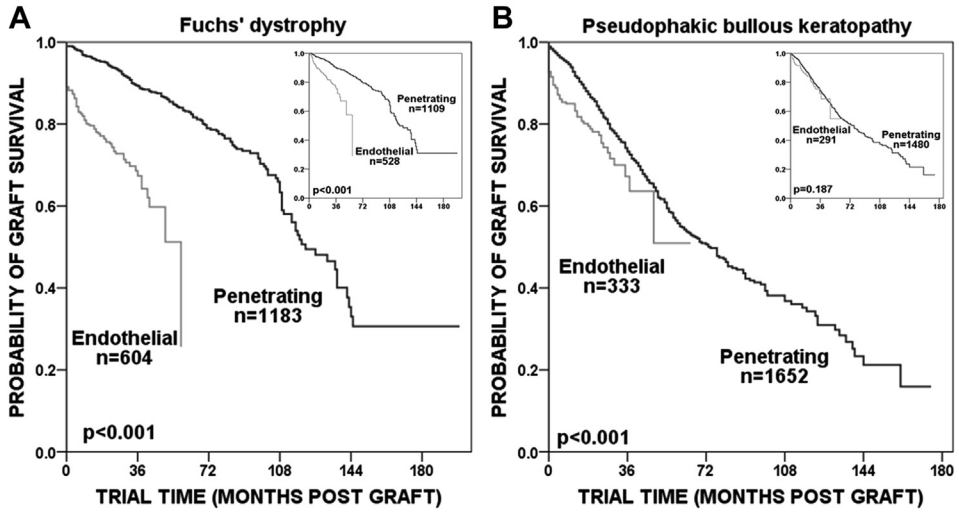

Fig. 9. Kaplan–Meier survival plots of observed penetrating corneal grafts (penetrating) and endokeratoplasties (endothelial) performed from 1996 through 2013, stratified by indication for graft. The numbers on the plot represent the number of grafts at risk in each stratum. A, Grafts for Fuchs' dystrophy; B, Grafts for pseudophakic bullous keratopathy. The differences between the curves in (A) and (B) are significant at $P < 0.001$ (log-rank test). The insets show the data with all grafts that failed within the first postoperative month in each stratum that were removed from analysis. Penetrating grafts for Fuchs' dystrophy fared better than did endokeratoplasties for the same indication ($P < 0.001$), even when early graft failures were removed from analysis. Reprinted from Ophthalmology, Vol. 121/5, DJ Coster, MT Lowe, MC Keane, KA Williams, A comparison of lamellar and penetrating keratoplasty outcomes: a registry study, pp. 979–987, Copyright 2014, with permission from Elsevier.

Association (European Eye Bank Association, 2010). However, a report by BISLIFE, based in the Netherlands, reported that 22% of the implanted corneas from centers they are collaborating with, were being HLA matched in 2009 (BIS Foundation, 2009). In the Australian Corneal Graft Registry Report of 2012, and the Cornea Donor Study published in 2012 (Stulting et al., 2012), HLA-matching rates and methods are not mentioned at all and are supposed to be performed rarely, even for corneal regrafts, while graft failure as an indication for corneal transplantation is increasing and immune rejection remains the main reason of graft failure. The Dutch Organ Transplantation Registry report of 2013 mentioned that of the 1286 transplanted corneas, only 46 were HLA-typed (Leiden van et al., 2014).

Currently, graft failure is the third or fourth indication for keratoplasty (both penetrating and lamellar), depending on the country, and ranges from 12 to 21%, while the percentage is gradually increasing (Keenan et al., 2012; Konijn-Janssen et al., 2011; Tan et al., 2014; Williams et al., 2012) overall, the number of grafts for Fuchs endothelial keratoplasty is increasing, and this may lead to more regrafts (Claesson and Armitage, 2013; Keenan et al., 2012). This latter study compared first and regrafts for keratoconus, Fuchs endothelial dystrophy and bullous keratopathy in Sweden and demonstrated that not only the risk for failure was increased, but also the resulting visual outcome was worse when one needed a regraft for keratoconus and Fuchs endothelial dystrophy. One should thus look for ways to reduce the need for retransplantations.

One wonders whether more frequent HLA matching might help to prevent retransplantations. Matching is more expensive and cumbersome than the random distribution of corneal grafts. We looked at the papers that describe the effect of HLA matching on corneal transplant survival (Table 1), and while studies before 2000 have shown contradictory results for HLA matching for corneal allografts, the overall conclusion from more recent studies favours HLA matching for Class I for high risk cases (see below). However, the book has not been closed on the effect of HLA-DR typing and matching (Baggesen et al., 1991; Beekhuis et al., 1991; Ehlers and Kissmeyer-Nielsen, 1979; Foulks et al., 1983; Hargrave et al., 2004; Hoffmann and Pahlitzsch, 1989; Reinhard et al., 2003; Stark et al., 1978; The Collaborative Corneal Transplantation Studies Research Group, 1992; Vail et al., 1997; Vannas, 1975). For example with regard to HLA-DR, the collaborative corneal transplantation studies (CCTS) found no beneficial effect of HLA-DR matching (The Collaborative Corneal Transplantation Studies Research Group, 1992), while another study found that HLA-DR matching decreased graft survival (Vail et al., 1994). A third study on the other hand found that matching for HLA-DR could inhibit or even obliterate the effect APCs have in initiating immune rejection (Baggesen et al., 1996). The CCTS study was a major study performed in the USA, and showed no positive effect of HLA-DR typing and matching in corneal allografts for HLA-A and -B either. However, when one looks closely at the HLA typing data of the CCTS, the outcome of the CCTS study was greatly affected by the lack of repeatability of the HLA typing: in a separate study, peripheral blood samples that had been used to determine the different HLA alleles in the CCTS were retyped, and it was noticed that there were especially problems with regard to HLA-DR (Hopkins et al., 1992). As there was only 55% concordance between the original result and the outcome of retyping, this must clearly have prevented proper matching (Hopkins et al., 1992; Volker-Dieben et al., 2000). Völker-Dieben et al. analyzed the effect of erroneous HLA-DR typing in a study where they determined the beneficial effect of HLA-DR typing on corneal allograft survival (Volker-Dieben et al., 2000). Even with 5% erroneous typing results, the effect of matching for HLA-DR was lost. The conclusion of the CCTS study, i.e. that matching did not lead to better survival, was therefore based on incorrect typing data, and cases that were considered well matched, may well have been badly matched. Apart from the influence of erroneous matching, the rather aggressive immunosuppressive regimens used in this study possibly prevented any positive findings as well (Armitage, 2004; The Collaborative Corneal Transplantation Studies Research Group, 1992; Volker-Dieben et al., 2000). One may wonder whether proper matching might reduce the amount of immunosuppression needed, or whether the use of aggressive immunosuppressive treatment overcomes the need for HLA matching in corneal transplantation. As corticosteroids may have many side effects, we will ask the question whether other studies support the use of HLA matching in corneal transplantation.

Table 1
Overview of clinical studies of HLA-matching and corneal graft survival (details).

Author, year	Study setup	Patients (n)	Mean follow up	Antigens analyzed	Risk groups	Blood cross-matched before Tx	Typing method	Measured event
Allansmith et al., 1974	Random selected prospective Single center	43	12 months (range 0–26 months)	A	Overall	No (afterwards in 11 pt)	Broad Serological	Immunoreaction Non-immunological failure
Gibbs et al., 1974	Retrospective Single center	155	Unknown (observation period 24 months)	A	Based on degree of vascularization (absent, mild, severe)	No	Broad Serological	Rejection episode Opaque graft
Vannas, 1975	Prospective Single center	80	Unknown	A,B,C	Overall High (vascularized bed)	Yes	Both not mentioned (serological likely)	Rejection (not further defined)
Batchelor et al., 1976	Retrospective Single center	200	Unknown (range 7–44 months)	A, B	Overall Severe vascularization	No	Broad Serological	Irreversible immune rejection
Stark et al., 1978	Prospective/ *retrospective* Single center	84/*19*	14 months (range 3–45 months)	A, B, C	High (<2 quadrants vascularized + regraft and/or previous foreign HLA exposure)	Yes/*No*	Broad Serological	Immunoreaction
Ehlers and Kissmeyer-Nielsen, 1979	Retrospective Single center	222	Unknown (range 0–138 months)	A, B	Overall	Unknown	Broad, typing method unknown (serological)	Rejection episode and/ or opaque graft
Foulks and Sanfilippo, 1982	Prospective Singe center	46	16 months (range unknown)	A, B	High (>1 quadrant vascularized and/or regraft)	Yes	Broad Serological	Graft failure
Ozdemir, 1986	Prospective Single center	40	24 months (range unknown)	A, B	High (>1 quadrant vascularized and/or regraft)	Yes	Broad Serological	Graft failure
Sanfilippo et al., 1986	Blind prospective, Two center	97	Unknown (range 1–77 months)	A, B	High (>1 quadrant vascularized and/or regraft)	Yes	Broad Serological	Immunoreaction and irreversible reaction
Keyserlingk et al., 1987	Retrospective One center	57	22 months (range unknown)	A, B, DR	Normal High (significant vascularization, prior rejection episode, corneal perforating ulcer)	Unknown	Broad Serological	Immunoreaction and graft failure
Volker-Dieben et al., 1987	Prospective Two center	1218/*123*	Unknown (range 12–121 months, end point Kaplan Meier 60 months)	A, B/*DR*	Overall High (>1 quadrant vascularized)	Unknown	(Broad) Serological	Clear graft
Hoffmann and Pahlitzsch, 1989	Prospective, Single center	137	Unknown	B, DR	Normal High (based on preoperative diagnosis, postoperative AC irritation, graft diameter > 7.5 mm, less than 2 compatible B or DR)	Unknown	Not mentioned	Endothelial immunoreaction
Boisjoly et al., 1990	Masked prospective Single center	435	36 months follow up for all	A, B	Overall	Yes	Broad Serological	Endothelial immunoreaction Graft failure
Baggesen et al., 1991 Study updated (extended) in 1996	Prospective Multi center	74	Unknown (range 1–40 months)	DR	High (vascularization >0 quadrant and/or regraft)	Unknown	Broad and DNA, Serology and RFLP (DNA)	Graft failure
Beekhuis et al., 1991	Prospective (A, B), *Retrospective (DR)*, Single center	107	Unknown (at least 36 months)	A, B/*DR*	High (>1 quadrant vascularized and/or regraft)	Unknown	Unknown	Immunoreaction as cause of graft failure
The Collaborative Corneal Transplantation Studies Research Group, 1992	Double blind prospective Multi center	419	Unknown (at least 18 months, and for 69% at least 36 months)	A,B, DR	High (>1 quadrant vascularized)	Yes, but unknown whether for all	Broad Serological	Immunoreaction and graft failure (due to rejection and due to all causes separately)
Hoffmann et al., 1994	Retrospective Single center	248	Unknown (at least 18 months)	A,B, DR	Normal High (regraft)	Unknown	Serological	Immunoreaction

(*continued on next page*)

Table 1 (*continued*)

Author, year	Study setup	Patients (n)	Mean follow up	Antigens analyzed	Risk groups	Blood cross-matched before Tx	Typing method	Measured event
Vail et al., 1994	Prospective Multi center	542	Unknown (range 7–66 months)	A, B, DR	Low (Normal) High (based on recipients age, regraft, diagnosis, vascularization, surgeon experience)	Unknown	Not mentioned	Immunoreaction and graft failure
Baggesen et al., 1996	Masked, randomized retrospective Multi center	74	Unknown (at least 36 months)	DR	High (vascularization >0 quadrant and/or regraft)	Unknown	High resolution RFLP (DNA)	Graft failure
Vail et al., 1997	Retrospective Multi center	602	Unknown	A, B, DR	Overall	Unknown	Not mentioned	Immunoreaction
Munkhbat et al., 1997	Retrospective Single center	81	Unknown (1 year follow up)	DRB1, DQB1, DPB1	Low (normal) High (>2 quadrant vascularized and/or regraft)	Unknown	Broad RFLP (DNA)	Immunoreaction
Munkhbat et al., 1999	Retrospective Single center	79	Unknown (1 year follow up)	A, B	Low (normal) High (>2 quadrant vascularized and/or regraft)	Unknown	Broad RFLP (DNA)	Immunoreaction
Volker-Dieben et al., 2000	Prospective/ *retrospective* Single center	1681/*558*	58 months (range unknown)	A, B/*DR*	Low (Normal) High (>1 quadrant vascularized)	Yes	Broad, Serological	Immunoreaction
Khaireddin et al., 2003 (Update on study Hoffmann et al., 1994)	Retrospective Single center	459	31 months low risk (range 2–159 months) 45 months high risk (range 2–182 months)	A, B, DR	Low (normal) High (>1 quadrant vascularized and/or previous ulcer, burn, or regraft)	Unknown	Broad and splits Serological	Immunoreaction
Bartels et al., 2003	Prospective Single center	303	50 months (median; range unknown)	A, B	High (>1 quadrant vascularized and/or regraft)	Yes	Broad (matching) and split (analysis) Serological (complement-depended cytotoxicity)	Immunological graft failure (graft not clear in 2 months with treatment) Overall graft failure
Reinhard et al., 2004a	Prospective Single center	418	40 months (not 60 months as reported; range unknown)	A, B, DR	Low (normal)	Unknown	Broad Serological (A, B) Immunogenetical (DR)	Immunoreaction Rejection-free clear graft
Bohringer et al., 2004	Prospective Single center	545	24 months (range unknown; standard deviation 18 months)	A, B, DR	Low (normal) (Avascular and first transplant)	Unknown	Broad (matching) and splits with HLA Matchmaker (analysis) Serological (A, B) Molecular (DR)	Immunoreaction
Minor antigens								
Inoue et al., 2000b	Retrospective Single center	396	49 months normal risk (range 6–122 months) 45 months high risk (range 6–123 months)	H-Y	Low (normal) High (>1 quadrant vascularized and/or regraft)	Unknown	Gender based	Immunoreaction
Reinhard et al., 2004a	Prospective Single center	418	40 months (not 60 months as reported)	H-Y	Low (normal)	Unknown	Gender based	Immunoreaction
Böhringer et al., 2006 (Reinhards group)	Retrospective Unknown amount of centers	291	31 months for H-Y (range unknown; standard deviation 22 months) 37 months for HA-3 (range unknown; standard deviation 22 months)	H-Y, HA-3	Low (normal) High (oversized grafts, glaucoma, herpetic scars, and/or regraft)	Unknown	Gender based (H-Y) and by PCR (HA-3)	Immunoreaction

When we take into account the differences between low- and high-risk patient populations with a focus on recent findings, a positive effect of serological HLA-matching is certainly found for corneal allografting, and is most profound in the high risk patient group. The diversity in the reported results can be explained by the fact that in many studies, serological HLA typing was performed, which has been reported to be erroneous in 16–35% of the cases, and not the DNA-based HLA typing techniques which are much more accurate and came gradually into use around 2000 (Bozon et al., 1997; Yu et al., 1997; Zafar et al., 2003). The standardization of HLA typing techniques has been essential in improving results. While typing for HLA Class I antigens was well developed around 1978 (Albert et al., 1976; Terasaki et al., 1978), problems with HLA-DR typing were still encountered in 1992 (Volker-Dieben et al., 2000). The application of DNA-methods to determine HLA gene polymorphisms should be able to produce conclusive results on the benefit of HLA matching in corneal grafting. The influence of minor H antigens could be another factor explaining the contradictory results (Nicholls et al., 1991).

3.1. Early studies on HLA matching

In 1986, a major study was published in the New England Journal of Medicine (Sanfilippo et al., 1986). This study reported on a prospective masked study at two hospitals, using ABO-compatible, crossmatch-negative recipients, matched for HLA-A and -B antigens. Only patients with vascularization of two or more quadrants or having a history of prior corneal-graft loss due to immune-mediated rejection were included. Post-operatively, topical corticosteroids were applied two to three times per day. The well-matched recipients developed a rejection episode in 21% of cases, versus 49% in the poorly-matched group.

According to a report published in 1995 (Beekhuis, 1995), cornea transplant centers in the Eurotransplant area (The Netherlands, Germany, Belgium and Austria) used HLA-matched grafts in 19% of cases. However, between centers, this percentage varied between 0 and 64%, and there was no consistency in the weighing of the importance of the HLA-A and -B versus the HLA-DR antigens. The views on what constituted a high-risk cornea for which HLA matching was advantageous were fairly consistent: a previous immunological rejection in the same eye, deep stromal vascularization in two or more quadrants, and chemical burns. There was no consensus on whether a regraft in the same eye after failure without a rejection, or a previous immunological rejection in the fellow eye constituted an indication for HLA matching. In Germany, HLA-DR compatibility was considered more important than matching for the Class I antigens, while in the Netherlands, only matching for HLA-A and -B was applied (Beekhuis, 1995).

3.2. HLA matching using HLA-A, -B and -DR

Matching donor and recipient can be performed for HLA Class I only, where two A's and two B's can be matched. When also looking at HLA-DR antigens, the total number of potentially-matchable antigens goes up to six. In 2000, Völker-Dieben et al. reported on all corneal transplantations performed in a single center between 1976 and 1996, that had been matched on the basis of HLA-A and -B antigens, and retrospectively typed for HLA-DR (558 cases) (Volker-Dieben et al., 2000). A beneficial effect of HLA-A and -B matching was found in both non-vascularized and vascularized cases, and a very significant influence was noticed for 0 mismatches for HLA-DR versus 1 or 2 mismatches for HLA-DR in moderately and severely vascularized recipients. No effect of DR matching on non-vascularized cases was noticed. Surprisingly, the paper does not provide information which technique was used for HLA-DR typing, serology or PCR.

Studies performed since 2000, which mostly use modern methods of HLA typing, show a clear beneficial effect of HLA matching in (high-risk) patients (Bartels et al., 2003; Hargrave et al., 2004; Khaireddin et al., 2003; Osawa and Streilein, 2005; Reinhard et al., 2003; Volker-Dieben et al., 2000). Khaireddin, using serological HLA typing, observed that a donor-recipient match of two or more of the six alleles in HLA-A, -B or -DR reduced the rejection rate by at least 10% in low-risk (at 10 years after PKP) cases, and by 40% in high-risk patients, at three years after PKP. An analysis at the split antigen level (highly detailed HLA typing) showed no improved results. In the low-risk group, matching for either the HLA-B or -DR locus was found to reduce the number of rejections, while in the high-risk group matching for any of the loci reduced rejections. In 2001, Bartels et al. performed retrospective DNA-based HLA typing of donors and of recipients with or without a rejection episode (Bartels et al., 2001). An increased rejection-free survival time of the patients (low- and high risk combined) was observed in graft recipients with one or two HLA-A matches, while no difference was observed for HLA-B and -DR. However, when one specifically looked at the high-risk group and studied the split level typing (identifying HLA subtypes), less rejection was observed in graft recipients with one or two HLA-DR matches. HLA-B matching had no significant effect on graft rejection, which may be due to the low number of HLA-B matches observed in this retrospective study (as the B locus is the most polymorphic one) (Bartels et al., 2001). In 2003, Bartels repeated the study for only HLA-A and -B, looking at the split-level HLA typing in a larger group of high-risk patients (Bartels et al., 2003), taking graft failure instead of graft rejection as end-point. Still, a better survival was found when there was no or only one split-mismatch for HLA-A/HLA-B.

In 2004, Reinhard et al. reported on a study in 418 first keratoplasties with an avascular recipient cornea, in which matching took place if a cornea with 0–2 mismatches of the six HLA-A, -B and -DR antigens could be found within 6 months (Reinhard et al., 2004a). If a match had not been found during that time period, a graft with 3–6 mismatches was allocated. HLA typing was performed serologically for the Class I antigens, and by PCR for Class II; corticosteroids had been given topically as well as systemically. Even in these low-risk cases, a clear influence of matching was observed: at 4 years postoperatively, 92% of the matched group and 72% of the badly-matched group were rejection free. Most of these rejections were reversible; nevertheless, a positive effect on corneal clarity was present. Interestingly, Reinhard et al. did not find a clear influence of the number of (mis)matches on chronic endothelial cell loss in a study of 223 normal risk transplantations. However, as only first PKPs in an avascular host cornea were included in their study, the occurrence of immunological responses was limited (although no numbers were reported) and strangely, cases with an identifiable immune response during follow up were excluded from the study (Reinhard et al., 2004b). Böhringer et al. reported in a prospective single center study of 545 normal risk corneal transplants, matched for HLA-A, and -B at the split level with a HLAMatchmaker algorithm (triplet string matching), that having 13 or less mismatches led to 85% rejection-free graft survival at 3 years, which significantly differed from the 76% rejection free survival when having more than 13 mismatches (Bohringer et al., 2004). This was despite having significantly more HLA-DR mismatches in the first group. Conventional matching for HLA-A and -B resulted in 92% rejection-free survival when only having 0–1 mismatches compared to 76% rejection-free survival when having 3–4 mismatches. Although this last finding was not significant, these studies clearly state the benefit of HLA-A and -B

matching. An overview of the studies found in literature on HLA matching is shown in Tables 1 and 2.

The unclear effect of HLA-DR may be due to different outcomes in relation to the degree of Class I matching: in kidney transplantation, HLA-DR matches lead to increased graft survival, but the otherwise significant effect of HLA-A and -B matching disappears when HLA-DR is incompatible (Doxiadis et al., 2007; Johnson et al., 2010).

3.3. Approaches to allo-antibodies

Although cell-mediated immunity is considered the dominant cause of corneal allograft rejection (Boisgerault et al., 2001; Hegde et al., 2005; Krensky et al., 1990; Niederkorn, 2001, 2007), complement-activating allo-antibodies may also contribute to corneal allograft rejection, especially once sensitization has occurred. In contrast to patients awaiting a renal transplant, patients on the corneal transplant waiting list are not routinely screened for the presence of HLA-specific antibodies. Serum of only high-risk or retransplant patients is screened for such antibodies, using either the complement-dependent cytotoxicity assay and Elisa (LAT, One Lambda) or the luminex screening assay (Lifecodes® SSO; Immucor, Nijlen, Belgium). Patients with a negative screening result (Panel Reactive Antibodies (PRA) $\leq$5%) are identified as non-immunized. For corneal transplant patients, pretransplant crossmatches are not being performed. In patients that are immunized (PRA >5%), the complement-dependent cytotoxicity test is used to characterize the specificities of the HLA antibodies. In the few immunized patients, one thoroughly looks for acceptable mismatches, first by selecting HLA antigens which gave negative results in the complement-dependent cytotoxicity screening and by applying the HLAMatchmaker program as described by Duquesnoy (2002).

3.4. Current strategy for HLA screening and typing

In the Netherlands, only high-risk recipients (mostly retransplants) are typed for the HLA antigens: for the LUMC, on average only five patients per year. In contrast, a considerable number of cornea donors (around 250 a year) happen to be typed for HLA in the LUMC for other organ donation purposes. The HLA class I antigens are currently typed using oligonucleotide probes, that provide a low resolution result with names that follow the HLA nomenclature that was developed in 2010 by the WHO Committee for Factors of the HLA System. HLA-DRB1 and DQB1 are similarly genotyped using the sequence-specific oligonucleotide probe (PCR/SSOP) technique as previously described (Verduyn et al., 1993).

HLA typing of the donors is used for allocation of these corneas to high risk patients and is done on basis of HLA-A, -B and -DR antigen matching, taking broad specificities into account first. Furthermore, in immunized patients, unacceptable mismatches are reckoned with and donors are selected after exclusion of these HLA mismatches. The allocation rules are ordered on HLA-A, -B, -DR broad level, country of the recipient, urgency, and HLA-A, B antigen split level. Currently, 60 Dutch patients are on the Dutch waiting list for an HLA-typed cornea, as well as 150 patients from abroad (Germany, Belgium, Austria, Italy, and Scandinavia). Around 600 Dutch patients are waiting for a non-HLA typed cornea. Annually, around 1250 Dutch inhabitants receive a non HLA-typed cornea transplant and around 50–60 a HLA-typed cornea transplant (data provided by the Dutch Transplantation Society).

3.5. HLAMatchmaker

HLAMatchmaker is a matching algorithm which was originally introduced to explain why many mismatched transplants do well. HLAMatchmaker determines histocompatibility at the epitope rather than antigen level in terms of the humoral alloimmune response. An epitope has two characteristics namely antigenicity, i.e. the reactivity with antibody, and immunogenicity, i.e. the ability of inducing an antibody response. Immunogenicity depends on the structural difference between an immunizing protein and the antibody responder's homologous proteins. Certain structural differences lead to immunodominant epitopes whereas others are associated with low immunogenicity. The elucidation of three-dimensional molecular structures and amino acid sequence differences between HLA antigens made it possible to view each HLA antigen as a string of short linear sequences (triplets) involving polymorphic amino acid residues in antibody-accessible positions. These triplets are considered key elements of epitopes that can induce the formation of specific antibodies.

The triplet-matching concept has clinical relevance because HLA-A, B mismatched kidney transplants that are compatible at the triplet level have practically the same graft survival rates as the zero HLA-A, B antigen mismatches defined by conventional criteria. Triplet matching has been shown to benefit platelet transfusions of refractory thrombocytopenic patients. HLAMatchmaker is also useful in the determination of acceptable mismatches for highly-sensitized patients that are considered for kidney transplantation.

Recent studies have led to an updated version of HLAMatchmaker: stereo-chemical modelling of crystallized complexes of antibodies with different protein antigens revealed that antigenic proteins have functional epitopes consisting of amino acid residues that are about 3 Å apart from each other and at least one of them is non-self. The term now used to describe patches of polymorphic residues within a radius of 3.0–3.5 Å is “eplet”. By using this updated version of HLAMatchmaker (using eplets) a more complete repertoire of structurally-defined HLA epitopes can be made, which also provides a more detailed assessment of HLA compatibility.

Using HLAMatchmaker (Duquesnoy, 2002) to identify the number of mismatched HLA Class I eplets (polymorphic amino acid configurations), Böhringer et al. were able to show an additional value of the HLAMatchmaker on top of HLA Class I matching in preventing immunorejection after keratoplasties (Bohringer et al., 2010).

4. Matching minor histocompatibility antigens

Even in fully HLA-matched cases, corneal survival, after excluding the non-immunological causes for graft failure, is well below 100% (Baggesen et al., 1991; Beekhuis et al., 1991; Ehlers and Kissmeyer-Nielsen, 1979; Foulks et al., 1983; Hargrave et al., 2004; Hoffmann and Pahlitzsch, 1989; Reinhard et al., 2003; Stark et al., 1978; The Collaborative Corneal Transplantation Studies Research Group, 1992; Vail et al., 1997; Vannas, 1975). This indicates that aside from major histocompatibility antigens, minor H antigens could play a role. Studies in murine models in the 1990s observed that minor H antigens were of higher relevance for alloimmunity leading to corneal graft rejection than HLA antigens (Nicholls et al., 1991; Sano et al., 1996; Sonoda and Streilein, 1992; Yamada and Streilein, 1998). In humans, one study found that mismatching for the minor HY antigen, which is only expressed on male cells, seemed not to influence graft survival (Inoue et al., 2000b). However, an influence of HY in this study might well been obscured as they did not correct for HLA-antigen mismatches. A more recent clinical study on corneal graft survival, with a mean follow up of two years and taking HLA matching into account, showed that mismatches for the minor H antigen HY indeed led to more graft rejection. Mismatches in the minor antigen HA-3 also had a negative effect on graft survival, although not significantly (Böhringer et al., 2006).

Table 2
Overview of clinical studies of HLA-matching and corneal graft survival (outcome).

Author, year	Antigens analyzed	Outcome (n = group size)
Allansmith et al., 1974	A	**No effect overall** (immunoreaction); no p-value mentioned 2 matches 14% immunoreaction (n = 7); 1 match 9% immunoreaction (n = 22); 0 matches 0% immunoreaction (n = 14) NB: effect of confounding factors not mentioned.
Gibbs et al., 1974	A	**No effect overall** (rejection episode); no p-value mentioned 2 matches 41% rejection (n = 29); 1 match 52% rejection (n = 62); 0 matches 41% rejection (n = 64) **Beneficial severe vascularization** (opaque graft with or without rejection); $p < 0.05$ 2 matches 47% opaque graft (n = 19); 0 matches 76% opaque graft (n = 29) NB: effect of other confounding factors not mentioned.
Vannas, 1975	A,B,C	**Beneficial overall** (rejection); no p-value mentioned 5–6 matches 4% rejection (n = 27); 3–4 matches 21% rejection (n = 19); Untyped 26% rejection (n = 34) **Beneficial high risk** (rejection); no p-value mentioned 5–6 matches 8% rejection (n = ?); Untyped 39% rejection (n = ?) NB: effect of confounding factors not mentioned.
Batchelor et al., 1976	A, B	**Beneficial severe vascularization** (irreversible immune rejection) Two year follow-up (n = 73): 2 matches 27% rejection (n = 12); 1 match 72% rejection (n = 30); $p < 0.05$ 2 matches 27% rejection (n = 12); 1 or 0 matches 70% rejection (n = 60); $p < 0.01$ Confounding factors (unknown whether equally distributed per group): 120 first grafts and 80 regrafts; graft sizes differ 5–10 mm;
Stark et al., 1978	A, B, C	**No effect high risk** (immunoreaction); no p-value mentioned 4 matches 0% immunoreaction (n = 1); 3 matches 40% immunoreaction (n = 5); 2 matches 9% immunoreaction (n = 11); 1 match 26% immunoreaction (n = 35); 0 matches 27% immunoreaction (n = 51) Confounding factors (unknown whether equally distributed per group): 20 first grafts and 64 regrafts.
Ehlers and Kissmeyer-Nielsen, 1979	A, B	**Beneficial overall** (rejection episode and/or opaque graft); $p < 0.005$ 2–4 matches 12% rejection (n = 49); 0–1 matches 35% rejection (n = 173) Confounding factors (having impact on outcome and not corrected for): Transplant indication, p-value unknown.
Foulks and Sanfilippo, 1982	A, B	**Beneficial high risk** (graft failure); trend, no p-value mentioned 3–4 matches 0% graft failure (n = 7); 0–2 matches 21% graft failure (n = 39) NB: effect of confounding factors not mentioned.
Ozdemir, 1986	A, B	**Beneficial high risk** (graft failure): $p < 0.05$ Five year follow-up: 2–3 matches 15% graft failure (n = 20); 0–1 matches 45% graft failure (n = 20) NB: effect of, or possible confounding factors not mentioned.
Sanfilippo et al., 1986	A, B	**Beneficial high risk** (immunoreaction); $p < 0.01$ (Chi-square Test), $p < 0.01$ (Multivariate Cox Regression) 2–3 matches 21% immunoreaction (n = 38); 0–1 matches 49% immunoreaction (n = 59) **Beneficial high risk** (irreversible immunoreaction); $p < 0.02$ (Multivariate Cox Regression) For the irreversible immunoreactions, group size and percentage are unknown. NB: p-values of multivariate analysis adjusting for age and graft size.
Keyserlingk et al., 1987	A, B, DR	**Beneficial high risk** (graft survival); $p = 0.001$ 2–4 matches 0% graft failure (n = 7); 0–1 matches 46% graft failure (n = 13) **No data for normal risk** (n = 20) NB: effect of, or possible confounding factors not mentioned.
Volker-Dieben et al., 1987	A, B/*DR*	**A, B** **Beneficial overall** (graft clarity); $p < 0.001$ 2–4 matches (A,B) ± 40% opaque graft (n = 497); Untyped ± 50% opaque graft (n = 721) **Beneficial high risk** (graft clarity); $p < 0.001$ 2–4 matches (A,B) ± 45% opaque graft (n = 397); Untyped ± 60% opaque graft (n = 343) ***DR*** **No effect overall** (graft clarity); ns 2 matches (DR) ± 12% opaque graft (n = 45); 1 match (DR) ± 25% opaque graft (n = 64); 0 matches (DR) ± 28% opaque graft (n = 14) **No effect high risk** (graft clarity); ns 2 matches (DR) ± 18% opaque graft (n = 34); 1 match (DR) ± 34% opaque graft (n = 44); 0 matches (DR) ± 30% opaque graft (n = 11) Confounding factors (having influence on graft clarity, but not corrected for): degree of vascularization, $p < 0.001$; number of regrafts, $p < 0.001$; diagnosis, $p < 0.001$; patient age, $p < 0.001$; graft diameter, $p < 0.001$ NB: unknown whether the 123 DR-typed patients were in the matched or untyped group for A,B.
Hoffmann and Pahlitzsch, 1989	B, DR	**Beneficial overall** (immunoreaction); $p < 0.05$ 2–4 matches 11% immunoreaction (n = 46) vs. 0–1 matches 29% immunoreaction (n = 91) **No effect normal risk** (immunoreaction); $p < 0.10$ 2–4 matches 0% immunoreaction (n = 15); 0–1 matches 13% immunoreaction (n = 68) **Beneficial high risk** (immunoreaction); $p < 0.01$ 2–4 matches 16% immunoreaction (n = 31); 0–1 matches 74% immunoreaction (n = 23) NB: effect of confounding factors not mentioned.
Boisjoly et al., 1990	A, B	**Beneficial overall** (immunoreaction); $p < 0.001$ (multivariate analysis correcting for vascularization, graft diameter, HLA-DR, regrafting, and age) 2–4 matches 19% immunoreaction (n = 174) (0 or 1 mismatch at both loci); 0–2 matches 40% immunoreaction (n = 261) (2 mismatches at either loci) **Beneficial overall** (graft failure); $p = 0.04$ (unclear whether corrected for confounders) 2–4 matches 18% graft failure (n = 174) (0 or 1 mismatch at both loci); 0–2 matches 29% graft failure (n = 261) (2 mismatches at either loci) Analysis for DR and high risk group had too less power.

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Table 2 (*continued*)

Author, year	Antigens analyzed	Outcome (n = group size)
Baggesen et al., 1991 Study updated (extended) in 1996	DR	**Beneficial high risk** (graft failure); p = 0.003 18 month follow-up 1–2 matches 7% graft failure (n = 51); Untyped 50% graft failure (n = 23) Confounding factors (having influence on outcome, but not corrected for): regrafts, p < 0.01; NB: RFLP detected mismatches were serology did not
Beekhuis et al., 1991	A, B/*DR*	**Unknown, no statistical comparisons made** NB: More grafts failed from non-immunological reasons than from immunoreactions: 13 out 33 due to immunoreaction
The Collaborative Corneal Transplantation Studies Research Group, 1992	A,B, DR	36 months follow-up: **A, B** **No effect high risk** (graft failure all causes); p = 0.59 3–4 matches 33% graft failure (n = 137); 0–2 matches 37% graft failure (n = 282) **No effect high risk** (immunoreaction); p = 0.83 3–4 matches 64% immunoreaction (n = 137); 0–2 matches 66% immunoreaction (n = 282) **No effect high risk** (failure due to immunoreaction); p = 0.66 3–4 matches 21% failure due to immunoreaction (n = 137); 0–2 matches 26% failure due to immunoreaction (n = 282) **DR** **No effect high risk** (graft failure all causes); p = 0.99 2 matches ± 43% graft failure (n = 199); 0–1 match ± 41% graft failure (n = 220) **No effect high risk** (immunoreaction); p = 0.53 2 matches ± 69% immunoreaction (n = 199); 0–1 match ± 70% immunoreaction (n = 220) **No effect high risk** (failure due to immunoreaction); p = 0.87 2 matches 25% failure due to immunoreaction (n = 199); 0–1 match 24% failure due to immunoreaction (n = 220) **Beneficial high risk** (failure due to immunoreaction); p = 0.02 When using only donor-recipient pairs in which four distinct DR antigens were identified. NB: p-values of multivariate analysis adjusting for ABO, vascularization, and regrafts
Hoffmann et al., 1994	A,B, DR	**A** **No effect normal risk** (immunoreaction); ns 1–2 matches 11% immunoreaction (n = 109); 0 matches 13% immunoreaction (n = 56) **No effect high risk** (immunoreaction); ns 1–2 matches 56% immunoreaction (n = 59); 0 matches 54% immunoreaction (n = 24) **B** **Beneficial normal risk** (immunoreaction); p < 0.01 1–2 matches 4% immunoreaction (n = 80); 0 matches 19% immunoreaction (n = 85) **Beneficial high risk** (immunoreaction); p < 0.01 1–2 matches 47% immunoreaction (n = 64); 0 matches 84% immunoreaction (n = 19) **DR** **Beneficial normal risk** (immunoreaction); p < 0.05 1–2 matches 10% immunoreaction (n = 122); 0 matches 16% immunoreaction (n = 43) **Beneficial high risk** (immunoreaction); p < 0.05 1–2 matches 49% immunoreaction (n = 68); 0 matches 87% immunoreaction (n = 15) NB: postoperative regimen changed during the years
Vail et al., 1994	A, B, DR	**A, B** **Beneficial overall** (graft failure?); trend, not significant **DR** **Bad effect overall** (graft failure?); p = 0.02 NB: unclear which groups were compared, no data on amount of immunoreaction/graft failure per HLA loci, DR groups not corrected for HLA-A,B differences and visa versa.
Baggesen et al., 1996	DR	**Beneficial high risk** (graft failure); p = 0.03 2 matches 23% graft failure (n = 38); 0–1 matches 42% graft failure (n = 36) Confounding factors (having influence on outcome, but not corrected for): Regrafts, p < 0.05;
Vail et al., 1997	A, B, DR	At 12 months follow-up: **A, B** **Beneficial overall** (immunoreaction); trend, not significant due to too small groups **DR** **Bad effect overall** (immunoreaction); no p-values mentioned NB: unclear which groups were compared, no data on amount of immunoreaction/graft failure per HLA loci, DR groups not corrected for HLA-A,B differences and visa versa.
Munkhbat et al., 1997	DRB1, DQB1, DPB1	12 months follow-up: **DRB1, DQB1, DPB1 together** **No effect overall** (immunoreaction); ns **No effect normal risk** (immunoreaction); ns **Beneficial high risk** (immunoreaction); p = 0.02 1–4 matches 17% immunoreaction (n = 23); 0 matches 50% immunoreaction (n = 28) **Separate analysis of the loci for overall, normal, high risk** **DRB1 no effect** (immunoreaction), although trend for benefit in high risk group **DQB1 no effect** (immunoreaction), although trend for benefit in high risk group **DPB1 beneficial high risk** (immunoreaction): p = 0.01 1–2 matches 7% immunoreaction (n = 14); 0 matches 46% immunoreaction (n = 37) NB: other factors of influence are not mentioned or corrected for.

Table 2 (*continued*)

Author, year	Antigens analyzed	Outcome (n = group size)
Munkhbat et al., 1999	A, B	12 months follow-up: **A, B** **Beneficial overall**; p = 0.03 1–4 matches 19% immunoreaction (n = 42); 0 matches 41% immunoreaction (n = 37) **Beneficial high risk**; p = 0.008 1–4 matches 17% immunoreaction (n = 24); 0 matches 52% immunoreaction (n = 25) **A separate** **Beneficial overall**; p = 0.001 1–2 matches ± 12% immunoreaction (n = 26); 0 matches ± 39% immunoreaction (n = 54) **Beneficial high risk**; p = 0.02 1–2 matches ± 14% immunoreaction (n = 16); 0 matches ± 48% immunoreaction (n = 34) **B separate** **No effect overall**; p = 0.60 1–2 matches ± 25% immunoreaction (n = 24); 0 matches ± 31% immunoreaction (n = 55) **No effect high risk**; p = 0.17 1–2 matches ± 17% immunoreaction (n = 12); 0 matches ± 40% immunoreaction (n = 37) NB: other factors of influence are not mentioned or corrected for.
Volker-Dieben et al., 2000	A, B/*DR*	**A, B** **Beneficial normal risk** (immunoreaction); p = 0.05 2–4 matches 9% immunoreaction (n = 480); 0–1 matches 13% immunoreaction (n = 349) **Beneficial high risk** (immunoreaction); p < 0.001 2–4 matches 25% immunoreaction (n = 642); 0–1 matches 42% immunoreaction (n = 207) **Corrected for vascularization status: beneficial: p = 0.01** ***DR*** **No effect normal risk** (immunoreaction); p = 0.14 2 matches 7% immunoreaction (n = 69); 0–1 matches 12% immunoreaction (n = 209) **Beneficial high risk** (immunoreaction); p = 0.02 2 matches 15% immunoreaction (n = 66); 0–1 matches 29% immunoreaction (n = 214) **Corrected for vascularization status: beneficial: p = 0.03** Confounding factors (having influence on outcome, but not corrected for): graft size, p = 0.002; organ culture, p = 0.001; gender, p = 0.01; HLA-A,B and DR
Khaireddin et al., 2003 (Update on study Hoffmann et al., 1994)	A, B, DR	**Beneficial normal risk** (immunoreaction); p = 0.04 2–6 matches 12% immunoreaction (n = 249); 0–1 matches 26% immunoreaction (n = 46) **Beneficial high risk** (immunoreaction); p < 0.001 2–6 matches 41% immunoreaction (n = 130); 0–1 matches 92% immunoreaction (n = 13) NB 1: analysis at split level added no advantage NB 2: other factors of influence are not mentioned or corrected for.
Bartels et al., 2003	A, B	**Split level analysis** **Beneficial high risk** (immunological graft failure); p = 0.002 3–4 matches ± 15% immunological graft failure (n = 216); 0–2 matches ± 31% immunological graft failure (n = 87) NB: the beneficial effect remained significant with an odds-ratio of 0.41, after multivariate analysis correcting regrafts and indication for transplantation. **Beneficial high risk** (overall graft failure); p = 0.04
Reinhard et al., 2004a	A, B, DR	Four year follow-up **A, B, DR** **Beneficial normal risk** (immunoreaction); p = 0.048 4–6 matches 8% immunoreaction (n = 66); 0–3 matches 28% immunoreaction (n = 352) **Beneficial normal risk** (rejection-free clear graft); p = 0.03 4–6 matches 8% no rejection-free/clear graft (n = 66); 0–3 matches 34% no rejection-free/clear graft (n = 352) Confounding factors (having influence on outcome, but not corrected for): organ storage time, p = 0.03.
Bohringer et al., 2004	A, B, DR	**Split level analysis A, B (triplet-string matching)** **Beneficial normal risk** (immunoreaction); p < 0.05 <13 triplet mismatches 15% immunoreaction (n = 147); >13 triplet mismatches 24% immunoreaction (n = 398) NB 1: groups differed significantly for DR matches (p = 0.02). NB 2: Cox multivariate regression analysis was used, yet it is unknown which confounding factors were included. **Broad level analysis A, B** **No effect normal risk** (immunoreaction); p = 0.08 3–4 matches 8% immunoreaction (n = 57); 0–2 matches 24% immunoreaction (n = 488) NB: groups differed significantly for DR matches (p < 0.01). **DR** Analysis not performed due to too small group size.
Minor antigens		
Inoue et al., 2000b	H-Y	**No effect normal risk** (immunoreaction); p = 0.71 Matched (male–male) ± 25% immunoreaction (n = 175); Mismatched (male–female) ± 27% immunoreaction (n = 53) **No effect high risk** (immunoreaction); p = 0.70 Matched (male–male) ± 40% immunoreaction (n = 127); Mismatched (male–female) ± 40% immunoreaction (n = 41) NB: no assessment or correction for possible confounding effect of HLA type.

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Table 2 (*continued*)

Author, year	Antigens analyzed	Outcome (n = group size)
Reinhard et al., 2004a	H-Y	**No effect normal risk** (immunoreaction); ns Matched (male–male) (n = 291); Mismatched (male–female) (n = 418) No other data mentioned except relative risk of 1.1. NB: no data provided on possible confounding effect of HLA type.
Böhringer et al., 2006 (Reinhards group)	H-Y, HA-3	**H-Y** **Beneficial overall** (immunoreaction); p = 0.02 Matched 12% immunoreaction (n = 148); Mismatched 23% immunoreaction (n = 81) **H-Y** **No effect overall** (immunoreaction); p = 0.52 Matched 15% immunoreaction (n = 148); Mismatched 27% immunoreaction (n = 81) NB 1: p-values are of multivariate analysis adjusting for risk group, HLA-A1 mismatch and patient age. NB 2: no assessment or correction for possible confounding effect of HLA type (although data was available).

Since both the HY and HA-3 minor H antigens are expressed on a variety of tissues, including corneal tissue, it justifies a potential role for both in corneal transplantation (de Bueger et al., 1992; Dierselhuis and Goulmy, 2009; Goulmy et al., 1995), and especially for HY, as larger solid organ transplant studies found increased graft loss for HY mismatches (Gratwohl et al., 2008); gender mismatches are known to be an independent risk factor in HLA-matched Stem Cell Transplantations (Gratwohl et al., 2001; Stern et al., 2006). More research in this area is warranted, as we do not yet know which other polymorphic tissue-specific antigens are expressed in the cornea, and more specifically, from which proteins these peptides have been derived.

5. Other approaches to prevent rejection

5.1. Potential of biologics to prolong human corneal graft survival

Most of the recent studies indicate that matching donor and recipient for HLA-A and -B antigens may improve graft survival in high risk cases, and probably in low risk cases as well. However, when this is not possible, one may try to reduce the chance of rejection using a variety of techniques. Knowing the degree of mismatching may help to decide on the post-transplant protocol. One of the new classes of drugs that may help to improve corneal graft survival is that of the biologics, which have especially been applied experimentally, but are increasingly being used clinically. Biologics, which in this context are primarily recombinant antibodies or fusion proteins, are an important and widely-used class of drugs for the treatment of many ocular conditions including inflammatory eye disease (Durrani et al., 2011; Jap and Chee, 2008), diabetic macular edema (Ho et al., 2012), and neovascular age-related macular degeneration (Holz et al., 2014). The choice of route of administration of biologics is a complex issue that is of direct relevance to prevention and treatment of clinical corneal graft rejection. Unmodified protein drugs cannot be delivered orally, because they will be rapidly degraded in the gastrointestinal tract. In humans, the common routes of administration of biologics for amelioration of eye disease include intravenous injection (used for example to deliver infliximab in some cases of Behçet's disease), and intravitreal injection (used to deliver eg. bevacizumab, ranibizumab or aflibercept to moderate aberrant angiogenesis and reduce macular edema in the posterior segment).

Reports on the systemic use of immunomodulatory biologics for the prophylaxis and treatment of human corneal graft rejection are relatively few. In a small study reported in the German-language literature, basiliximab (a chimaeric monoclonal antibody with specificity for CD25) in combination with corticosteroids was found to be moderately effective for the prophylaxis of corneal graft rejection (Birnbaum et al., 2008). Alemtuzumab, originally known as Campath-1 (a humanized monoclonal antibody with specificity for CD52) was used systemically to reverse corticosteroid-resistant corneal allograft rejection in several recipients, with some success (Dick et al., 2000; Newman et al., 1995). However, administration of these specific biologics is not without risk in either the short or long term and their side-effects profiles, and need for continuous monitoring by non-ophthalmologists together with their limited efficacy, probably explain why neither has as yet found widespread favour as an immunosuppressant for keratoplasty.

In recent times, the use of topical or subconjunctival administration of biologics to target the cornea has generated some interest. Large biologics of the size of intact antibody molecules will not pass across the human ocular surface over any reasonable timeframe (Allansmith et al., 1979), although it is possible to enhance the penetration of engineered small antibody fragments into the cornea (Thiel et al., 2002, 2013). However, topical administration of a biologic for the successful prophylaxis or treatment of human corneal graft rejection has not yet been achieved. Subconjunctival administration is a well-established method for producing a depot of a drug that may influence disease processes in the cornea and anterior segment. The application of anti-vascular endothelial growth factor (VEGF) biologics delivered by subconjunctival injection is actively being examined as a means to limit corneal neovascularization in humans, especially in cases of trauma or infection. Aside from reducing the direct consequences of vascularization upon vision, the rationale is to increase the chances of success of a corneal graft (should one subsequently be required), given that neovascularization is a well-established risk factor for rejection-mediated corneal graft failure (Coster and Williams, 2005).

Cursiefen and co-workers have demonstrated the importance of lymph vessels in corneal transplantation. Immunohistochemistry was applied to human corneas to determine the presence of LYVE-1 and podoplanin- (two markers of lymphangiogenesis) positive vessels. About 8% of vessels in vascularized corneas were lymph vessels. There was a strong correlation between the presence of lymphatic vessels and hemangiogenesis (Cursiefen et al., 2002). Anti-angiogenesis treatments that decreased both blood and lymph vessel ingrowth were able to restore the immune privilege. VEGF inhibitors such as bevacizumab were found to effectively block corneal angiogenesis as well as lymphangiogenesis (Bock et al., 2007). Blocking lymph- and hemangiogenesis with an inhibitor of VEGF (VEGF Trap (R1R2)) greatly improved corneal transplant survival (Cursiefen et al., 2004). Similarly, blocking VEGF-A or VEGFR-3 post-transplantationally resulted in

less vessels and subsequently less APC trafficking, ending in improved graft survival in an experimental model (Bachmann et al., 2008; Chen et al., 2004; Niederkorn and Larkin, 2010). A recent randomized, placebo-controlled clinical trial of three subconjunctival injections of 2.5 mg bevacizumab for new-onset corneal neovascularization demonstrated a significant *reduction* in the area of corneal vessels in the treated group, compared with an *increase* in the area of new vessels in the control group, at 3 months after initiation of treatment (Petsoglou et al., 2013). The intervention was reportedly well tolerated, with no major safety concerns, and could as such provide a good measure to reduce the risk of corneal graft rejection. Another approach may be the inhibition of the VEGF-receptor related kinases, such as VEGFR-tyrosine kinase. Application of a VEGFR-tyrosine kinase inhibitor led to improved survival of corneal transplants in a murine model (Hos et al., 2008).

As antigens from the AC drain to preauricular and submandibular lymph nodes (Camelo et al., 2005), local lymphadenectomy in mice helped to prevent antigens from reaching the local lymphoid tissue, and prolonged corneal graft survival (Plskova et al., 2004; Yamagami and Dana, 2001). One may also attack the lymph vessels: CD11b+ macrophages express LYVE-1 and Prox-1 under inflamed conditions in the murine cornea, and these cells were able to form vessel-like structures in vivo. Blocking such cells may help in preventing (lymph)angiogenesis (Maruyama et al., 2005).

Depletion of antigen-presenting cells may be an alternative for HLA Class II matching. This may effectively block antigen-presentation by APC depletion. It has been shown that blocking corneal and conjunctival antigen-presenting cells by local depletion with clodronate-containing liposomes completely inhibited graft rejection in a murine model (Slegers et al., 2000). A problem with this approach is the simultaneous blocking of local innate immune protection against pathogens, such as Acanthamoeba (Van Klink et al., 1996). On the other hand, this approach is inherent to using grafts that have been kept in culture for a while: reduction of HLA Class II expression was found in corneas stored for two weeks in organ culture (Al-Fakih et al., 2012; Mayer et al., 2007). However, time and type of storage may influence the quality of the graft. A disadvantage of storing corneas for a longer time is that it may adversely affect reepithelialization, even if storage in Optisol-GS lasted less than 14 days (Lam et al., 2013).

Other immunological treatments such as blocking of CD4+ T cells will, unless topically applicable, likely result in significant systemic immunosuppression, which is therefore unlikely to be used clinically as preventive treatment in clinical corneal transplantation. Many different immunomodulatory biologics have been examined for their efficacy in prolonging corneal graft survival in experimental animals. Because mice and rats have open lymphatics in the peritoneal cavity, intraperitoneal injection has been the favoured means of delivery in these species, but such administration is clearly not applicable for humans. Furthermore, the immune systems of mice and men differ substantially in many respects (Mestas and Hughes, 2004). Ophthalmologists have naturally had reservations about administering any of these potent immunosuppressants systemically to their patients with corneal grafts, without a great deal more evidence of efficacy. Corneal graft rejection is a so-called “orphan disease” (Aronson, 2006), so that large pharmaceutical companies are unlikely to expend resources on the discovery of new biologic drugs for this relatively rare condition. The role of biologics in the future is thus likely to be restricted to agents that have already been licensed for use in other, more common diseases. In such instances, the safety profile at least will already be reasonably well-established, as was the case with bevacizumab, licensed initially for use in colon cancer.

5.2. Potential of gene therapy to prolong human corneal graft survival

The potential of gene therapy to modulate corneal diseases in general and corneal graft rejection in particular has been extensively – even exhaustively – studied and reviewed (Borras, 2003; George et al., 2000; Jun and Larkin, 2003; Kampik et al., 2012; Mohan et al., 2005; Parker et al., 2009; Qazi and Hamrah, 2013; Ritter et al., 2013; Williams et al., 2004) over the past 15 years. The usual approach is to modify the donor corneal endothelium *ex vivo*, prior to transplantation, with a transgene designed to influence the afferent or efferent arm of the allograft response. This is a clinically-relevant scenario, as donor corneas are preserved in the eye bank for varying periods prior to release for keratoplasty.

Increasing the expression of the factors that mediate immune privilege by gene transfer of immunomodulatory molecules such as TNF-receptor, TGF-beta, IL-10, IL-12, and NGF (Nerve Growth Factor), may increase or restore corneal immune privilege and prolong graft survival. This has been investigated in several animal models (Beutelspacher et al., 2006; Comer et al., 2002; Gong et al., 2007; Klebe et al., 2001a, 2005; Rayner et al., 2001; Ritter et al., 2007); however, most factors were not able to prolong corneal survival when applied alone, with the exception of NGF (Gong et al., 2007).

The choice of the animal model is especially important in studying *ex vivo* gene transfer to corneal endothelium, because unlike the situation in humans and larger mammals, the endothelial cells of small rodents can replicate (Tuft et al., 1986). A positive outcome in a small animal model thus demands confirmation in a larger, preferably outbred animal model, several of which are available (Klebe et al., 2001b; Nicholls et al., 2012). Despite a number of partial successes, overall outcomes have thus far been somewhat underwhelming. Indefinite and biologically-significant, long-term prolongation of corneal graft survival has seldom been achieved in the majority of animals tested, a likely requirement of the gene technology regulators before permission would be forthcoming for any clinical trial. Not surprisingly, then, *ex vivo* gene therapy directed at a donor cornea prior to clinical transplantation, with the ultimate goal of prolonging the survival of that graft, has not been reported. This is in stark contrast to success in the use of gene therapy for inherited monogenic retinal disorders such as Leber's congenital amaurosis (Bainbridge et al., 2008; Maguire et al., 2008). One likely reason lies in the remarkable degree of redundancy that is evident in the immune response to an allograft. Possibly not one transgene will prove sufficiently broad-acting enough on its own to modulate the immune response to a corneal graft completely. Whether a combinatorial approach will be more successful has yet to be thoroughly tested. Topical, rather than systemic therapies, and lamellar keratoplasty, are the current approaches that are being applied to reduce corneal allograft rejection. The most successful future therapies would be those that target more than one pathway.

5.3. Cellular suppression

A potential new approach to prevent or treat rejection may be found in the use of mesenchymal stem cells. Bone marrow-derived mesenchymal stem cells (MSCs) are non-hematopoietic cells, that are capable of a range of anti-inflammatory functions, and can reduce corneal rejections in mouse and rat transplantation models (Lan et al., 2012; Oh et al., 2012), reviewed by Li and Zhao (2014). They have been found to stimulate tissue repair (Lan et al., 2012) by increasing the expression of anti-inflammatory cytokines such as TGF-beta and IL-1Ra. Intravenously injected MSCs home to damaged (inflamed) tissues, including a cornea that has undergone a corneal transplant (Omoto et al., 2014). When injected at the time

of a corneal transplantation in mice, these cells are able to bring down the number of APCs in the cornea and draining lymph nodes, as well as the number of induced Th1 cells, while improving graft survival. Earlier, Oh et al.(Oh et al., 2012) reported that injection of MSCs at the time of corneal transplantation in mice would reduce rejection. On day 7 post-transplantation, the number of dendritic cells and macrophages was reduced in corneas of mice that had received MSCs. The injection of MSCs reduced not only the aspecific early inflammation induced by surgery but also the allo-specific response observed on day 28 post transplantation. The production of a soluble factor, tumor necrosis factor-alfa stimulated gene/protein 6 (TSG6) was involved and infusion of recombinant TSG6 was also capable of reducing rejection rate, by reducing the local corneal immune response. Using an immunosuppressive factor would make the use of cells redundant. However, if cells would be more efficient, it raises the question whether the MSCs need to be compatible with the cornea donor. Obtaining donor-derived human MSCs or cells derived from other sources such as the umbilical cord might be feasible for human use.

6. Discussion

Corneal transplantation is considered one of the most successful form of transplantation in humans. Long-term survival rates in low-risk non HLA-matched patients are higher than those seen in HLA-matched solid organ transplants (Cecka, 2010; Claas et al., 2005; Coster and Williams, 2005; Gondos et al., 2013; Ing et al., 1998; Inoue et al., 2000a; Thompson et al., 2003; Williams et al., 1997). A prospective study on PKPs for normal risk cases with a median follow-up of 18 months showed rejection episodes in 11% of the grafts in the first 18 months. Risk factors for rejection were atopic dermatitis, clinically-manifest tear insufficiency and short storage of the graft (Kuchle et al., 2002). This shows that in normal risk cases, graft rejection can occur, but is not common. Next to penetrating keratoplasty, other allografting methods such as ALK and deep-ALK for anterior corneal disease are more frequently applied, improving the survival rates even further by leaving the endothelium intact. Lamellar transplants are usually performed in diseases where vascularization is absent. A survival rate of 99% over 9 years has been reported in deep-ALK performed for stromal disease (Sarnicola et al., 2012). The group of 806 eyes consisted of keratoconus (74%), postherpetic keratitis scarring (15%), and other stromal opacities. This excellent success is mainly attributed to the diminished endothelial cell loss for deep-ALK compared to PK (Borderie et al., 2012; Kubaloglu et al., 2012). However, one should take into account that ALK is especially used in keratoconus, a disease that consists of thinning and malformation of the cornea, usually without inflammation or blood vessel formation, which would also have excellent survival in perforating grafts. PKPs performed in keratoconus are hardly ever rejected, so it is logical that laminar grafts for this disease are also not prone to rejection (Thompson et al., 2003). The influence of ALKs on the overall corneal survival rates to date is minimal as 81–94% of the corneal allografts still replace the endothelium (Konijn-Janssen et al., 2011; Lichtinger et al., 2012; Williams et al., 2012). In the posterior transplants, one either replaces Descemet's membrane with a new Descemet membrane together with the endothelial cells, or with a combination of Descemet's with endothelium and an additional thin layer of corneal stroma (Melles et al., 2000, 2008). In both cases, the target of endothelial rejections is the donor tissue; although long-term results remain to be determined, the first prospective studies are reporting 5-year survival rates around 72.5% for DLEK (Deep Lamellar Endothelial Keratoplasty) (Mashor et al., 2010), and 89% for DSEK (Descemet's Stripping Endothelial Keratoplasty) (Anshu et al., 2012a), while the 5-year survival for full thickness grafts for Fuchs' dystrophy is 83–87% (Cheng et al., 2013; Fasolo et al., 2011; Thompson et al., 2003). Although these grafts are especially performed for corneas with primary or secondary Fuchs' dystrophy, a fast loss of endothelial cells may also occur following the transplantation, independent of the technique applied (Chan et al., 2012). A study that compared DMEK (Descemet's Membrane Endothelial Keratoplasty, n = 141), DSEK (n = 598) and PK (n = 30) in patients with similar demographics and indications for surgery, showed a 2-year survival rate of 99% for DMEM, 88% for DSEK, and 82% for PK (Anshu et al., 2012b). However, recent data from the Australian Corneal Graft Registry Study, comparing the graft survival of 1643 lamellar and 9875 penetrating keratoplasties between 1996 and 2013, showed that the survival of DALKs (deep anterior lamellar keratoplasty) and endokeratoplasties is worse than the survival of penetrating keratoplasties performed for the same indications (mostly keratoconus for DALK, and mostly Fuchs' dystrophy or pseudophakic bullous keratopathy for endokeratoplasties) (Coster et al., 2014). When focusing on irreversible rejection as the reason for graft failure, PK was the least favourable with 30% (635/2094) of the failures due to irreversible rejection, compared to only 2% (1/53) for DALK and 12% (37/318) for endokeratoplasty. Rejection can thus still occur, although at a lower rate, but the current results show a lot of failures due to other causes. When focusing on DALK for keratoconus, the rejection rate for anterior lamellae was very low. Overall survival was better for DALK at three years, but when early failures were excluded, survival was similar (Jones et al., 2009).

Despite (technical) advances, immunological rejection remains a major reason for graft failure in all types of grafts, both in low- and high-risk patients. Strikingly, for the high-risk patients, the prognosis of corneal graft survival is similar to that of solid organ transplants (Cecka, 2010; Claas et al., 2005; Thompson et al., 2003; Williams et al., 1997), and patients may receive systemic treatments to prevent their corneal graft from being rejected (Joseph et al., 2007; Nguyen et al., 2010). Considering this, it seems that transplanted corneas are ‘forgotten grafts’ with regard to the number of adequate studies investigating the reasons leading to corneal allograft rejections (George and Larkin, 2004). Furthermore, notwithstanding that in corneal allografting, HLA typing and proper allocation is generally assumed to be unnecessary, most studies show that outcomes in high risk cases are better for increasingly-matched grafts and best for those grafts which have no HLA differences with the recipient at all. Obviously, finding a good match can be difficult due to the high polymorphism of the HLA system. In solid organ transplantation where the benefit of HLA matching is evident, there is not always time to wait for a completely compatible donor, as waiting might prove fatal to the patient. Corneal transplantation however, is performed to restore or improve sight and not to save life, and with the exception of emergency transplants, this often provides the valuable time needed to find a compatible donor. With longer cornea storage times, possibly up to one year (He et al., 2012), even more time will be available for proper matching. Worldwide, a great shortage exists in the number of grafts available, and this is even the case in countries with a long tradition of cornea banking. With the increasing number of elderly patients, more corneas will be necessary, and the lower the number of necessary re-grafts, the more patients can be helped. Therefore, it would be a waste not to invest in studies to determine properly the benefit of matching corneal allografts for HLA Class II and minors in addition to HLA Class I antigens.

From an immunological point of view, studies on the effect of HLA matching should ideally take immunological rejection, not necessary leading to graft failure, as end point. This would help to better differentiate between the causes of graft failure, and thus

help to develop preventive measures. Comparing this graft rejection-free survival time is more appropriate than using graft failure-free survival and might give more adequate results, even more so when DNA-based HLA matching at broad or split-level is used instead of the less accurate serological matching. A system identifying acceptable HLA mismatches and only matching for the most relevant and significant HLA alleles, could be a valuable, and time-and money-saving approach (Claas et al., 2005, 2009; Opelz and Dohler, 2007). Currently, the FANCY trial (Functional Antigen Matching in Corneal Transplantation, NCT00810472), running from 2009, is investigating the results of HLA matching in a blind randomized study. Patients are being matched using the HLAMatchmaker algorithm, identifying acceptable mismatches for sensitized patients, and the end-point is first endothelial graft rejection. In the UK, the CTFS II/Corneal Transplant follow-up Study II is a multicenter study that was commenced in 1998 (ISRCTN 25094892). In this study, a waiting list of HLA-typed patients was established, to maximize the chances of patients getting a matched graft. This study should provide information on the role of HLA-DR in graft survival. Efforts to improve corneal graft rejection-free survival need also take into account the effect of minor H antigens, as in mice these seem to be able to negate the MHC matching effect. The advantage of better matching is a better graft survival without side effects, which is not going to be the case for medical interventions.

An interesting question is whether the genetic make-up of the recipient influences the rate of rejection: the CTFSII study in the UK also investigates the role of different gene polymorphisms that may influence the outcome of cornea transplants. The genotypes of different cytokine genes were determined in 384 patients undergoing a full-thickness transplant, and related to the occurrence of rejection during the first three years after transplantation. Specific haplotypes of the *TNFalfa* gene were either associated with a reduced or an increased risk of rejection (Winton et al., 2014a). Similar results were reported for polymorphisms in VEGF-A and the IL-17F gene, which together with a specific TNFalfa polymorphism seem to make up a “high inflammatory haplotype”, that leads to a higher chance of rejection (Winton et al., 2014b). It is fascinating that these genes are located on chromosome 6. It does however mean, that a determination of the genetic make-up of the recipient, independent of the corneal recipient status, may be used to assess the risk of rejection, and therefore the need to perform HLA matching or not.

The cost-effectiveness of HLA matching for keratoplasties also plays a role in the decision to perform HLA matching. Baumler et al. performed a retrospective analysis and used information that typing donor and recipient cost 1200 euro, and came to the conclusion that proper matching would give a prolonged rejection-failure-free survival of more than 1000 days (Baumler et al., 2014). The incremental cost of HLA matching would than range from 2.10 euro to 6.71 euro per additional day of graft survival, which is very acceptable given the high cost of regrafting and the shortage of corneal donors worldwide.

7. Conclusion and future directions

Despite controversial results of the effect of HLA matching on corneal allograft survival in older studies, we should not ignore that recent studies, using more accurate typing methods, provide consistent evidence that HLA matching is beneficial to corneal allograft survival in general and even more in high-risk allografts. The corneal transplantation field can take advantage of the results coming from solid organ HLA matching, as once the immune privilege has been compromised, the same immunological mechanisms apply. Obviously, and especially from a patient's view, successful prevention of immune rejection, rather then immune suppression by post-transplantation treatments or immune-modifying treatments, such as gene therapy, remains the best primary approach. Combining HLA matching with other preventive immunological therapies, thus targeting multiple pathways at the same time, may even further enhance long-term corneal graft survival.

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