

RETINAL DISEASE

Disrupting galectin-3 and NET-mediated microglia-neutrophil cross-talk suppresses pathological retinal angiogenesis

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Current research on pathological retinal neovascularization primarily focuses on growth factors, inflammation, and endothelial signaling pathways. However, increasing attention is being directed toward disruptions in the retinal immune microenvironment. Therefore, deciphering the immune-angiogenic interplay could uncover therapeutic avenues for neovascular disorders. Through integrative analyses of single-cell RNA sequencing from human fibrovascular membranes and multicohort clinical datasets, we identified neutrophil infiltration as an independent risk factor for diabetic retinopathy progression. Mechanistically, activated microglia preceded and potentiated neutrophil infiltration by secreting galectin-3 (GAL3), establishing a self-amplifying feedback loop that sustained microglial activation and drove pathological angiogenesis in mice with oxygen-induced retinopathy (OIR). To therapeutically disrupt this loop, we engineered a photocurable hydrogel for the sustained intravitreal delivery of GAL3 and vascular endothelial growth factor (VEGF)-neutralizing antibodies, which effectively suppressed aberrant angiogenesis in mice with OIR. Together, these findings reinforce the concept of retinal neovascularization as an immunovascular disorder and underscore the therapeutic potential of microenvironment-modulating strategies using biomaterials.

INTRODUCTION

Retinal angiogenesis is a key pathological mechanism underlying vision-threatening ocular disorders, including diabetic retinopathy (DR), retinopathy of prematurity, and age-related macular degeneration (1–3). These conditions are marked by abnormal neovascularization (NV) characterized by fragile vessels with insufficient pericyte coverage and dysregulated angiogenic signaling, leading to vascular leakage, hemorrhage, and irreversible vision loss. Current first-line treatments, particularly anti-vascular endothelial growth factor (anti-VEGF) agents, have shown essential clinical efficacy; however, their therapeutic benefit remains limited in a substantial subset of patients because of adverse effects and variable response rates (4). A more comprehensive understanding of retinal NV is therefore critical, not only to elucidate underlying pathogenic mechanisms but also to inform the development of therapeutic strategies.

Pathological angiogenesis is a tightly regulated, multistep process orchestrated by numerous cellular and molecular factors (5, 6). Although activated endothelial cells are central to this process, emerging evidence underscores the pivotal role of immune microenvironment dysregulation in driving aberrant vascular growth (7–9). The retinal immune milieu comprises resident and infiltrating immune cells, including microglia (MG), perivascular macrophages, neutrophils, and T cells, and a complex network of glial cells, cytokines, chemokines, complement proteins, and specialized vascular elements (10, 11). Interactions among these components are critical for maintaining

ocular immune privilege (12). In this context, immune cells modulate angiogenesis through the secretion of proangiogenic mediators and direct ligand-receptor interactions (13). Nonetheless, the intricate interplay among immune constituents during pathological angiogenesis remains poorly understood.

Among retinal immune cells, resident MG are central to maintaining homeostasis under physiological conditions (7, 14–16). In parallel, recent studies have shown that peripheral immune cells can rapidly infiltrate the retina after disruption of the blood-retinal barrier (BRB), contributing to pathological responses (17–22). Neutrophils represent the most rapidly responding innate immune cells upon retinal insult (23). Beyond their antimicrobial functions, activated neutrophils release nuclear and granular contents to form neutrophil extracellular traps (NETs), weblike structures composed of double-stranded DNA, histones, and proteolytic enzymes (24, 25). NETs have been implicated not only in autoimmune pathologies (26) but also in retinal vascular remodeling and repair (27). Moreover, increasing evidence suggests that the activities of resident MG and infiltrating immune cells are not isolated; rather, they exhibit frequent and functionally marked cross-talk (28). However, the mechanisms underlying neutrophil infiltration and NET formation in the context of retinal NV remain to be fully elucidated.

In this study, we identified a self-amplifying feedback loop between MG and neutrophils, using integrated multiomic analyses of patient-derived clinical specimens and experimental oxygen-induced retinopathy (OIR) models. We further elucidated the progressive deterioration of immune microenvironment dysregulation and propose a mechanism by which this loop cross-talks with pathological angiogenesis, thereby aggravating NV, which might partially account for the poor response to anti-VEGF therapy observed in some patients. To therapeutically disrupt this detrimental loop, we developed a hydrogel-based delivery system encapsulating galectin-3 (GAL3)-neutralizing antibodies. This platform enabled sustained intraocular

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release, achieved substantial inhibition of pathological retinal NV, and obtained a better effect in alleviating the process of the disease together with anti-VEGF therapy, offering a promising strategy for restoring immune homeostasis in the retinal microenvironment.

RESULTS

Disturbance in the retinal immune microenvironment and recruitment of peripheral neutrophils were observed in patients with retinal neovascular disease

To investigate immune mobilization in retinal vascular diseases, we analyzed single-cell RNA sequencing (scRNA-seq) data from fibrovascular membranes (FVMs) of patients with advanced diabetes available in the Gene Expression Omnibus (GEO) database (series GSE165784). Cluster analysis identified nine distinct cell subpopulations (Fig. 1A). Furthermore, the nine subpopulations were defined as specific cell clusters according to the surface markers (Fig. 1B) and were annotated using lineage-specific markers (Fig. 1C). MG markers were prominently expressed, suggesting that MG played a central regulatory role during advanced disease progression. We also observed abundant myeloid cell populations in the FVM samples, indicative of peripheral immune cell infiltration into retinal tissues. To systematically explore this peripheral immune involvement, we collected blood samples from 12 patients with proliferative DR and 12 patients with idiopathic epiretinal membrane. A comparative analysis of peripheral leukocyte profiles revealed a significant increase in total white blood cell counts, with neutrophils exhibiting the most marked elevation (Fig. 1D, $P < 0.01$). Binomial logistic regression analysis revealed that among neutrophil, monocyte, and lymphocyte percentages, only the neutrophil percentage emerged as an independent risk factor for DR development (Fig. S1A). These findings suggested the potential contribution of systemic immune alterations as well as neutrophil involvement to retinal vascular pathology. An analysis of aqueous and vitreous humor samples showed elevated expression of inflammatory cytokines, including interleukin-6 (IL-6) and IL-8, indicating active intraocular inflammation in patients with retinal vascular disease (Fig. 1E). Immunofluorescence (IF) staining further confirmed neutrophil activation in FVMs, alongside the involvement of retinal-resident MG (Fig. 1F). To validate the changes in peripheral blood and the connection between them and the occurrence of DR, we conducted an independent analysis in a cohort comprising 165 patients with diabetes mellitus without retinopathy and 166 with DR (Fig. S1, B and C). Peripheral blood samples from patients with DR showed a significantly elevated neutrophil percentage, reinforcing the association between neutrophils and retinal pathology (Fig. 1G, $P < 0.001$). Binomial logistic regression analysis revealed that, among neutrophil, monocyte, and lymphocyte percentages, only the neutrophil percentage emerged as an independent risk factor for DR development (Fig. 1H). This relationship was further corroborated by large-scale validation using data from 4633 National Health and Nutrition Examination Survey (NHANES) participants with DR and non-DR (Fig. S1, D and E), confirming the persistent association between elevated neutrophil infiltration and DR progression (Fig. 1, I and J). Collectively, these multicohort analyses across diverse clinical settings consistently implicated neutrophils as potential contributors to DR pathogenesis. These findings suggested that peripheral neutrophil mobilization, in association with MG activation, disrupted the retinal immune microenvironment in retinal vascular diseases.

Recruitment of neutrophils toward retinal lesions and release of NETs exacerbate retinal NV

To investigate the recruitment and function of neutrophils, we established a mouse model of OIR, characterized by robust retinal NV. Mouse pups were exposed to 75% oxygen from postnatal day 7 (P7) to P12 to induce vascular occlusion and then returned to room air. Retinal NV peaked at P17 (Fig. 2A). In the OIR model, aberrant NV remodeling was initiated predominantly from the venous system, especially from venous-side capillaries and venules (Fig. S2, A and B). scRNA-seq of the retina isolated at P17 identified a distinct neutrophil cluster in the OIR group (Fig. 2B) with *Padi4* expression (Fig. 2C). In addition, increased retinal expression of inflammatory cytokines *Il6* and *Il1b* was also detected in OIR mice (Fig. 2D). Peripheral blood also showed elevated neutrophil counts despite retinal neutrophil depletion, suggesting the peripheral origin of neutrophil activation (Fig. 2E). Flow cytometry confirmed significant neutrophil mobilization in peripheral blood samples at P17 in OIR mice (Fig. 2F, $P < 0.001$). IF imaging showed neutrophil recruitment to the retina, with aggregation around neovascular clusters (Fig. 2G). In the OIR model (P17), we consistently observed that neutrophils were predominantly localized around the aberrant vascular sprouts (Fig. S2C). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted to elucidate the function of these neutrophils, and the results revealed up-regulated expression of genes involved in NET formation, cytoskeletal remodeling, and hematopoietic cell markers (Fig. 2H). Given the role of NETs in neutrophil function, we assessed their presence and detected NETs near neovascular regions (Fig. 2, I to L). To test the regulatory role of neutrophils and NETs, we induced neutrophil depletion through an intravitreal injection of anti-LY6G antibodies (Fig. S3A). NETs were absent after neutrophil depletion (Fig. S3B). This intervention reduced both neovascular and avascular areas (Fig. 2M). Correspondingly, treatment with the NET inhibitor GSK484 also reduced pathological NV and avascular areas (Fig. 2N). Under in vitro conditions, the endothelial cells capsulated with neutrophils showed stronger trends of sprouting and lumen formation (Fig. 2O and Fig. S4, A to C). Together, these findings highlighted that, during retinal NV, neutrophils were recruited from the periphery to the retina, where they released NETs. Similarly, neutrophils could also be found in other end organs affected by the diabetes pathology (Fig. S5A). Alongside MG, the neutrophils contributed to the angiogenic process, potentially through NET-mediated mechanisms.

Reciprocal regulatory interactions between MG and neutrophils that synergistically disrupt the retinal immune microenvironment

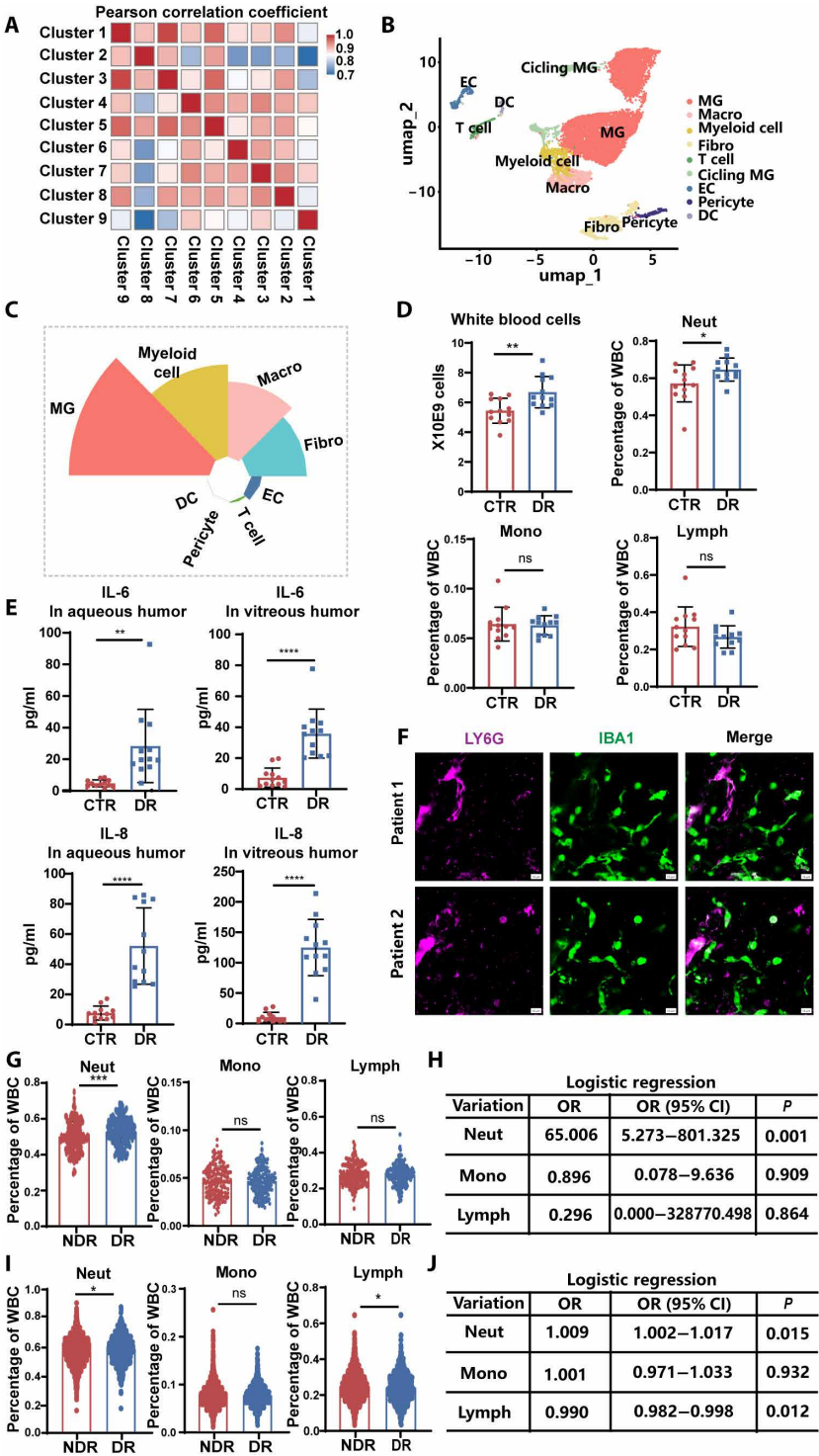
To elucidate the role of neutrophils in the retinal microenvironment, we examined the intercellular communication between major retinal cell types and neutrophils using scRNA-seq. The analysis revealed interactions not only among neutrophils themselves but also between neutrophils and MG (Fig. 3, A and B). Consistently, IF staining showed spatial proximity between MG and neutrophils, both of which were found to surround neovascular lesions (Fig. 3C). This was likely because of BRB disruption under pathological conditions, which permitted neutrophil infiltration and MG migration. However, it remained unclear which cell type initiated this pathological response. To address this, we assessed the quantity and activation status of MG and neutrophils in the retina of OIR mice from P12 to P17. By P13, MG exhibited an activated, amoeboid morphology characterized by enlarged somas and retracted processes. Neutrophils, however, were

Fig. 1. Immune microenvironment of patients with retinal NV was disturbed. (A) The correlation and clustering results of scRNA-seq of FVMs from patients with DR in the GEO database. The color scale represents the correlation coefficients between samples. (B) The uniform manifold approximation and projection (UMAP) plot shows major cell types and subclusters generated through Seurat. (C) The relative expression of markers of different cells in all of the cells from the FVMs from patients with DR. (D) The bar plot shows the numbers of white blood cells from the CTR (control) group and patients with DR ($n = 12$ per group). (E) The bar plots show the concentrations of inflammatory cytokines IL-6 and IL-8 in aqueous and vitreous humors from the CTR group and patients with DR ($n = 12$ per group). (F) IF staining of MG (IBA1 in green) and neutrophils (LY6G in purple) in FVMs collected from patients. Scale bars, 20 μm . (G) The bar plots show the percentages of neutrophils, monocytes, and lymphocytes among white blood cells in blood samples from outpatients. (H) The table shows logistic regression of the percentage of neutrophils among the white blood cells and the occurrence of DR in the outpatients with DR. (I) The bar plots show the percentages of neutrophils, monocytes, and lymphocytes among white blood cells in blood samples from 5514 patients included in the NHANES database. (J) The table shows logistic regression of the percentage of neutrophils in the white blood cells and the occurrence of patients with DR in the NHANES database. DM, diabetes mellitus; DC, dendritic cells; EC, endothelial cells; Fibro, fibroblast; Macro, macrophage; WBC, white blood cells; Neut, neutrophil; Mono, monocyte; Lymph, lymphocyte; OR, odds ratio; CI, confidence interval. Bars = means $\pm$ SD; Student's t test was used for (E), (F), (I), and (J). ns, no significance; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

not detectable until P15, suggesting that MG activation precedes neutrophil infiltration (Fig. 3, D and E, and fig. S6A). Further investigation demonstrated that MG regulated neutrophil activation and function. To eliminate resident MG by oral administration of the colony-stimulating factor 1 receptor inhibitor PLX5622 from P12 (29), we observed the complete absence of neutrophils and NETs in the retina (Fig. 3, F and G), confirming the regulatory role of MG. This interaction seemed reciprocal: Depletion of neutrophils in OIR mice also led to a significant reduction in MG numbers (Fig. 3H, $P < 0.0001$), indicating that neutrophils similarly influence MG. To validate these findings in vitro, we isolated primary neutrophils from mouse peripheral blood samples (fig. S6, B to D) and established two coculture systems. We assessed the effects of neutrophils and NET-inducing neutrophils on BV2 MG cells. Next, we cocultured primary neutrophils with either hypoxia-activated or nonactivated BV2 cells to investigate the influence of MG on neutrophils under pathological conditions. NETosis-induced neutrophils were most strongly affected by BV2 cell behavior, whereas hypoxia-activated BV2 cells exerted the greatest influence on neutrophils (Fig. 3, I and J). These results underscored a dynamic, bidirectional interaction between MG and neutrophils in the context of retinal pathology.

Microglial GAL3 triggers neutrophil infiltration and NET formation

To uncover the molecular mechanisms by which MG regulate neutrophils, we performed scRNA-seq on MG from the OIR retina. The



results revealed *Lgals3*, the gene encoding GAL3, as the highest fold change among differentially expressed genes in MG (Fig. 4A). Consistently, the expression of GAL3 was also up-regulated in the vitreous and aqueous humors in patients of the proliferative DR group compared with those in patients of the non-DR group (Fig. 4B). IF staining confirmed increased GAL3 expression in OIR mice (Fig. 4C). By using GAL3-neutralizing antibody (AbGAL3) intravitreally, the inhibition of GAL3 effectively reduced retinal angiogenesis. When

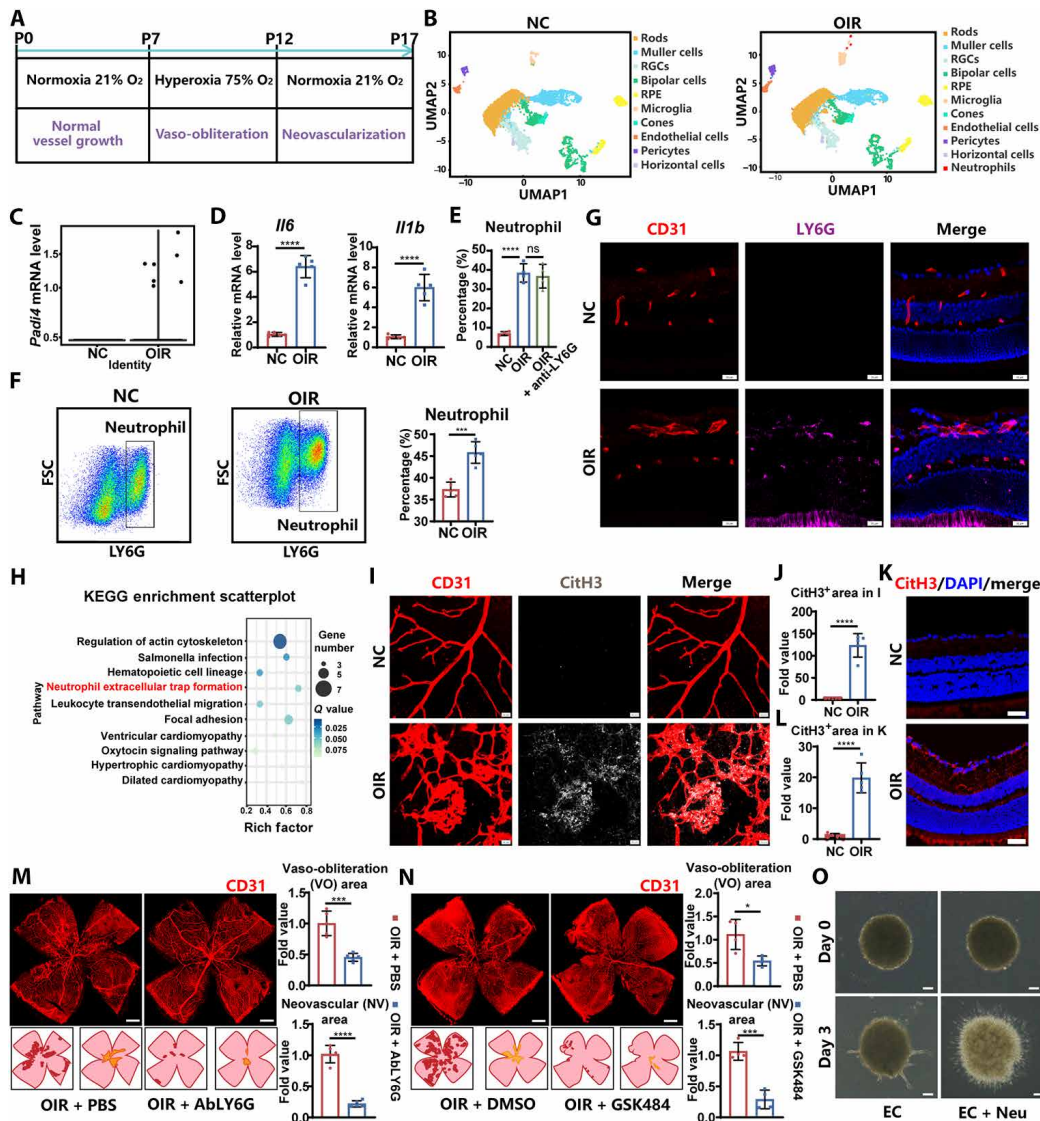


Fig. 2. Neutrophils and NETs played vital roles in retinal vascular diseases.

(A) The schematic of the OIR model in mice. Exposure to hyperoxia (75% O₂) in mice from P7 to P12 induced the dropout of retinal vascularization. Ischemia insult triggered pathological NV and peaked on P17. (B) The UMAP plots show major cell types and subclusters in the control and OIR groups. (C) The violin plot shows the mRNA level of *Padi4* related to neutrophil in MG of the OIR group (analyzed by R package). (D) The bar plots show inflammatory cytokines *Il6* and *Il1b* in the retinas from normal and OIR mice, normalized to *Actin* and shown relative to the normal group ($n = 5$ per group). (E) The percentages of neutrophils in the peripheral blood collected from untreated normal mice on P17, OIR mice on P17, and the mice after the intravitreal injection of anti-LY6G on P17 ($n = 4$ per group). Normalized to *Actin* and shown relative to the immunoglobulin G (IgG) group. (F) The number of LY6G⁺ neutrophils in the peripheral blood in the untreated normal control (NC) and OIR ($n = 5$ per group). (G) IF staining of retinal vessels (CD31, red) and neutrophils (LY6G, purple) on P17 in the cross sections from the normal and OIR groups. Scale bars, 20 μ m. (H) The enriched genes in the KEGG pathways in the neutrophils from the OIR group (analyzed by R package). (I) IF staining and (J) quantification of retinal vessels (CD31, red) and NETs (CitH3, white) on P17 in the whole mount. Scale bars, 20 μ m ($n = 5$ per group). (K) IF staining and (L) quantification of NETs (CitH3, red) and nucleus [4',6-diamidino-2-phenylindole (DAPI), blue] on P17 in the cross sections from normal and OIR groups. Scale bars, 20 μ m ($n = 5$ per group). (M) IF staining of retinal vessels

(CD31, red) on P17 in the flat-mounted section after the clearance of neutrophils. In the schematic below, red indicates neovascular (NV) area and yellow indicates vaso-obliteration (VO) area. The plots on the right show quantifications. Scale bars, 500 μ m ($n = 5$ per group). (N) IF staining of retinal vessels (CD31, red) on P17 in the flat-mounted section after the clearance of NETs. In the schematic below, red indicates the NV area, and yellow indicates the VO area. The plots on the right show quantifications. Scale bars, 500 μ m ($n = 4$ per group). (O) The images show the sprouting between spheroids composed solely of ECs and ECs cocultured with neutrophils. Scale bars, 100 μ m. Bars = means $\pm$ SD; Student's *t* test was used for (D), (F), (J), (L), (M), and (N). One-way ANOVA was used for (E). * $P < 0.05$, *** $P < 0.001$, and **** $P < 0.0001$. RGCs, retinal ganglion cells; DMSO, dimethyl sulfoxide; RPE, retinal pigment epithelium.

compared with the VEGF neutralizing antibody (AbVEGF) injection, the AbGAL3 injection yielded efficacy in inhibiting angiogenesis comparable to that with the AbVEGF injection (Fig. 4D). Moreover, when comparing the effects of AbGAL3 and AbVEGF in neutrophil participation, NET formation, and inflammation, AbGAL3 had a unique inhibitory capacity, an effect barely observed with AbVEGF treatment, suggesting that GAL3 may promote angiogenesis through distinct mechanisms from the VEGF pathway (Fig. 4E). Given that MG predominately expressed GAL3, MG-specific GAL3 knockout mice were generated and analyzed (fig. S7, A and B). After the OIR model was set up, neutrophil infiltration and NET formation were reduced in mice in which *Lgals3* was conditionally knocked out in MG [conditional knockout (CKO)], along with decreased expression

of associated molecular markers (Fig. 4, F and G). By contrast, when CKO mice received intravitreal injections of GAL3-expressing BV2 cells, neutrophil recruitment to neovascular lesions resumed, and pathological NV was aggravated (Fig. 4H and fig. S8A). These findings suggested that MG-derived GAL3 promotes neutrophil aggregation and NET formation, thereby facilitating retinal angiogenesis. To confirm the neutrophil-microglia-GAL3 axis as observed in OIR, a high-fat diet and streptozotocin-induced diabetic model and a db/db diabetic model were used for the assessment. GAL3 was found to modulate the infiltration of neutrophils and then the formation of NETs (fig. S9, A to H).

Stimulation of primary neutrophils with GAL3 resulted in up-regulation of NET-related genes and detectable NET release in the supernatant (Fig. 4, I and J). IF staining showed that GAL3-induced NETs

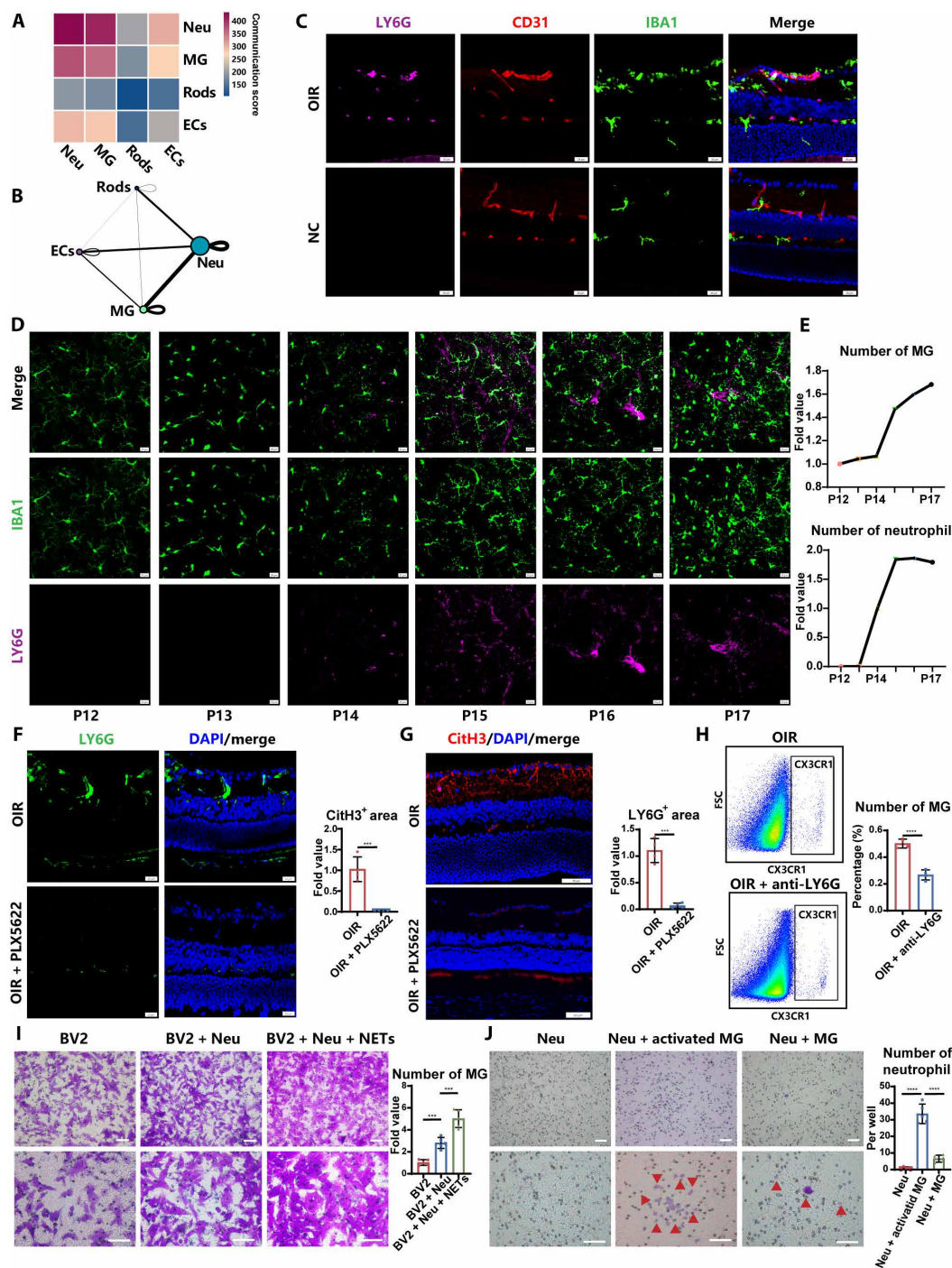


Fig. 3. MG and neutrophils were closely regulated by each other in the disturbance in immune microenvironment.

(A) Cellular interaction between neutrophils and several other retinal cells in the OIR model. Color bar corresponds to the communication score as indicated on the right side of the image. (B) Cellular interactions comparison between neutrophils and several other retinal cells in the OIR model analyzed using the data of scRNA-seq. Line thickness is proportional to the change in communication strength; loops indicate reactions with cells of the same type; larger circles represent more ligand-receptor pairs involved in the interactions with other cells. (C) IF staining of retinal vessels (CD31, red), neutrophils (LY6G, purple), MG (IBA1, green), and nuclei (DAPI, blue) in the cross sections from normal and OIR groups on P17. Scale bars, 20 μ m. (D) IF staining of retinal MG (IBA1, green) and neutrophils (LY6G, purple) in the flat-mounted sections from P12 to P17 in the OIR group. Scale bars, 20 μ m. (E) Fold values of the numbers of MG and neutrophils during the pathological process of NV in the OIR model from P12 to P17. P12 was used to normalize the number of MG, and P14 was used to normalize the number of neutrophils. Different colors refer to different time points. (F) IF staining of retinal neutrophils (LY6G, green) and nuclei (DAPI, blue) on P17 in the cross sections from OIR mice and the mice with removed MG by PLX5622. The plot on the right shows quantification of the left plots and is normalized to the OIR group. Scale bars, 20 μ m ($n = 4$ per group). (G) NETs (CitH3, red) and nuclei (DAPI, blue) on P17 in the cross sections from OIR mice and the mice with removed MG by PLX5622. The plot on the right shows quantification of the left plots and is normalized to the OIR group. Scale bars, 20 μ m ($n = 4$ per group). (H) The flow plots on the left show the number of CX3CR1⁺ MG before and after the inhibition of neutrophils in the OIR retinas. The plot on the right shows quantification of the left plots ($n = 4$ per group). (I) The plots on the left show the invasion of BV2 cells, BV2 cells cocultured with primary neutrophils and with NETs induced by PMA

from primary neutrophils by transwell invasion assay. Scale bars, 20 μ m ($n = 5$ per group). The plot on the right shows quantification of the left plots and is normalized to the BV2 group. (J) The plots on the left show the invasion of primary neutrophils and primary neutrophils cocultured with MG activated by hypoxia or with solely MG by transwell invasion assay (red triangles, invaded neutrophils). The plot on the right shows quantification of the left plots and is normalized to the Neu group. Scale bars, 20 μ m ($n = 5$ per group). Neu, neutrophils; FSC, forward scatter. Bars = means $\pm$ SD; Student's *t* test was used for (F) to (H). One-way ANOVA was used for (I) and (J). ****P* < 0.001 and *****P* < 0.0001.

were morphologically similar to those generated by the classical phorbol 12-myristate 13-acetate (PMA) pathway (fig. S10A), and the effect was reversed in the coculture system of neutrophils and MG in which the expression of GAL3 was knocked down by si*Lgals3* (Fig. 4K).

To further assess whether GAL3 acts as a central mediator of angiogenic-immune cross-talk, we injected recombinant GAL3 into

the vitreous of wild-type mice to mimic pathological conditions (fig. S11A). This resulted in vascular dilation and leakage (Fig. 4L), disruption of the characteristic trilaminar retinal vasculature, and accumulation of neutrophils and NETs around neovascular clusters in the inner retina (Fig. 4, M and N). Furthermore, GAL3 disrupted both the inner and outer layers of the BRB in vivo (fig. S11,

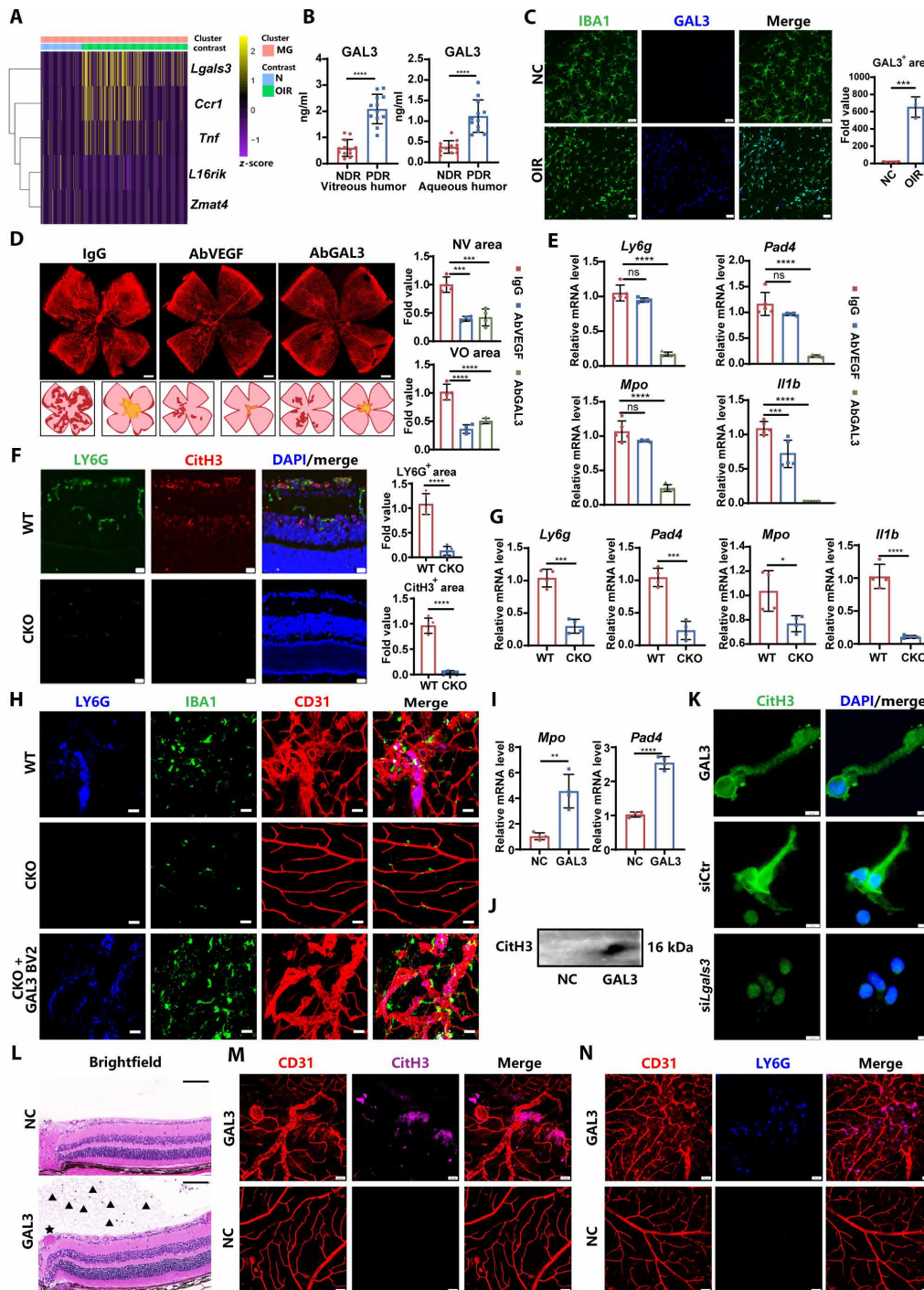


Fig. 4. MG-derived GAL3 regulated neutrophil recruitment and NET formation.

(A) Heatmap representing the differentially expressed genes of MG in the normal and OIR groups. The color bars above the heatmap indicate cell types and groups. Purple and yellow represent the corresponding z-scores shown on the right. (B) The expression of GAL3 from vitreous (left) and aqueous humors (right) ($n = 12$ per group). (C) IF staining of retinal MG (IBA1, green) and GAL3 (blue) in the cross sections from the normal and OIR groups ($n = 3$ per group). Normalized to the NC group. (D) IF staining of retinal vessels (CD31, red) on P17 OIR mice in the flat-mounted sections after IgG, AbVEGF, and AbGAL3 therapies. In the schematic below, red indicates the NV area, and yellow indicates the VO area. The plots on the right show quantifications ($n = 4$ per group). Scale bars, 500 μm . (E) mRNA expression of *Ly6g*, *Mpo*, and *Pad4* is shown for the mice subjected to IgG, anti-VEGF, and anti-GAL3 therapies. Normalized to *Actin* and shown relative to the IgG group ($n = 5$ per group). (F) IF staining of neutrophils (LY6G, green), NETs (CitH3, red), and nucleus (DAPI, blue) in the cross sections from wild-type and Cx3cr1-Lgals3^{f/f} OIR mice ($n = 5$ per group). Normalized to the wild-type group. Scale bars, 20 μm . (G) mRNA expression of *Ly6g*, *Mpo*, *Pad4*, and *Il1b* from wild-type and Cx3cr1-Lgals3^{f/f} OIR mice is shown. Normalized to *Actin* and shown relative to the WT group ($n = 4$ per group). (H) IF staining of retinal neutrophils (LY6G, blue), vessels (CD31, red), and MG (IBA1, green) on P17 in the flat-mounted sections from wild-type, transgenic, and transgenic with GAL3^{BV2} cell injection groups. Scale bars, 50 μm . (I) mRNA expression of NET formation (*Mpo* and *Pad4*) in the untreated neutrophils (NC group) and neutrophils 4 hours after stimulated by GAL3 (GAL3 group) is shown. Normalized to *Actin* and shown relative to the NC group ($n = 4$ per group). (J) The blot shows the release of NETs in the supernatants in the untreated neutrophils (NC group) and neutrophils 4 hours after stimulated by GAL3 (GAL3 group). (K) IF staining determined the morphology of NETs (CitH3, green) and nuclei (DAPI, blue) generated from neutrophils stimulated by GAL3 and neutrophils cocultured with MG with siCtrl or siLgals3 under hypoxia. Scale bars, 5 μm . (L) Hema-

toxylin and eosin staining is shown 3 days after GAL3 intravitreal injection to the C57 mice. Scale bars, 100 μm . Black arrowheads refer to blood cells effused from leaky vessels; the black star refers to enlarged vessels. (M) IF staining of retinal vessels (CD31, red) and NETs (CitH3, purple) 3 days after the injection in the flat-mounted sections from the NC and GAL3 groups. Scale bars, 50 μm . (N) IF staining of retinal vessels (CD31, red) and neutrophils (LY6G, blue) 3 days after the injection in the flat-mounted sections from the NC and GAL3 groups. Scale bars, 50 μm . WT, wild type. Bars = means $\pm$ SD; Student's *t* test was used for (B), (C), (F), (G), and (I). One-way ANOVA was used for (D) and (E). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

B to E), with similar effects observed in vitro (fig. S11, F to I). We also examined the expression of surface proteins on neutrophils after GAL3 stimulation in vitro. The results showed up-regulation of *Cxcr2* and *Cd11b* expression, indicating enhanced transendothelial

migration ability of neutrophils. In addition, increased expression of *Tnf* and *Il1b* suggested heightened neutrophil activation (fig. S12A). Furthermore, we also assessed changes in endothelial cells, which are one of the primary cellular components of blood

vessels, after GAL3 stimulation. GAL3-treated endothelial cells presented high expression of adhesion molecules, which created favorable conditions for neutrophils to migrate across blood vessels into tissues (fig. S12B).

GAL3-TLR4 pathway promotes the release of NETs

We next investigated the mechanism by which GAL3 regulates NET formation. Gene expression analysis of neutrophils before and after establishing the OIR model revealed an up-regulation of Toll-like receptor 4 (*Tlr4*) (Fig. 5A). An intravitreal injection of neutralizing antibodies against GAL3 in OIR mice down-regulated the expression of both *Tlr4* and its adaptor protein *Trif* and also reduced the expression of *Casp8*, a key molecule implicated in NET release (Fig. 5B). Furthermore, GAL3 stimulation on primary neutrophils isolated from mouse peripheral blood samples increased the expression of *Tlr4*, *Trif*, and *Casp8* (Fig. 5C), suggesting that GAL3 may act through TLR4 to trigger downstream signaling. This hypothesis was supported by molecular docking models and immunoprecipitation assays, which confirmed a direct interaction between GAL3 and TLR4 (Fig. 5, D and E). IF colocalization analysis further showed that retinal GAL3 blockade effectively regulated the TLR4 pathway, thereby reducing NET formation (Fig. 5, F to M). To probe the functional role of TLR4, we administered the TLR4 inhibitor TAK-242 and its agonist neoseptin intravitreally. GAL3 inhibition attenuated retinal NV and vaso-obliteration, whereas TLR4 activation by neoseptin and GAL3 supplementation exacerbated these pathological features. By contrast, TLR4 inhibition reversed these effects (Fig. 5N). Under in vivo conditions, NET production was markedly reduced after TLR4 inhibition (Fig. 5O). Under in vitro conditions, TAK-242 blocked GAL3-induced NET formation, whereas neoseptin restored it, underscoring the pivotal role of the GAL3-TLR4 pathway in NET formation (Fig. 5, P and Q).

Neutrophil-derived NETs amplify MG activation, forming a positive feedback loop with MG

To further explore the regulatory interactions between neutrophils, NETs, and MG, we established a coculture system combining BV2 MG cells with primary peripheral blood neutrophils and PMA-induced NETs (fig. S13A). Bulk RNA sequencing of BV2 cells revealed substantial transcriptomic alterations across different treatment groups relative to the control (Fig. 6A). Gene Ontology (GO) enrichment analysis highlighted key changes in immune response, cell migration and adhesion, angiogenesis-related processes, and ligand-receptor interactions (Fig. 6B and fig. S13, B and C). We also examined the expression of the MG activation markers *Itgax*, *Lgals3*, *Cd9*, *Itgam*, *ApoE*, and *Cx3cl1*. These genes were highly up-regulated in the coculture group containing NETs, whereas they were down-regulated in cultures with neutrophils alone (Fig. 6C), emphasizing the critical role of NETs in MG activation. Pairwise pathway enrichment analysis showed that, compared with the BV2 monoculture control, coculture with neutrophils alone primarily altered cellular component-related pathways (Fig. 6D). By contrast, BV2 cells cocultured with NET-inducing neutrophils exhibited phenotypic changes consistent with those aligning with immune activation and proangiogenic MG (Fig. 6, E to G). Intravitreal administration of the NET inhibitor GSK484 in OIR mice revealed altered MG localization: In the untreated OIR retina, activated MG migrated to superficial lesion areas, whereas NET inhibition restricted MG to the outer plexiform layer, suggesting suppressed activation (Fig. 6H). Similarly, the extra intravitreal

injection of purified NETs extracted from stimulated neutrophils into natal mouse caused the changes in microglial morphology, which further indicated the activation of MG arising from NETs (Fig. 6I). The expression of *Lgals3* (encoding GAL3), proangiogenic factors (*Vegfa*), and angiogenesis and inflammation-related factors (*Ang2/Ang1* and *Tnf*) was up-regulated (Fig. 6J). Under in vitro conditions, morphological analyses of BV2 cells stimulated with NETs derived from primary neutrophils showed appreciable increases in the length-to-width ratio and nuclear area, indicating activation (Fig. 6K). GAL3 expression was elevated in both BV2 cells and their supernatants after exposure to neutrophil-derived NETs, suggesting that GAL3 may, in turn, promote further NET formation in an amplifying feedback loop (Fig. 6, L and M). Moreover, supernatants from PMA-induced NETs upgraded *Lgals3*, neutrophil chemotaxis-related genes (*Cxcl1* and *Cxcl2*), *Vegfa*, *Ang2/Ang1*, and *Tnf* in BV2 cells (Fig. 6N). Collectively, these findings demonstrated that NETs potentiate MG activation, triggering a GAL3-dependent cascade that enhances neutrophil recruitment and pathological angiogenesis. These data further supported the existence of a self-reinforcing loop between MG and neutrophils, wherein reciprocal activation amplified GAL3 accumulation and NET formation, thereby exacerbating disease progression (fig. S14A).

Hydrogel cross-linked with anti-GAL3 effectively blocks the positive feedback to alleviate the progression of retinal NV

Given the central role of GAL3 in sustaining immune dysregulation, we sought to disrupt this positive feedback loop by targeting MG-derived GAL3 to attenuate NV. To this end, we developed a photocurable hydrogel formulation capable of exerting sustained release of anti-AbGAL3 after a single intravitreal injection. Upon exposure to a 405-nm light source, the hydrogel solidified (Fig. 7, A and B), and cross-linking with the antibody did not markedly alter its pore size or elastic modulus (fig. S15, A and B). An intravitreal injection of the hydrogel precursor, followed by in situ photopolymerization, resulted in tight adherence of the gel to the retinal surface, as confirmed by hematoxylin and eosin staining (Fig. 7, C and D). We conjugated anti-GAL3 with the Alexa Fluor 488 (AF488) fluorophore and encapsulated it in the hydrogel (Ab@GelMA). This complex was intravitreally injected into the mouse eyes. Using an in vivo imaging system, we monitored fluorescence intensity and observed that, in contrast with the obvious fluorescence decrease by day 2 after injecting the drug, the hydrogel-encapsulated neutralizing antibody showed continued release until day 7 (Fig. 7, E and F). By detaching the hydrogel attached to the inner retinal surface and quantifying the fluorescence intensity (fig. S15C), we calculated the cumulative release profile of the hydrogel-encapsulated antibody (Fig. 7G). Concurrently, we observed that the fluorophore-labeled antibody reached the retina to exert therapeutic effects (fig. S15D). Under in vitro conditions, release kinetics also showed that the neutralizing antibody was stably released for more than 3 days (fig. S15E). This delivery system effectively suppressed the accumulation of neutrophils and NETs (Fig. 7, H to K) as well as inflammation in the retina (Fig. 7, L and M). Furthermore, we combined AbGAL3 and AbVEGF treatment through GelMA. AbGAL3@GelMA can effectively reduce the density of NV and the area of nonperfusion zones, and the combined application yielded even better results (Fig. 7L). In addition, we observed that the hydrogel was completely cleared by day 35 (fig. S15F), revealing great potential for translational relevance.

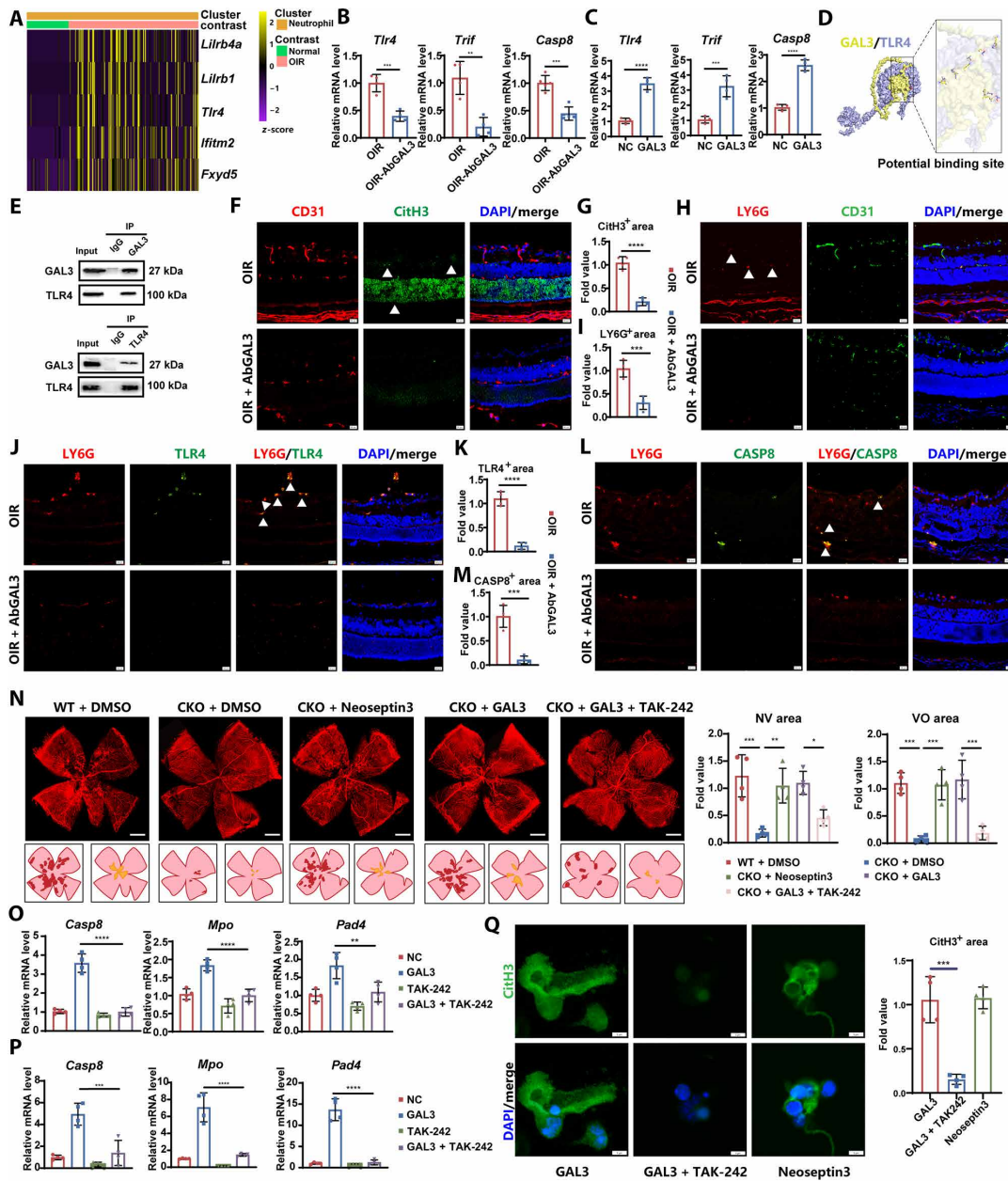


Fig. 5. GAL3 bound to TLR4 of recruited neutrophils to release NETs.

(A) Heatmap representing the differentially expressed genes of neutrophils in the normal and OIR groups. The color bars above the heatmap indicate cell types and groups. Purple and yellow represent the corresponding z-scores shown on the right. (B) mRNA expression of *Tlr4*, *Trif*, and *Casp8* in the OIR and OIR-AbGAL3 groups on P17. Normalized to *Actin* and shown relative to the OIR group ($n = 4$ per group). (C) mRNA expression of *Tlr4*, *Trif*, and *Casp8* in the normal and GAL3-treated neutrophils in vitro. Normalized to *Actin* and shown relative to the NC group ($n = 4$ per group). (D) Molecular docking prediction model of TLR4 (purple) with GAL3 (yellow). The plot on the right shows the possible binding sites. (E) Immunoprecipitation experiments between GAL3 and TLR4. The top shows that immunoprecipitation of GAL3 coprecipitated TLR4. The lower panel shows that immunoprecipitation of TLR4 coprecipitated GAL3. The IgG lane serves as a negative control, and the input lane serves as a positive control. (F) IF staining of retinal vessels (CD31, red), NETs (CitH3, red), and nuclei (DAPI, blue) on P17 in the cross sections from the OIR and OIR-AbGAL3 groups. White triangles refer to the positive area of NETs. (G) Quantification of (F). Normalized to the OIR group. Scale bars, 20 μm ($n = 4$ per group). (H) IF staining of retinal neutrophils (LY6G, red), vessels (CD31, green), and nuclei (DAPI, blue) on P17 in the cross sections from the OIR and OIR-AbGAL3 groups. White triangles refer to the positive area of neutrophils. (I) Quantification of (H). Normalized to the OIR group. Scale bars, 20 μm ($n = 4$ per group). (J) IF staining of retinal neutrophils (LY6G, red), TLR4 (green), and

nuclei (DAPI, blue) on P17 in the cross sections from the OIR and OIR-AbGAL3 groups. White triangles refer to the colocalized area of LY6G and TLR4. (K) Quantification of (J). Normalized to the OIR group. Scale bars, 20 μm ($n = 4$ per group). (L) IF staining of retinal neutrophils (LY6G, red), CASP8 (green), and nuclei (DAPI, blue) on P17 in the cross sections from the OIR and OIR-AbGAL3 groups. White triangles refer to colocalized area of LY6G and CASP8. (M) Quantification of (L). Normalized to the OIR group. Scale bars, 20 μm ($n = 4$ per group). (N) Retina samples were collected on P17, followed by immunostaining for retinal vessels (red) in the wild-type group, knockout (KO) group, and KO group subjected to Neoseptin 3, rGAL3, and TAK-242 ($n = 4$ per group). In the schematic below, red indicates the NV area, and yellow indicates the VO area. The plots on the right show quantifications. Scale bars, 500 μm . (O) mRNA expression of *Mpo*, *Pad4*, and *Casp8* on P17 in untreated adult C57 mice (NC group) and mice subjected to GAL3, TAK-242, and GAL3 plus TAK-242 intravitreal injection. Normalized to *Actin* and shown relative to the NC group ($n = 4$ per group). (P) mRNA expression of *Mpo*, *Pad4*, and *Casp8* in untreated neutrophils (NC group) and neutrophils stimulated by GAL3, TAK-242, and GAL3 plus TAK-242. Normalized to *Actin* and shown relative to the NC group ($n = 4$ per group). (Q) IF staining of NETs (CitH3, green) and nuclei (DAPI, blue) produced by primary neutrophils in vitro stimulated by GAL3, GAL3 plus TAK-242, and neoseptin ($n = 4$ per group). Scale bars, 5 μm . CASP, caspase. Bars = means $\pm$ SD; Student's t test was used for (B), (C), (G), (I), (K), and (M). One-way ANOVA was used for (N), (O), (P), and (Q). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. rGAL3, recombinant GAL3.

DISCUSSION

Our findings reveal an unrecognized immune-mediated mechanism that sustains pathological angiogenesis in the retina—a reciprocal, GAL3-dependent loop between infiltrating neutrophils and resident

MG. This poses a challenge to the traditional MG-centric view of retinal angiogenesis by demonstrating that neutrophils not only participate in immune dysregulation but also serve as potent amplifiers of disease progression through NET release. In addition, the hydrogel

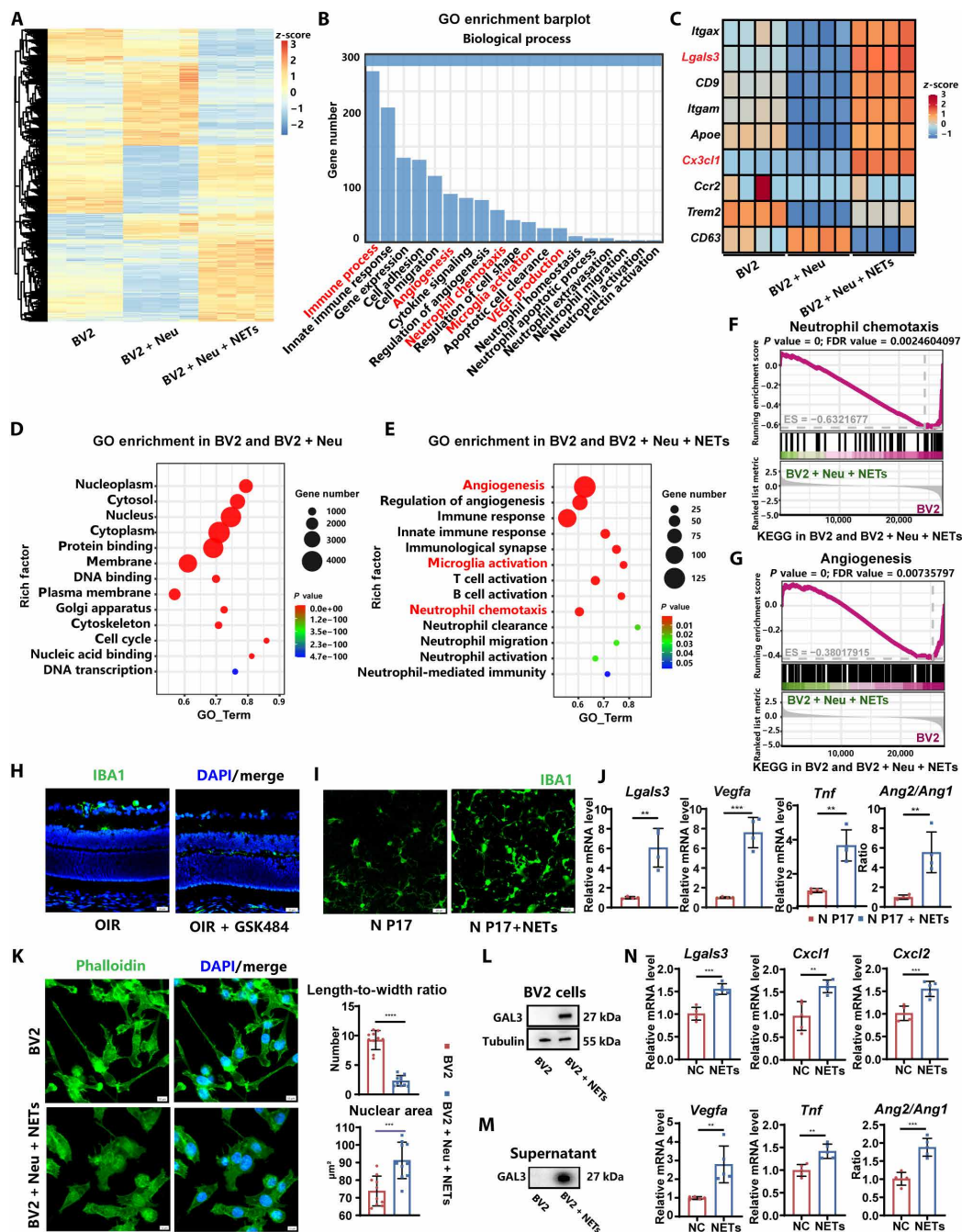


Fig. 6. NETs released by recruited neutrophils further activated MG. (A) The heatmap is shown for the difference in BV2 cells, BV2 cells cocultured with neutrophils, and BV2 cells cocultured with neutrophils releasing NETs ($n = 4$ per group). The black lines on the left represent a dendrogram, which groups molecules with highly similar expression patterns. (B) GO enrichment bar plot in biological process between BV2 cells, BV2 cells cocultured with neutrophils, and BV2 cells cocultured with neutrophils releasing NETs. The pathways related to the disturbance of immune microenvironment and angiogenesis are marked with red. (C) Heatmap representing the differentially expressed genes related to microglial activation in BV2 cells, BV2 cells cocultured with neutrophils, and BV2 cells cocultured with neutrophils releasing NETs. The gene *Lgals3* encoding GAL3 is marked with red. (D) GO enrichment dot plot between BV2 cells and BV2 cells cocultured with neutrophils. Dot size indicates the number of genes, and dot color corresponds to P value. (E) GO enrichment dot plot between BV2 cells cocultured with neutrophils and BV2 cells cocultured with neutrophils releasing NETs. Dot size indicates the number of genes, and dot color corresponds to P value. The pathways related to immune cell activation and angiogenesis were marked with red. (F) KEGG enrichment of neutrophil chemotaxis in BV2 cells cocultured with neutrophils and BV2 cells cocultured with neutrophils releasing NETs. (G) KEGG enrichment of angiogenesis in BV2 cells cocultured with neutrophils and BV2 cells cocultured with neutrophils releasing NETs. (H) IF staining of MG (IBA1, green) and nuclei (DAPI, blue) from OIR mice and OIR mice after GSK484 injection to inhibit NETs in the cross sections. (I) IF staining of MG (IBA1, green) from the normal P17 mice and mice subjected to NET injection in flat mount. (J) mRNA expression of genes related to GAL3 (*Lgals3*), angiogenesis (*Vegfa*), and inflammation (*Ang2/Ang1* and *Tnf*) in the retinas from the NC group and the group subjected to NET injection. Normalized to *Actin* and shown relative to the NC group ($n = 4$ per group). (K) IF staining of phalloidin (green) and nuclei (DAPI, blue) in BV2 cells and BV2 cells stimulated by NETs induced from primary neutrophils. Normalized to the untreated BV2 group ($n = 10$ per group). Scale bars, 10 μ m. (L) The Western blot shows the expression of GAL3 in the BV2 cells and BV2 cells cocultured with neutrophils releasing NETs. (M) The Western blot shows the expression of GAL3 in the supernatants from the BV2 cells and BV2 cells cocultured with neutrophils releasing NETs. (N) mRNA expression of genes related to GAL3 (*Lgals3*), neutrophil chemotaxis (*Cxcl1* and *Cxcl2*) and angiogenesis (*Vegfa*), and inflammation (*Ang2/Ang1* and *Tnf*) in BV2 cells and BV2 cells cocultured with NETs. Normalized to *Actin* and shown relative to the untreated BV2 group ($n = 5$ per group). Bars = means $\pm$ SD; Student's t test was used for (J), (K), and (N). ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. FDR, false discovery rate; ES, enrichment score.

platform overcomes the key limitations of current ocular drug delivery methods. Compared with the direct intravitreal injection of GAL3-neutralizing antibodies, as reported previously (15), our system achieved greater efficacy at lower concentrations with single administration (fig. S16, A and B).

Pathological angiogenesis is a hallmark of retinal vascular diseases. Although anti-VEGF therapy has revolutionized treatment and remains the clinical gold standard approach (30), its efficacy is limited—only approximately one-third of patients experience meaningful visual improvement (30–33). Moreover, long-term use is associated with

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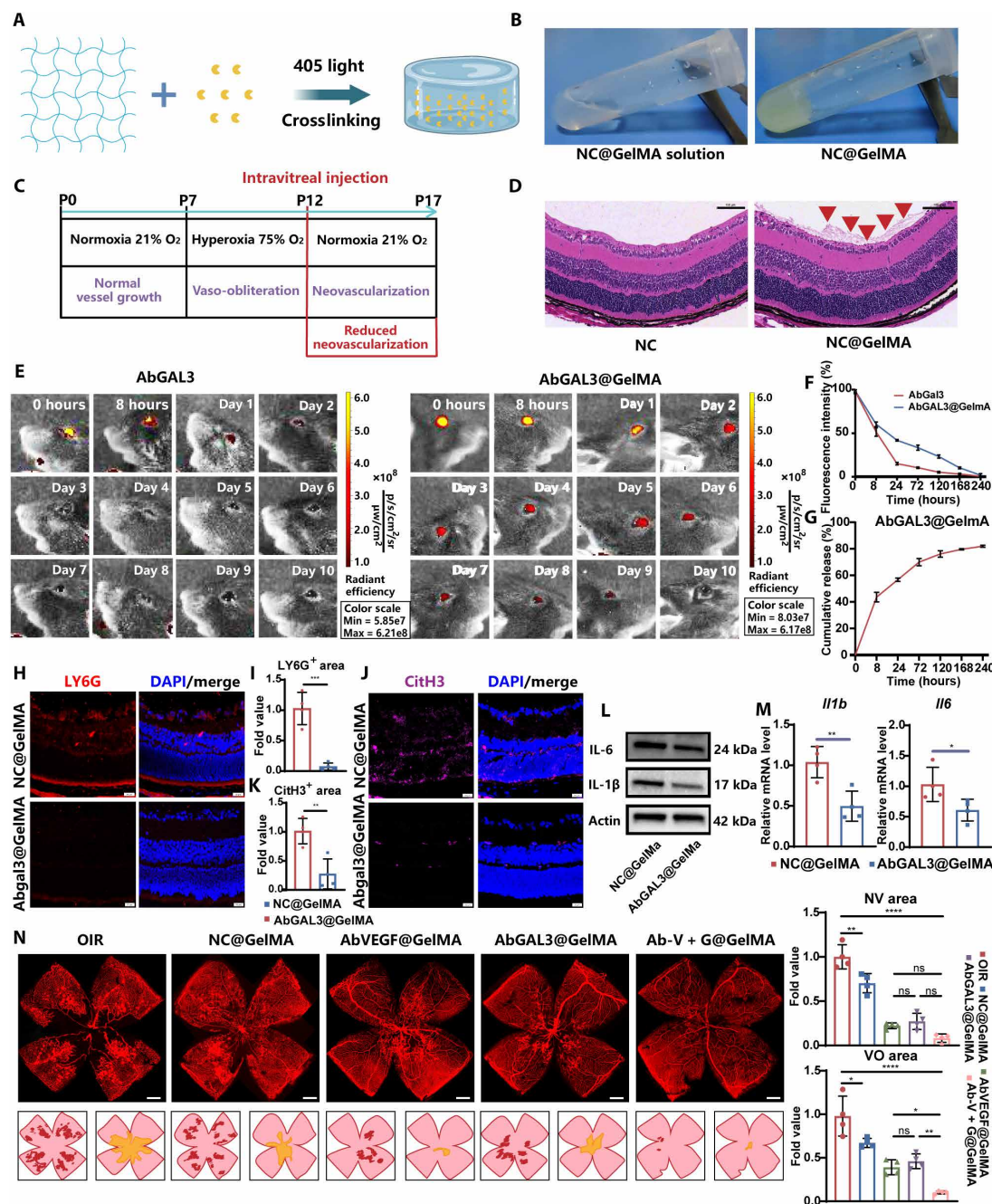


Fig. 7. A combined hydrogel inhibited the positive feedback and reduced retinal angiogenesis by inhibiting the function of GAL3.

(A) Schematic diagram of the preparation of the Ab@GelMA. The figure was created using BioRender.com. (B) Photograph of the GelMA solution and hydrogel formation after ultraviolet light exposure. (C) The schematic of C57 mice subjected to intravitreal injection of phosphate-buffered saline (PBS), NC@GelMA, and AbGAL3@GelMA on P12 when the OIR model was constructed. (D) Hematoxylin and eosin staining of C57 mice in the cross sections from the NC and AbGAL3@GelMA groups. Scale bars, 100 μ m. Red triangle, hydrogel attached to the surface of retina. (E) In vivo imaging system of adult mice subjected to intravitreal injection of AbGAL3 conjugated with AF488 and AbGAL3@GelMA conjugated with AF488. The color scales refer to radiant efficiency. (F) Quantification of fluorescence intensity in (E). Normalized to 0 hour of injection. (G) The calculated trend of cumulative release of antibody from hydrogel taken off from the surface of retina. (H) IF staining and (I) quantification of retinal neutrophils (LY6G, red) and nuclei (DAPI, blue) after the injection of GelMA in the cross sections from the NC@GelMA and AbGAL3@GelMA groups ($n = 4$ per group). Normalized to the NC@GelMA group. Scale bars, 20 μ m. (J) IF staining and (K) quantification of retinal NETs (CitH3, purple) and nuclei (DAPI, blue) after the injection of GelMA in the cross sections from the NC@GelMA and AbGAL3@GelMA groups. Normalized to the NC@GelMA group ($n = 4$ per group). Scale bar, 20 μ m. (L) The Western blot shows the expression of IL-6 and IL-1 β in the retina after intravitreal injection of NC@GelMA and AbGAL3@GelMA. (M) mRNA expression of *Il6* and *Il1b* in the retina after intravitreal injection of NC@GelMA and AbGAL3@GelMA. Normalized relative to the NC@GelMA group ($n = 4$ per group). (N) IF staining of retinal vessels (CD31, red) on P17 in the flat-mounted sections from the PBS, NC@GelMA, AbVEGF@GelMA, AbGAL3@GelMA, and both AbVEGF plus AbGAL3 (Ab-V + G)@GelMA groups. In the schematic below, red indicates the NV area, and yellow indicates the VO area. The plots on the right show quantifications normalized to the OIR group ($n = 4$ per group). Scale bars, 500 μ m. Ab, anti-GAL3 antibody. Bars = means $\pm$ SD; Student's *t* test was used for (I), (K), and (M). One-way ANOVA was used for (N). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

adverse effects and diminishing responses (30–32). These limitations underscore that anti-VEGF therapy addresses only part of the disease mechanism. Inspired by cancer immunotherapy strategies (34, 35), we propose that combining anti-angiogenic therapy with immunomodulation may yield synergistic benefits. We confirmed that the effect of the anti-GAL3/anti-VEGF combination substantially overpassed anti-VEGF alone. Our findings introduce an immunological mechanism, immune microenvironment dysregulation, as a key driver of retinal NV.

Neutrophils, the most abundant circulating leukocytes, play a central role in innate immunity, yet their functions in retinal angiogenesis remain poorly defined. Emerging studies have shown that, under pathological conditions, neutrophils are recruited through compromised vascular barriers (36–39) and release of NETs (40), which promote angiogenesis and vascular remodeling in the retina (27, 41–44). However, these findings are preliminary and require validation. In our study, scRNA-seq combined with OIR models

revealed that MG activation precedes neutrophil infiltration, yet the latter is not merely a passive consequence of vascular damage. Using myeloid cell-specific knockout mice and an in vitro BRB model, we demonstrated that MG-derived GAL3 directly activates circulating neutrophils through the TLR4 axis, triggering their transendothelial migration. Pioneer neutrophils then induce MG to release additional GAL3 through NET formation, creating a feed-forward loop that amplifies peripheral immune cell infiltration. NETs, instead of intact neutrophils, serve as active signaling vectors internalized by MG, inducing sustained GAL3 expression and proinflammatory gene activation. This cross-cellular epigenetic signaling may explain the prolonged immune disruption instigated by short-lived neutrophils. We propose that initial low-grade neutrophil infiltration primes MG through NETs, which, in turn, promotes further neutrophil recruitment, establishing a self-sustaining inflammatory circuit. This cascade positions neutrophils as early initiators of systemic immune-vascular dysregulation, linking localized MG activation to broader pathological angiogenesis.

The role of activated MG and MG-derived cytokines in retinal angiogenesis is well established; however, the multifaceted functions of individual cytokines in the broader retinal immune microenvironment remain underexplored. In this study, we identified GAL3 as a pivotal cytokine beyond its previously reported effects on endothelial regulation (15). GAL3, a well-characterized immunomodulatory lectin, has been implicated in diverse inflammatory and angiogenic pathologies through its interactions with hypoxia-inducible factor, VEGF pathways (45–51), and various membrane ligands (52–54). In this study, we demonstrated that GAL3 also contributes to maintaining immune homeostasis in the retina, and its dysregulation facilitates NV. To achieve a longer effect targeting this pathway, we developed a photocurable hydrogel capable of encapsulating an AbGAL3, enabling sustained drug release. This platform effectively disrupts the early phase of the GAL3-driven positive feedback loop, achieving favorable therapeutic outcomes. Its strong retinal adhesion minimizes dispersion-related complications, offering a promising adjunct to anti-VEGF therapies.

Despite these advances, some limitations of the study should also be acknowledged. Our analyses were limited to neutrophil-related parameters in peripheral blood, comparing healthy individuals and patients with retinal vascular diseases. Further investigations are necessary to characterize neutrophils and other immune cell subsets in ocular tissues. Given that GAL3 has also been reported to influence T cell differentiation and natural killer cell migration and homing (55–57), it is plausible that these and other immune populations participate in regulating the GAL3-driven feedback loop. Elucidating this broader immune regulatory network is essential for understanding immune microenvironment instability in retinal diseases.

In summary, our work reframes retinal NV as a consequence of immune dysregulation, introducing an immunomodulatory therapeutic paradigm. By targeting the MG-neutrophil feedback axis, GAL3 inhibition transitions treatment from reactive angiogenesis suppression to proactive restoration of immune equilibrium. Continued research into the underlying mechanisms will be crucial for refining this strategy and expanding its relevance to other immune-mediated vascular disorders.

MATERIALS AND METHODS

Study design

This study aimed to investigate the role of immune microenvironment dysregulation in modulating pathological NV in DR, with a focus on the cross-talk between resident MG and peripherally recruited neutrophils

and its underlying mechanisms. In human studies, we collected blood samples, vitreous humor, and aqueous humor to confirm the upgraded inflammation in patients with DR. In animal studies, a hypoxia-induced OIR mouse model was used to mimic pathological retinal angiogenesis in proliferate DR. Antibody-mediated depletion strategies and *Cx3cr1-Lgals3^{fl/fl}* transgenic mouse models were used to assess the respective roles of MG and neutrophils in pathological angiogenesis. For in vitro mechanistic studies, we used primary isolated murine neutrophils, along with human umbilical cord endothelial cells, the human HL-60 cell line, and the murine BV2 cell line.

We performed at least three technical replicates in all experiments, and the number of biological replicates for each experiment is indicated in the corresponding figure legends. Mice were randomly assigned to treatment groups, and all analyses were conducted by investigators blinded to genotype or treatment.

Study approval

Studies involving human donor samples were approved by Xijing Hospital, Fourth Military Medical University (KY20243268-1), and informed consent was obtained from all participants. All animal experiments were approved by the Institutional Animal Care and Use Committee and the Laboratory Animal Welfare and Ethics Committee of the Fourth Military Medical University (20240464).

Statistical analysis

Statistical analyses were conducted using Image-Pro Plus 6.0 and GraphPad Prism 5 software. Quantitative data are presented as mean $\pm$ SD. For comparisons between two groups, statistical significance was assessed using either a parametric unpaired Student's *t* test or a nonparametric Mann-Whitney *U* test. For comparisons involving three or more groups, one-way analysis of variance (ANOVA) was performed, followed by Šidák's multiple comparisons test to determine statistical significance. In ratio analyses, the control group was normalized to 1. *P* < 0.05 was considered statistically significant.

Supplementary Materials

The PDF file includes:

Materials and Methods

Figs. S1 to S16

Tables S1 to S5

Other Supplementary Material for this manuscript includes the following:

Data file S1

MDAR Reproducibility Checklist

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Disrupting galectin-3 and NET-mediated microglia-neutrophil cross-talk suppresses pathological retinal angiogenesis

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Editor's summary

How the immune microenvironment affects pathological retinal neovascularization is not fully understood in the context of diabetic retinopathy. Zhou *et al.* found that microglial activation and subsequent neutrophil infiltration were risk factors for diabetic retinopathy progression in human sequencing datasets. Microglial secretion of galectin-3 caused pathological angiogenesis in a mouse model of oxygen-induced retinopathy. Delivering neutralizing antibodies against galectin-3 and vascular endothelial growth factor via a photocurable hydrogel suppressed angiogenesis in this mouse model. Together, these data indicate an interplay between the immune microenvironment and pathological angiogenesis that may be investigated as a therapeutic avenue for diabetic retinopathy. —Brandon Berry

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