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# Antibody-based immunosuppressive agents for corneal transplantation

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**Abstract**

**The progress in antibody engineering over the last 20 years has created the tools for the development of novel antibody-based drugs and constructs, such as small antibody fragments, suitable for topical administration. In rheumatology, oncology, transplantation medicine and ophthalmology, therapeutic antibody constructs, and antibody fragments have been responsible for the clinical progress seen over the last decade. Although antibody-based therapies have become a well-established immunosuppressive option in solid organ transplantation, there are only very few reports with regard to corneal transplantation. The following review explains some of the important aspects of engineered antibody-based therapeutic agents and summarises the current use of such immunosuppressive therapies in transplantation medicine and corneal transplantation.**
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## Introduction

Until recently, the use of immunosuppressive antibodies in corneal transplantation was limited to a small number of anecdotal case reports. Antibodies have to be administered systemically and the morbidities associated with immunosuppressive antibodies, such as prolonged T-lymphocyte depletion, cytokine activation, and allergic reactions upon repeated administration have often been unacceptable even for patients receiving organ transplantation. New techniques in molecular engineering have allowed the construction of better-tolerated and more effective immunosuppressive antibodies. Over the last decade, these antibody constructs have become an important tool in organ transplantation with up to 80% of recipients being treated with such antibody-based agents.[1] There are growing efforts to design antibody-based drugs for ocular diseases. This review summarises some important aspects of antibody-based immunosuppressive agents in transplantation from an ophthalmic perspective.

## Antibody constructs

There has been enormous progress in the techniques of antibody engineering over the last two decades.[2] Originally, immunosuppressive polyclonal and monoclonal antibodies were generated either by immunizing animals or by culturing murine hybridoma cell lines. These cumbersome techniques have been replaced by DNA engineering combined with very effective antigen-binding selection techniques, such as phage or ribosomal display.[3] The modern techniques in antibody engineering that have evolved over the last two decades allow the customised design of chimeric, humanized, or even fully human antibodies from synthetic DNA libraries (Figure 1). In addition, antibody engineering allows for the construction of small antibody fragments, such as single-chain variable region (scFv) fragments.[4] ScFv fragments have approximately half the molecular size of regular antigen-binding fragments (Fab), which enable the former to penetrate into the anterior chamber and the vitreous when administered topically.[5–7]

In contrast to IgG antibodies, which have two antigen-binding sites per molecule, Fab and scFv antibody fragments have a single-binding site, permitting monovalent binding only. Divalent binding allows formation of a tighter bond and cross-linking of non-soluble antigens,

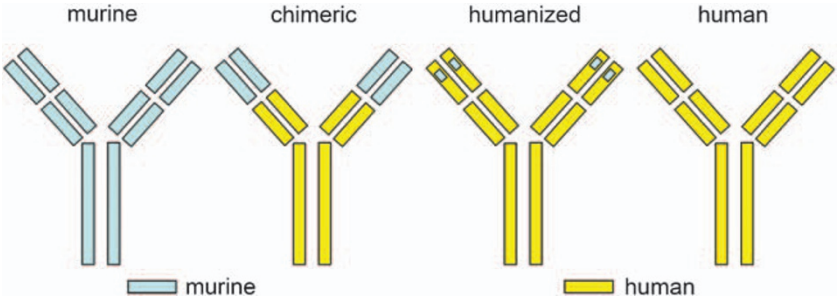


**Figure 1** Various composites of human and non-human elements in antibody constructs.

such as receptors on cell surfaces. For soluble antigens, such as tumour necrosis factor-α (TNF-α) or vascular endothelial growth factor (VEGF), divalent binding by a single large antibody has a similar effect to monovalent binding by twice as many small antibody fragments. This is shown by the similar clinical effect observed with the use of the divalent bevacizumab (avastin, humanised antibody) compared with the monovalent ranibizumab (lucentis, Fab fragment) in age-related macular degeneration.[8] On the premise that antibody-based immunosuppressive therapies in corneal transplantation should be administered topically to reduce the risk of systemic side effects, the ideal target antigens are small soluble antigens, which can be blocked by small monovalent antibody fragments.

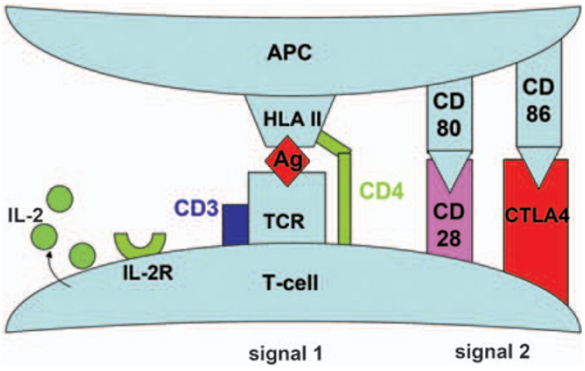


**Figure 2** Receptors involved in the interaction between the T lymphocyte and the antigen-presenting cell (APC). TCR = T-cell receptor, Ag = antigen, HLA = human leukocyte antigen, IL = interleukin, CD = cluster of differentiation, CTLA4 = cytotoxic T-lymphocyte antigen 4.

## Antibody-based immunosuppressive therapies in transplantation

Although the ultimate goal in transplantation immunology remains the induction of donor-specific tolerance in the recipient, the realistic goals of antibody-based immunosuppressive therapies today are more modest to improve the long-term graft survival rate by slowing or reducing sensitisation of the recipient to the donor, to reduce the dose and thereby the side effects of conventional immunosuppressive therapies, or to rescue grafts from acute or chronic rejection. As the immune processes associated with these aims differ, the choice of an individual antibody strongly depends on the specific goal, the timing, and the organ being transplanted.

The key player in the process of graft rejection is the T lymphocyte, and therefore various receptors on T cells are the targets of most antibody-based therapies (Figure 2). The T-cell receptor and two adjacent molecules, CD3 and CD4, are involved in antigen recognition (signal 1). The receptors CD80 and CD86 on antigen-presenting cells and the corresponding receptors CD28 and CTLA4 are responsible for the activation of the T cell (signal 2). Once activated, interleukin 2 (IL-2) secreted by the T cell serves as an autostimulant, further activating the T cell through the IL-2 receptor (IL-2R, signal 3). All three signals are required to stage a normal T-cell response to transplantation antigens (Table 1).

## Antibody therapies during the induction phase

The use of antibody-based therapies during the induction phase of an immune response is designed to increase the long-term graft survival by modifying the process of donor-specific sensitisation at the time of transplantation. Today, in organ transplantation, the most common therapeutic strategies during the induction phase are anti-IL-2R antibodies. There are two commercially available anti-IL-2R antibodies: basiliximab and daclizumab. Antibody names featuring the suffix '…x…mab' refer to the chimeric antibodies, whereas those featuring the suffix '…z…mab' refer to the humanised antibodies. Both anti-IL-2R antibodies have been used successfully in renal, lung, and liver transplantations.[8] The concept of anti-IL-2R antibody therapies are primarily to target the initial wave of T cells activated by migrating APC from the donor organ to the recipient and by chemokines liberated from vascular endothelium of the graft. This early wave of T-cell activation is initiated primarily by reperfusion injury of

**Table 1** Immunosuppressive antibodies and constructs, and the experience in ophthalmology

| *Treatment strategy* | *Target antigen/ cell* | *Antibody* | *Experience in ophthalmology* |
|---|---|---|---|
| Induction therapy | CD25/T cells | Daclizumab | No published reports in corneal transplantation |
| | CD25/T cells | Basiliximab | One RCT indicating equivalent effect to systemic cyclosporine |
| | CD52/T cells | Alemtuzumab | Two case reports with preventative and therapeutic use in corneal graft rejection |
| Maintenance therapy | CD80, CD86/ APC | Abatacept | Weak effect in animal models of corneal transplantation |
| | CD80, CD86/ APC | Belatacept | No published reports in corneal transplantation |
| Rescue treatment for graft rejection | CD3/T cells | Muronomab/ ATG | No published reports in corneal transplantation |
| | TNFα | Infliximab | Effective in uveitis, no published reports in corneal transplantation |

the transplanted organ.[1] The protective effect of these antibodies depends on starting treatment early, that is, before donor T cells reach draining lymph nodes (eg, dosing on day 0 and +4 with respect to the day of transplantation). Although basiliximab and daclizumab are very effective in reducing the risk of organ graft rejection during the first year, they are ineffective for the treatment of acute graft rejection. Both antibodies are very frequently prescribed in organ transplantation, as they are well tolerated with very few side effects.[9] As there are no donor derived T cells migrating from the donor cornea to the recipient lymph node, the conceptual argument for the use of these antibodies in corneal transplantation is less evident. So far, there is one case series and a small randomised controlled trial reporting the effect of basiliximab in corneal transplantation.[10,11] In an uncontrolled group of seven high-risk keratoplasty patients treated with basiliximab combined with cyclosporine, no rejection was observed.[10] In a small randomised controlled trial, a 2-year graft survival did not differ between high-risk patients treated with basiliximab or cyclosporine. However, there were fewer side effects reported with basiliximab than with cyclosporine.[11]

A third antibody used in the induction phase is the anti-CD52 antibody alemtuzumab (Campath-1H; humanised antibody).[12] CD52 is a receptor on T cells, B cells, monocytes, and natural killer cells. Its function is unknown. Treatment with alemtuzumab results in prolonged, severe T-cell depletion. Although this treatment is highly effective in reducing the risk of short- and long-term graft rejection in kidney, lung, and liver transplants, its associated comorbidity seems to justify its use in corneal transplantation only in special circumstances. So far, there are two case reports where alemtuzumab given at the time of corneal transplantation (one case)[13] or during corneal graft rejection (two cases)[14] resulted in a long-lasting graft survival.

## Antibody-based therapy during the maintenance phase

CTLA4-Ig (abatacept and the affinity-matured belatacept) are antibody-based constructs combining the extracellular part of the immunomodulatory CTLA4 receptor with a human IgG Fc region. CTLA4-Ig was extensively tested in animal models of solid organ, skin, and corneal grafts during the 1990s. In rodents, a short treatment during the induction phase often resulted in a long-lasting donor-specific tolerance to skin, heart, and kidney grafts. The results reported with CTLA4-Ig in corneal transplants were generally less promising than in other transplant models.[15–17] Phase III clinical studies with belatacept in human kidney recipients showed no effect in reducing the rate of acute graft rejection, but a considerably reduced risk of chronic graft rejection.[18] However, the mechanisms involved in chronic renal graft rejection differ considerably from the mechanisms involved in corneal graft rejection. Thus far, there are no reports on the use of belatacept in corneal transplantation.

## Treatment of acute graft rejection with antibody-based therapies

Antibody treatment of corticosteroid-resistant acute rejection of solid organ graft is most often based on ATG (antithymocyte globulin) or the anti-CD3 antibody muronomab (OKT3®). [19] Anecdotal reports about OKT3 injections directly into the anterior chamber during acute corneal graft rejection described a devastating intraocular inflammatory response.

There are promising reports of the use of anti-TNF-α antibodies (infliximab) for the treatment of acute intestinal graft rejection[20] and graft-versus-host disease.[21] Systemically administered infliximab has been used successfully and with few side effects in severe uveitis.[22] TNF-α is involved in cell death during corneal graft rejection, and blocking TNF-α may have a profound

immunosuppressive effect. As TNF-α is a soluble antigen, it can be targeted by monovalent antibody fragments.[7,23] Phase I and II trials with an anti-TNF-α scFv prepared for topical administration to the eye (ESBA105) are currently being undertaken.

## Summary

Immunosuppressive antibodies are frequently used in organ transplantation. With the exception of anti-IL-2R antibodies, which may have some benefit in high-risk keratoplasty patients, the use of conventional antibodies is not justified routinely. Small antibody fragments, suitable for topical administration as eye drops, are currently under development and may in the future allow ophthalmologists to adopt some of the immunosuppressive strategies used in organ transplantation with fewer risks of systemic side-effects.

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