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Research article

Promotion of vessel regression by local VEGF-A blockade improves the survival rate of corneal transplants

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ABSTRACT

To primarily evaluate the efficacy of different topical vascular endothelial growth factor (VEGF) blocking treatments, namely Aflibercept and Bevacizumab, in promoting regression of pathologic corneal neovascularization (NV) and secondarily to investigate their effects on graft survival in a high-risk mouse corneal transplantation model. In addition, the immune modulatory effects were assessed.

After 14 days of suture-induced, inflammatory corneal NV, sutures were removed. Anti-VEGF eye drops or vehicle controls were applied topically 3 times daily for one week. After 21 days, the regression of blood vessels (BVs) and lymphatic vessels (LVs) was assessed via immunofluorescence staining. In addition, corneal grafting was performed in another set of mice. Therefore, allogeneic donor corneas from C57BL/6N mice were used and graft survival was monitored weekly for 8 weeks. Finally, hem-and lymphangiogenesis as well as the phenotype of antigen-presenting cells (APCs) in draining lymph nodes (dLNs) and corneas were analyzed by immunohistochemistry and FACS, respectively.

Both, Aflibercept and Bevacizumab eye drops supported the regression of both blood and lymphatic vessels. Aflibercept-treated corneas demonstrated decreased APC activation in the dLNs after treatment at day 21. In the high-risk transplantation model, both therapeutics could improve graft survival rates (Aflibercept: 60 %, $P = 0.0091$; Bevacizumab: 50 %, $P = 0.0547$) compared to control (10 %). Eight weeks post-transplantation, both treatment arms displayed decreased APC recruitment and an increased expression of Foxp3 on $CD4^+CD25^+$ T cells.

Our study demonstrates that different topically applied anti-VEGF eye drops effectively reduce pre-existing corneal neovascularization and thereby improve subsequent high-risk corneal transplantation. This translational approach could become a promising strategy to improve the clinical outcome of high risk keratoplasty in patients.

1. Introduction

In clinical practice, preexisting corneal stromal hem- and lymphangiogenesis in the recipient cornea has been identified as a strong risk factor for increasing immune rejections after corneal transplantation (Bachmann et al., 2010). These pathological vascular changes can significantly increase the risk of graft rejection, reducing the 5-year corneal transplant survival rates sharply from 90 % to less than 50 % (Bachmann, 2008; Di Zazzo et al., 2020). To regress corneal vessels is therefore important to improve graft survival, as lymphatic vessels provide pathways for donor-derived antigenic material to access regional lymph nodes, where they facilitate accelerated sensitization to graft antigens (Clahsen et al., 2023). Several modes of vessel regression have previously been tested, both pharmacologically as well as physically (Cursiefen et al., 2009; Hou et al., 2018). Simultaneously, blood vessels serve as efferent pathways for immune effector cells and host antigen-presenting cells, further exacerbating rejection (Cursiefen et al., 2004b).

Two commonly used anti-angiogenic drugs in clinical use for age-related maculopathy, aflibercept (Eylea) and bevacizumab (Avastin), share the common strategy of antagonizing vascular endothelial growth factor (VEGF) (Bock et al., 2007; Holash et al., 2002), a key driver of

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blood and lymphatic vessel development (Ciombor and Berlin, 2014). Anti-VEGF agents have demonstrated remarkable efficacy in suppressing pathological vessel formation in cancer treatment (Boucher et al., 2021; Sun, 2012) and managing neovascular eye diseases (Kim et al., 2016; Krizova et al., 2014; Yonekawa and Kim, 2015). Despite their similar therapeutic targets, these drugs function via different mechanisms. Bevacizumab is a monoclonal antibody that blocks VEGF by forming large, multimeric complexes that exhibit enhanced binding of VEGF-A in the presence of heparin and neuropilin on cell surfaces (MacDonald et al., 2016). In contrast, aflibercept acts as a VEGF trap, forming monomeric (1:1) complexes with VEGF. Unlike bevacizumab, aflibercept does not bind endothelial cell surfaces but antagonizes placental growth factor (PlGF) in addition to VEGF (Ciombor and Berlin, 2014). These mechanistic differences may influence their ability to inhibit tube formation, clear VEGF from the local environment (Yazdanyar et al., 2023), and regulate the immune microenvironment (Di Tacchio et al., 2019; Osada et al., 2008; Thomas et al., 2017). However, their precise effects in the transplant immune microenvironment remain unclear.

Newly formed vessels respond well to anti-VEGF treatments (Bock et al., 2008; Krizova et al., 2014; Zhang et al., 2021). In a well-defined mouse model of corneal transplantation, anti-angiogenic therapy has been shown to improve corneal graft survival by effectively inhibiting neovascularization and modulating the microenvironment of the graft bed (Bachmann, 2008; Dohlman et al., 2015; Salabarria et al., 2019; Zhang et al., 2022). However, mature vessels associated with chronic neovascularization present a greater therapeutic challenge (Kim et al., 2008), since they are covered by pericytes and depend less on VEGF supply (Cursiefen, 2003).

We could recently show, that corneal crosslinking as well as fine needle diathermy are effective in regressing already existing corneal blood and lymphatic vessels and thereby can improve graft survival (Hou et al., 2017, 2018; Le et al., 2018).Nevertheless, both methods are invasive and can cause rebound effects (Le et al., 2020).

In contrast, anti-VEGF therapies are routinely used in the eye in age-related maculopathy and are tolerated well. Furthermore, the application of eye drops is non-invasive and therefore does not cause inflammation and subsequent vessel regrowth. Thus, this study for the first time aims to compare the effects of topical eye drops of bevacizumab and aflibercept on the regression of preexisting vessels and the subsequent effects on graft survival in high-risk keratoplasty.

2. Materials and methods

2.1. Animals and anesthesia

Female C57BL/6N and Balb/c mice, aged 6–8 weeks, were purchased from Charles River Laboratories (Sulzfeld, Germany) and respectively used as graft donors and recipients. For surgical procedures, mice were anesthetized using a mixture of 120 mg/kg ketamine (Pfizer) and 20 mg/kg xylazine (Bayer AG). All animal procedures followed the Association for Research in Vision and Ophthalmology Statement.

2.2. Mouse model of regression of suture-induced, inflammatory corneal vessels

As previously described, mouse inflammatory Corneal Neovascularization (CNV) was induced in female Balb/c mice by placing three evenly distributed figure-of-eight sutures as described before (Zhang et al., 2021). The sutures remained in place for 2 weeks. Subsequently, Aflibercept (Eylea; Bayer) and Bevacizumab (Avastin, Genentech) were administered as eye drops as indicated. Drug concentrations (Aflibercept 10 mg/ml, Bevacizumab 5 mg/ml) were chosen based on prior validations demonstrating efficacy and safety while minimizing corneal damage (Salabarria et al., 2019; Zhang et al., 2021, 2022b; Bock et al., 2008; Hos et al., 2013). Alternatively, human IgG Fc was applied as control eye drops. These treatments were administered three times per day for one week after suture removal.

2.3. Allogeneic corneal transplantation in high-risk recipients

Corneal transplantation (C57BL/6N to Balb/c) was performed right after vessel regression (day 21 after suture placement). Donor corneas (diameter 2 mm) were transplanted into the recipient bed (diameter 1.8 mm)and secured with 11–0 nylon sutures. These sutures were removed after 1 week. Graft rejection was evaluated by a slit lamp once a week according to a 0 to 5+ scale from 2 weeks after transplantation until week 8 (Cursiefen et al., 2004a), with a score above 2 being defined as rejected.

2.4. Immunohistochemistry analysis of corneal whole-mounts

The hemangiogenesis and lymphangiogenesis of the corneal whole mounts were quantified 3 weeks after suture placement and 8 weeks after keratoplasty. Corneas were fixed in acetone and stained with CD31 for blood vessels and LYVE-1 for lymphatic vessels. Finally, the areas of BVs and LVs were quantified by using the Cell^F program as previously described (Bock et al., 2008).

2.5. Flow cytometry

Draining lymph nodes were collected for single-cell suspension to analyze flow cytometry. Corneal single-cell suspension was obtained by digestion with Librase (Merck, USA) for 1 h 2 corneas were pooled for one sample. Single cells were stained with CD11c, F4/80, MHC-II, CD3, CD4, and CD25 (Supplement Table 1).Some samples were fixed and permeabilized by Fix/Perm kit (Thermo Fisher Scientific) and stained for Foxp3. Subsequently, the stained cells were measured by a Canto II flow cytometer (BD, Franklin Lakes, NJ, USA), and the obtained data were then analyzed using FlowJo (version 10.7.1; FlowJo LLC, Ashland, OR, USA).

2.6. Statistical analysis

For multiple group comparisons, a one-way ANOVA test was used after testing for normal distribution and equal standard deviation. Corneal graft survival curves were compared using the Kaplan-Meier log-rank test. Data are shown as mean ± SD. $P < 0.05$ was considered statistically significant. All statistical analyses and graphs were performed with Prism 10 (version 10.1.1).

3. Results

3.1. Anti-VEGF-eye drops promote regression of neovascularization

To evaluate whether different anti-VEGF treatments could promote neovascular regression, Bevacizumab, Aflibercept, or Fc control eye drops were administered onto prevascularized corneas for an additional week (Fig. 1A). Immunofluorescence staining identified BVs as $CD31^+$ and LVs as LYVE-1+ (Fig. 1B). Compared with the control (BVs: 20.80 % ± 3.74 %), both Aflibercept (BVs: 16.28 % ± 3.61 %) and Bevacizumab (BVs: 15.20 % ± 5.08 %) groups showed significant reductions in hemangiogenesis ($P = 0.0189$, $n = 10$; one-way ANOVA).

When analyzing lymphatic vessel morphology, the Bevacizumab group (LVs: 3.68 % ± 1.49 %) demonstrated a significantly enhanced effect on lymphatic vessel regression relative to the vehicle control group (LVs: 5.19 % ± 1.57 %) ($P = 0.045$, $n = 10$). Also, the aflibercept group exhibited a reduction in lymphatic vessels (4.00 % ± 1.20 %); however, this decrease was not statistically significant ($P = 0.1265$, $n = 10$).Although the comparison between Control and Aflibercept did not reach statistical significance after adjustment, the medium-to-large effect size (Cohen's $d = 0.86$) suggests a biologically meaningful trend.

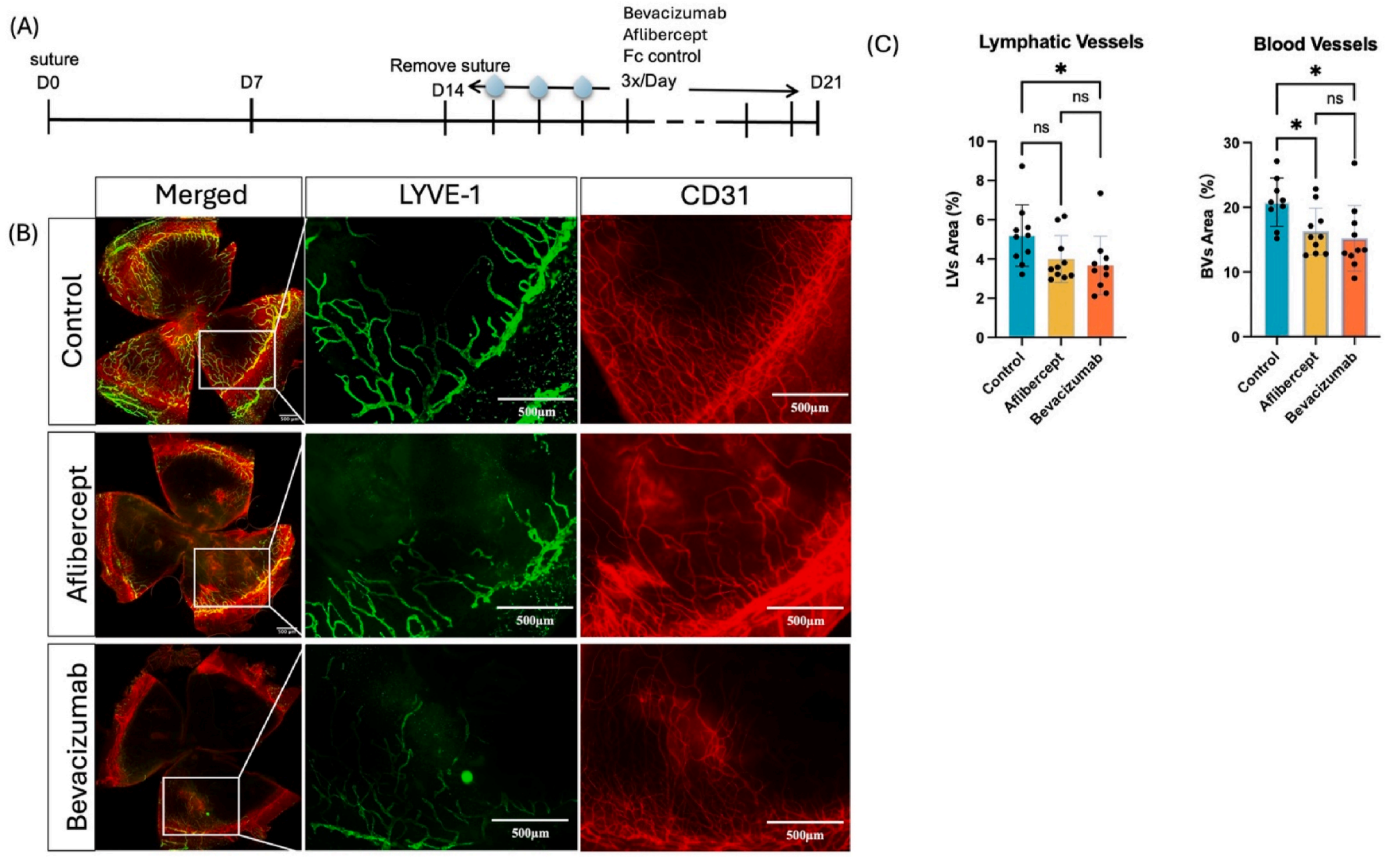

Fig. 1. Different anti-VEGF drugs promote blood and lymphatic vessel regression.
(A) Setup of the vessels regression experiments. Corneal (lymph)angiogenesis was induced by suture placement in Balb/c for 2 weeks. After suture removal, mice were treated topically with aflibercept eyedrops (0,087 mol/ml), bevacizumab eyedrops (0,033 mol/ml), or IgG Fc (10 mg/ml) as control three times daily for 1 additional week. (B) Representative immunofluorescence staining of corneal $CD31^{+}$ blood vessels and LYVE-1+ lymphatic vessels after one week of regression. (C) Area covered by blood/lymphatic vessels (%) in corneas. Data are shown as mean ± SEM. Statistical analysis was performed using one-way ANOVA. n = 10, *P < 0.05.

3.2. Regression of corneal vessels by topical anti-VEGF therapeutics affects antigen-presenting cells (APCs) in lymph nodes

Having shown that anti-VEGF treatment in the prevascularized corneas could effectively promote the regression of these pathological vessels, the question arose if this effect also influences the presence or activity of antigen-presenting cells in the draining lymph nodes.

To address this, we collected dLNs after regression treatment and analyzed the APC populations by flow cytometry.

We could show that in F4/80+ macrophages, MHCII + expression was significantly decreased in the aflibercept treatment group compared to the other groups (Fig. 2a) (P = 0.0282). Additionally, the number of CD11c + dendritic cells (DCs) in the dLNs was significantly reduced in the aflibercept group compared to the control and bevacizumab group (Fig. 2b) (Control: 2.84 % ± 0.53 %, vs. Aflibercept: 1.89 % ± 0.75 %, vs. Bevacizumab: 3.15 % ± 0.80 %; P = 0.0021). These findings suggest that at least the topical Aflibercept treatment of prevascularized corneas not only promotes vessel regression but additionally reduces the drainage and activation of APCs in the draining lymph nodes.

3.3. Preoperative, anti-VEGF mediated vessel regression improves graft survival after high-risk transplantation

Since the anti-VEGF eye drops could promote vessel regression and Aflibercept could even alter APC activity, we next assessed the long-term survival of allogeneic corneal grafts after anti-VEGF therapy promoted vessel regression. Graft survival was monitored weekly by opacity grading until week eight after transplantation. As illustrated in Fig. 3B, by week 8, the survival rate in the Aflibercept group was significantly increased to 60 % (P = 0.0091) compared to the 10 % survival rate observed in the control group. Also, the Bevacizumab group showed an increased survival with 37.5 % survival after 8 weeks compared to control with 22.22 % survival after 8 weeks. But the difference was not significant (P = 0.0547).

3.4. Topical aflibercept pretreatment of vascularized corneas decreases long-term APC recruitment and increases Foxp3 expression after corneal transplantation

To obtain further changes regarding the cellular mechanisms leading to the observed increased graft survival, we harvested the whole cornea and analyzed the frequency of APCs and T cells. In line with the graft survival the administration of Aflibercept reduced the F4/80, CD11b double-positive macrophages (Control: 30.6 % ± 1.65 %, vs. Aflibercept: 20.28 % ± 6.20 %; P = 0.02), and CD11c + dendritic cells (Control: 21.03 % ± 0.95 %, vs. Aflibercept: 14.48 % ± 6.20 %; P = 0.01) in the cornea 8 weeks after keratoplasty (Fig. 4). The administration of Bevacizumab could reduce the number of macrophages and dendritic cells, too, although not yet significant (DCs: Control: 21.03 % ± 0.95 %, vs. Bevacizumab: 18.28 % ± 1.40 %, P = 0.24; Macrophages: Control: 30.6 % ± 1.65 %, vs. Bevacizumab: 24.7 % ± 2.53 %, P = 0.19). These results suggest that Aflibercept especially inhibits the APC recruitment to the target area on a long-term scale. In addition, the Aflibercept and Bevacizumab groups showed higher Foxp3 expression

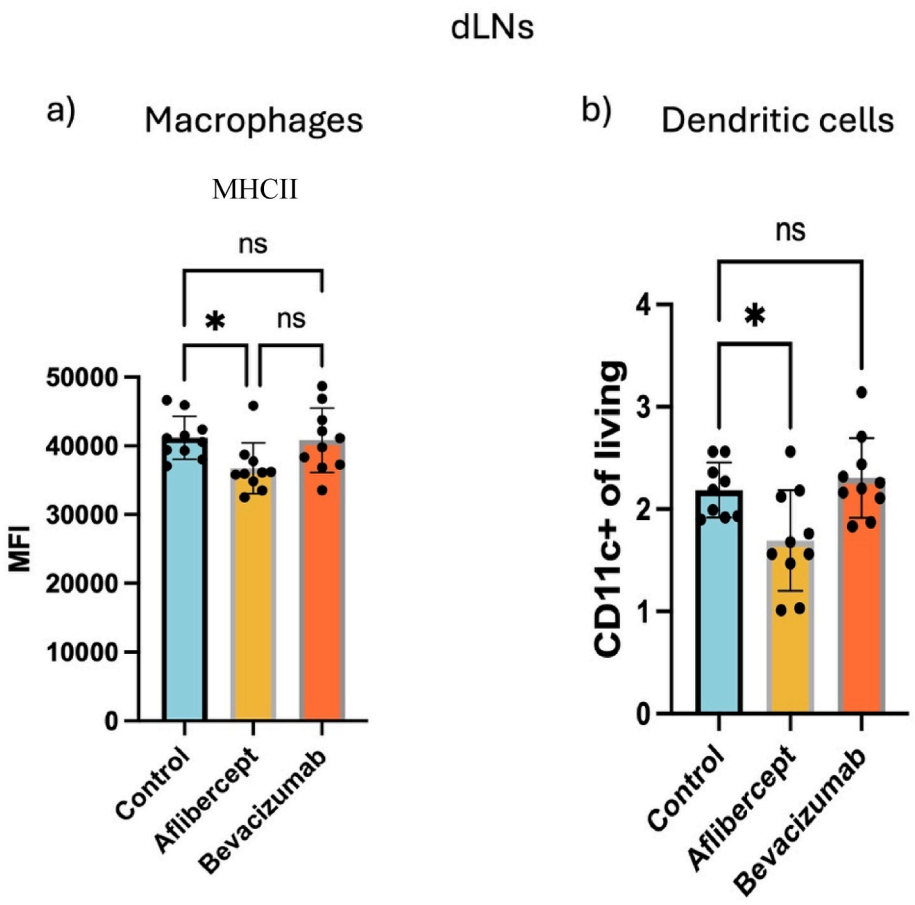

Fig. 2. Angioregressive VEGF-A blockade reduces dendritic cell and macrophage activity.
A) Analyses of the surface expression of MHC class II on F4/80+ macrophages from dLNs by MFI. B) Frequency of CD11c + dendritic cells in the draining lymph nodes. MFI: mean fluorescence intensity. Data are represented as mean ± SEM. Statistical analysis was performed using one-way ANOVA. n = 10; *P < 0.05.

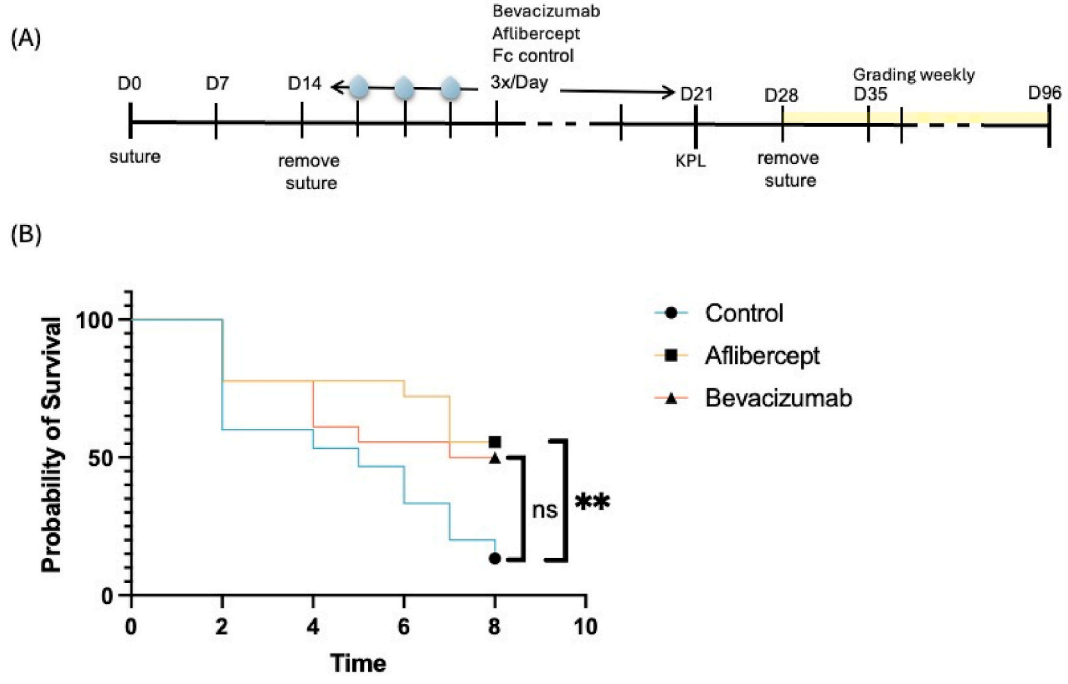

Fig. 3. Preoperative vessel regression by anti-VEGF therapy improves corneal graft survival. (A) Experimental set-up. One-week topical anti-VEGF treatment was applied to pre-vascularized corneas; subsequently, corneal grafts from C57BL/6N mice were transplanted into BALB/c recipient mice. (B) Graft survival was evaluated weekly. Statistical significance was assessed via the Log-rank test. n = 20, *P < 0.05; **P < 0.01.

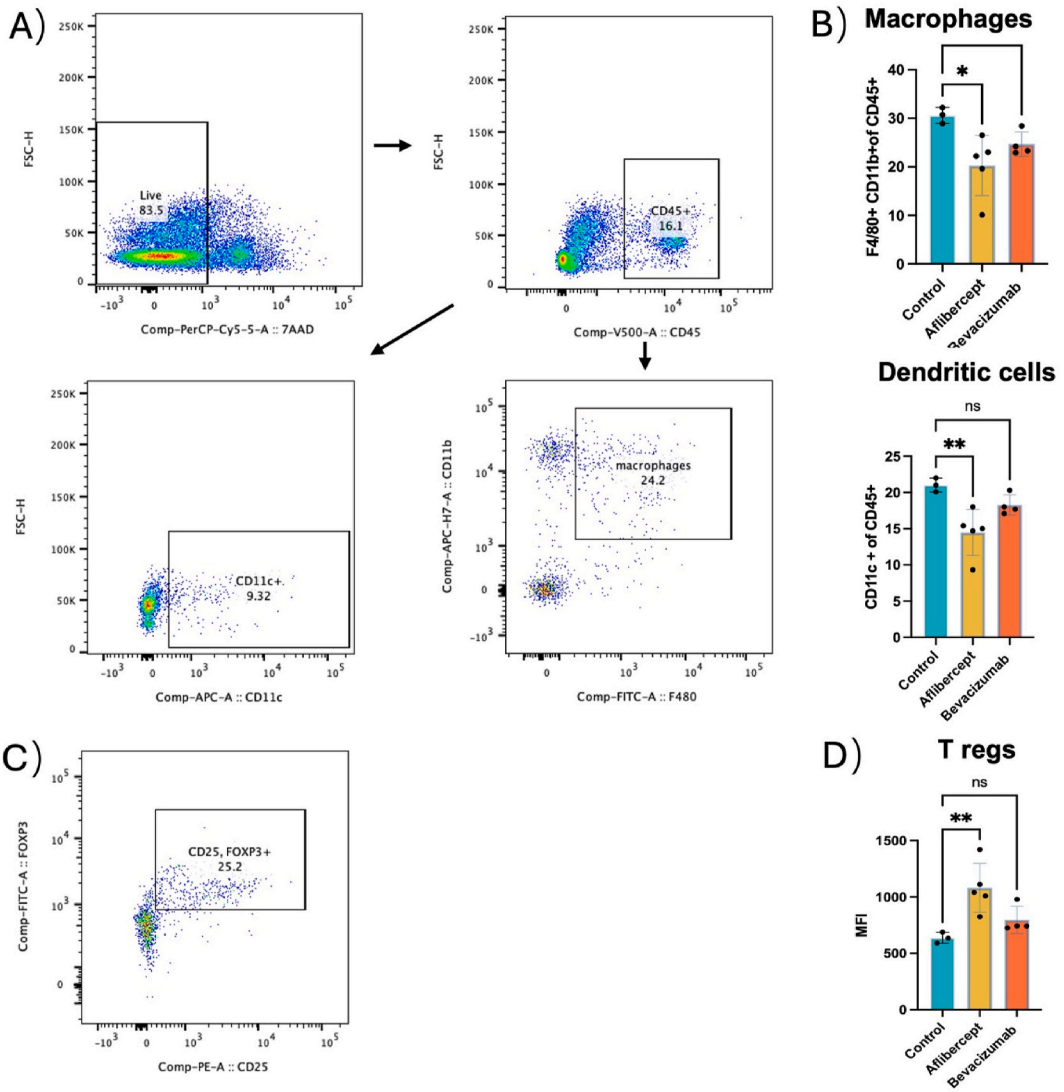

Fig. 4. Long-term reduction of local antigen-presenting cells and induction of tolerogenic mechanisms in the cornea following Aflibercept treatment. (A) Flow cytometry gating strategy: (1) viable cells were identified as 7AAD-; (2) lymphocytes were gated as $CD45^+$; (3) dendritic cells were defined as CD11c+; (4) macrophages were identified as CD11b + F4/80+. (B) Analysis of surface expression of CD11c and F4/80 in corneal antigen-presenting cells. (C) Regulatory T cells were identified as CD25+Foxp3+. (D) Analysis of Foxp3 expression in $CD4^+$ T cells. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA (n = 10); *P < 0.05.

on $CD4^+CD25^+$ regulatory T cells (MFI: Control: 638.33 ± 48.09 vs. Aflibercept: 1081.4 ± 217.35 vs. Bevacizumab: 797.5 ± 121.25, P = 0.01).

We further assessed the recruitment and activation of antigen-presenting cells in the dLNs at 8 weeks post-high-risk keratoplasty. As shown in Fig. 5, even 8 weeks after grafting, the frequency of CD11c + dendritic cells remained lower in both the aflibercept and bevacizumab treated groups compared to control groups (Control: 4.00 % ± 0.63 %, vs. Aflibercept: 2.92 % ± 0.87 %, vs. Bevacizumab: 3.40 % ± 0.71 %; P = 0.03). In addition to the reduced quantity of dendritic cells, their activation level was also lower in the aflibercept group. Specifically, the CD11c + dendritic cells exhibited a reduced mean fluorescence intensity (MFI) of MHC class II (Control: 20653.875 ± 6351.64, vs. Aflibercept: 15523.875 ± 4198.18, P = 0.05) in the aflibercept group compared to the control group.

These findings indicate that the regression of preexisting corneal vessels by topical anti-VEGF therapy exerts a sustained inhibitory effect on APC recruitment and activation in the dLNs, even long term after keratoplasty.

4. Discussion

Our study used local administration of different anti-VEGF drugs commonly used in clinical practice, proofing 1. that local anti-VEGF treatment of corneas with pre-existing neovascularization can help to accelerate the regression of neovascularization and 2. that these pre-treatments improve corneal graft survival. In addition, we could show that the preoperative, angioregressive blockade of VEGF exerts a regulatory effect on antigen-presenting cells both immediately after regression and long-term after keratoplasty.

Corneal neovascularization is known as the main risk factor for corneal graft rejection. Therefore, the anti-VEGF antibody bevacizumab is nowadays used off-label as eye drops to inhibit the outgrowth of blood and lymphatic vessels. The same is true for the more effective VEGF-trap aflibercept. Again, we could show that Aflibercept could inhibit the active corneal neovascularization.

Nevertheless, most patients present with already established vessels. To address these already established vessels different approaches are used. One very promising method is the fine needle diathermy to coagulate feeder cells. We and others could show that this method

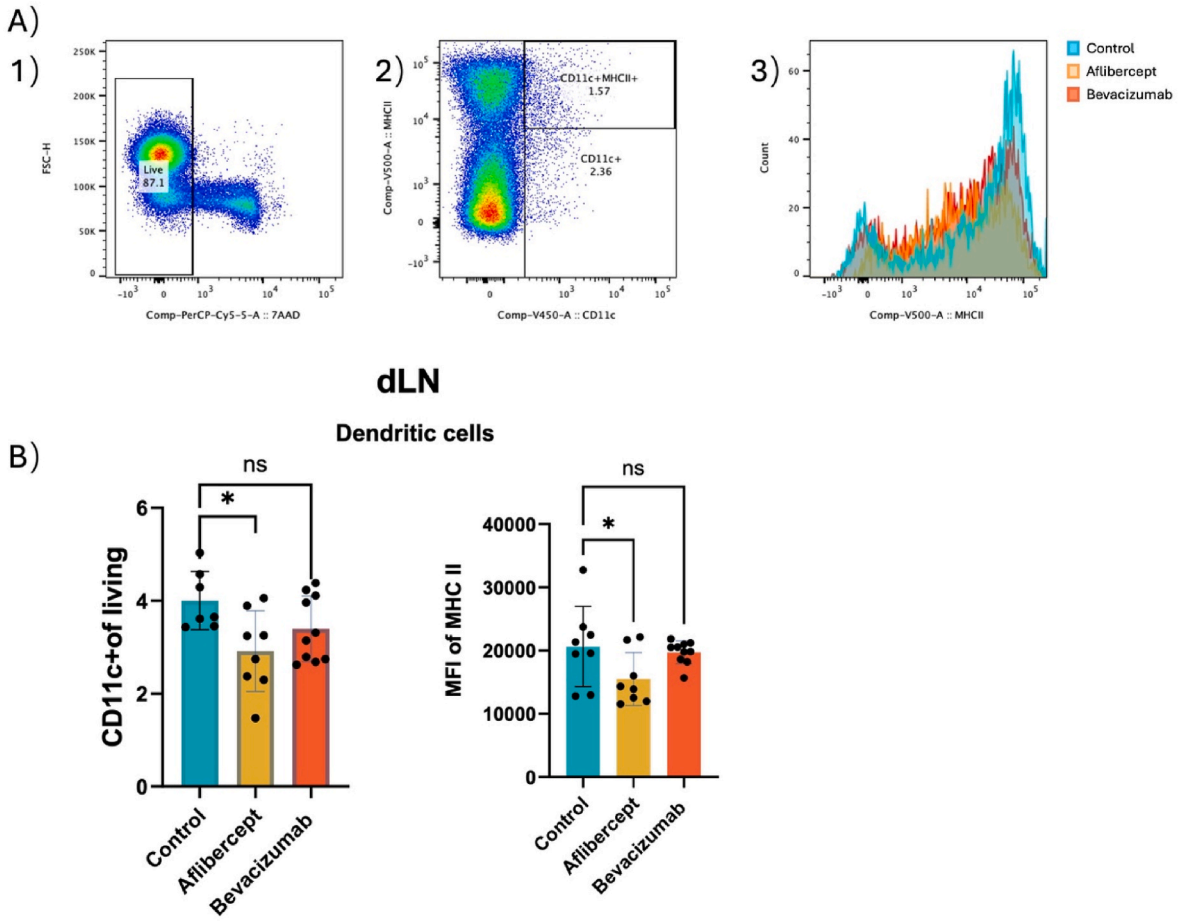

Fig. 5. Long-term reduction in the number and activation of antigen-presenting cells in draining lymph nodes following Aflibercept treatment. (A) Flow cytometry gating strategy for single-cell suspensions from draining lymph nodes: (1) viable cells were gated as 7AAD-; (2) MHC II+ and CD11c + cells were defined by antibody staining. (3) Represents MHCII fluorescence intensity (B). Flow cytometric analysis of CD11c and MHC II surface expression in dLNs. The frequencies and geometric mean fluorescence intensity are shown in the graphs. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA (n = 10); *P < 0.05.

indeed regresses existing vessels very effectively (Faraj et al., 2014; Le et al., 2018; Spiteri et al., 2015). Unfortunately, this treatment can cause a rebound neovascularization effect, so it has to be combined with antiangiogenic treatments like bevacizumab (Le et al., 2020). Common experimental approaches such as PDT or fine-needle cauterization also demonstrate clear vascular regression at one week but diminished effects at later time points (Hou et al., 2017). Consistently, we observed partial spontaneous regression after suture removal, with vessels poorly perfused. Moreover, anti-VEGF treatment reduced immune cell infiltration as early as day 7 in murine models (Cursiefen et al., 2004b), whereas in high-risk transplantation with mature vessels, Treg function is downregulated and the immunomodulatory capacity of VEGF blockade is limited (Inomata et al., 2016). Based on these findings, we chose one week after vascular regression for keratoplasty to best represent a clinically relevant high-risk setting where vessels and inflammation are still present.

When analyzing the effect of serum eye drops on the antiangiogenic effect of topical bevacizumab, our group could previously show that bevacizumab can support corneal vessel regression (Hos et al., 2013). In our current study, we could validate these findings and, in addition, could show for the first time that aflibercept can also support corneal vessel regression.

These findings are consistent with previous studies employing more invasive methods, such as intrastromal or subconjunctival injections (Peng et al., 2023; Ucgul et al., 2021) and with a case report describing the use of aflibercept eye drops to regress corneal neovascularization after failed steroid therapy (Aksoy, 2019). Our study underscores the potential of both drugs as less invasive and more patient-friendly approaches to support the regression of preexisting corneal vessels.

In terms of lymphatic vessel regression, both bevacizumab and aflibercept could regress lymphatic vessels, whereas aflibercept could not reach significance. Previous studies have shown that bevacizumab not only inhibits angiogenesis but also induces endothelial cell apoptosis through multiple mechanisms (Gerber and Ferrara, 2005). Additional studies have provided evidence that bevacizumab not only binds cell surface-bound VEGF on RPE cells but that this binding may trigger the complement cascade, resulting in cell death (Zeng et al., 2010). In contrast, aflibercept's ability to induce apoptosis appears to be cell-type dependent (Derakhshan et al., 2021; Papadopoulos et al., 2012; Şahiner et al., 2018). Further experimental studies are warranted to elucidate the effects of both drugs on the apoptosis of blood endothelial and lymphatic endothelial cells.

By limiting the outgrowth of the vessel, we and others could demonstrate an increased graft survival. In studies Bevacizumab has been tested as a post-surgery treatment to again inhibit the surgery mediated neovascularization (Dohlman et al., 2024). We could show a similar effect for Aflibercept in a preclinical model of high risk corneal transplantation (Zhang et al., 2022). In line with this we could show in our current study, that both drugs demonstrated similar efficacy in promoting vessel regression and subsequently promoted graft survival with aflibercept being slightly more effective than bevacizumab (Fig. 3). The fact, that Bevacizumab could not yet reach significance could be due

to its lower affinity to murine VEGF (Bock et al., 2007). In addition, Datjerdi et al. compared the effects of topical and subconjunctival administrated bevacizumab in a mouse model of high-risk corneal transplantation. Only the subconjunctival injection significantly improved the success rate of transplantation (Dastjerdi et al., 2010). Clinically, bevacizumab has also shown variable outcomes in corneal transplantation: some case studies reported reduced graft rejection over two to three years in high-risk patients (Derakhshan et al., 2021; Fasciani et al., 2015), while multi-center studies indicated no significant improvement in one-year graft survival rates (Dohlman et al., 2022). Aflibercept has been explored in experimental models for corneal keratoplasty. Various animal studies, including high-risk corneal transplantation models, have demonstrated improved graft survival with aflibercept administered via intraperitoneal injection, subconjunctival injection, pre-incubation, or topical eye drops (Dohlman et al., 2015; Salabarria et al., 2019; Zhang et al., 2021, 2022). Though long-term clinical data on aflibercept's use in corneal transplantation remains limited.

Our group has previously reported a reduced frequency of DCs in the dLNs by topical anti-VEGF treatment post-corneal transplantation (Zhang et al., 2022). Consistent with this, we observed a reduction in CD11c + DCs after one week of anti-VEGF-induced vascular regression. Additionally, another key antigen-presenting cell subset, F4/80+ macrophages, exhibited lower MHC class II expression. In our previous work (Zhang et al., 2022a), we demonstrated that MHC II expression increases concomitantly with the growth of neovascularization. In the regional dLNs, the activation of naive T lymphocytes requires their interaction with antigen-presenting cells expressing MHC class II(Roche and Furuta, 2015). This suggests that topical angioregressive VEGF-A blockade can modulate the recipient's immune response, effectively preparing for corneal graft acceptance.

In subsequent corneal transplantation, even after 8 weeks, the angiogressive VEGF-A blockade demonstrated fewer CD11c + DCs and F4/80+CD11b + macrophages in the cornea, accompanied by increased Foxp3 expression in the $CD3^{+}CD4^{+}$ T cell populations. It has been reported that regulatory T cells are already present in the draining lymph nodes of naïve mice (Inomata et al., 2016) and that Foxp3 expression is directly associated with sustained immune tolerance (Bock et al., 2013; Chauhan et al., 2009). This provides a plausible explanation for the higher graft survival observed (Fig. 3). We could show previously, that Aflibercept could deplete local VEGF in the cornea, following reduced CD11c + DC recruitment and enhanced Foxp3 expression, strengthening immune tolerance (Salabarria et al., 2019). Notably, at eight weeks after transplantation and post-treatment, the lymph nodes of the treatment groups exhibited fewer DCs with reduced MHC class II expression, reflecting an attenuated immune response at this critical checkpoint. In our previous study we did not find these changes by topical administration of Aflibercept during active, inflammatory neovascularization, although we could improve graft survival in this study, too. However, when applying this treatment after keratoplasty, we also found less CD11c+ and MHC class II expression on CD11c+ in dLNs after KPL (Zhang et al., 2021). In combination with our recent data this suggests that in order to induce long-term systemic immune tolerance, the method and timing of VEGF-A blockade could be important for the induction of long-term immune deviation.

In summary, we identified differences in the immune modulation and impact on corneal transplantation outcomes of two different anti-VEGF drugs, suggesting mechanisms beyond vascular regression alone. These findings highlight the need to further analyze how anti-VEGF therapies enhance transplant success. Our results provide valuable insights and suggest that Aflibercept and Bevacizumab may offer advantages in high-risk corneal transplantation by preoperative, non-invasive regression of blood and lymphatic vessels.

CRediT authorship contribution statement

Zhe Chen: Writing – original draft, Formal analysis, Data curation. **Wei Zhang:** Methodology. **Mert Mestanoglu:** Methodology. **Claus Cursiefen:** Writing – review & editing. **Felix Bock:** Writing – review & editing, Supervision.

Statement

During the preparation of this work, Zhe Chen used Chat GPT (GPT-5, OpenAI, 2025 version) in order to polish the language and check the grammar. After using Chat GPT, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of interest statement

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors of this manuscript have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exer.2025.110652.

Data availability

Data will be made available on request.

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